International Journal of Drug Policy 74 (2019) 236–245

Contents lists available at ScienceDirect

International Journal of Drug Policy

journal homepage: www.elsevier.com/locate/drugpo

Research Paper

Utility of a one-step screening and diagnosis strategy for viremic HCV infection among people who inject drugs in Catalonia

Verónica Saludes[a,b,c,d], Adrián Antuori[a,b,c,1], Cinta Folch[c,d,e,1], Noemí González[f], Núria Ibáñez[g], Xavier Majó[g], Joan Colom[h], Lurdes Matas[a,b,d], Jordi Casabona[c,d,e], Elisa Martró[a,b,c,d,*], on behalf of the HepCdetect II Study Group[2]

[a] Microbiology Department, Laboratori Clínic Metropolitana Nord, Hospital Universitari Germans Trias i Pujol, Crta. del Canyet s/n, Badalona, 08916 Barcelona, Spain
[b] Genetics and Microbiology Department, Universitat Autònoma de Barcelona, Badalona, Spain
[c] Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain
[d] Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[e] Centre for Epidemiological Studies on Sexually Transmitted Infections and HIV/AIDS of Catalonia (CEEISCAT), Public Health Agency of Catalonia (ASPCAT), Badalona, Spain
[f] El Local, Fundació IPSS, Sant Adrià del Besòs, Barcelona, Spain
[g] Program on Substance Abuse, ASPCAT, Barcelona, Spain
[h] Program for the Prevention, Control and Care of HIV, Sexually Transmitted Infections and Viral Hepatitis, ASPCAT, Barcelona, Spain

ARTICLE INFO

Keywords:
Hepatitis C virus (HCV)
People who inject drugs (PWID)
Dried blood spots (DBS)
One-step testing strategy
Unawareness
Harm-reduction services (HRS)

ABSTRACT

Background: In Catalonia (Spain), people who inject drugs (PWID) face numerous barriers to access to mainstream healthcare services for hepatitis C confirmatory diagnosis and treatment, so simplified testing strategies for viremic infection are urgently needed. Among PWID attending harm-reduction services in Catalonia, we aimed (i) to assess the utility of an *in-house* HCV-RNA detection assay on dried blood spots (DBS) as a one-step screening and confirmatory diagnosis strategy for hepatitis C, (ii) to estimate the prevalence of viremic HCV infection, and (iii) to identify factors associated with unawareness of viremic infection.

Methods: A cross-sectional study of current PWID ($N = 410$) was performed in four harm-reduction services. All participants underwent HCV antibody point-of-care testing and parallel DBS collection for centralized RNA testing. An epidemiological questionnaire was administered. Paired EDTA-plasma samples were additionally collected for HCV viral load testing in 300 participants.

Results: HCV-RNA testing from DBS was feasible and showed 97.2% sensitivity and 100% specificity for viral loads > 3000 IU/mL in real-life conditions. No significant differences in the performance when detecting viremic infections were observed between this one-step testing strategy vs. the conventional two-step algorithm involving venepuncture. Overall HCV seroprevalence was 79.8%, and prevalence of viremic infection was 58.5%. Importantly, 35.8% of viremic HCV participants were unaware of their status, and no specific socio-demographic or bio-behavioral factors independently associated with unawareness of viremic infection were identified. Among participants reporting a past or current HCV infection, 29.0% stated having received HCV antiviral treatment.

Conclusion: The high viremic HCV infection burden among PWID attending HRS, estimated for the first time in Catalonia, together with the low levels of awareness of viremic status and access to treatment, suggest that scaling up this one-step screening and diagnosis strategy to the network of harm-reduction services would help to achieve HCV elimination targets set by the World Health Organization.

* Corresponding author at: Microbiology Department, Laboratori Clínic Metropolitana Nord, Hospital Universitari Germans Trias i Pujol, Crta. del Canyet s/n, Badalona, 08916 Barcelona, Spain.
E-mail address: emartro@igtp.cat (E. Martró).
[1] These authors contributed equally to this article.
[2] Members of the HepCdetect II Study Group are included in the Acknowledgements section.

https://doi.org/10.1016/j.drugpo.2019.10.012

Introduction

The World Health Organization (WHO) has set main targets of reaching 90% diagnosis and 80% treatment rates to be achieved by 2030 to eliminate hepatitis C virus (HCV) infection as a major public health threat (World Health Organization, 2016a). In Spain, since the first National Plan against hepatitis C was approved in 2015, treatment rates have improved remarkably (Ministerio de Sanidad, Consumo y Bienestar Social, 2018), and recent modeling studies suggest that WHO elimination goals could be achieved before 2030, as long as 2017 levels of diagnosis and treatment can be maintained (Razavi, Sanchez, Pangerl & Cornberg, 2019). Recently, the proportion of viremic individuals unaware of their infection has been estimated at 29.4% in the general population attending primary care in Spain (Ministerio de Sanidad, 2019), while this data is unknown for certain populations who are at risk of HCV infection. Alternative testing strategies suitable for reaching undiagnosed cases need to be implemented before this situation leads to a stage where no more newly diagnosed people are available for treatment, which could be as early as 2022 ("diagnostic burn-out") (Hill, Nath & Simmons, 2017).

As HCV infection can be spontaneously cleared, the mainstay in diagnosing HCV infection is to initially screen for HCV antibodies (evidence of past or current HCV infection), and if reactive, HCV-RNA amplification tests are used to confirm the presence of HCV viremia in venous blood (conventional two-step diagnosis algorithm) (World Health Organization, 2017b). While reflex RNA testing upon a positive serological test is being implemented in Spanish hospitals (Crespo et al., 2019), several visits to mainstream healthcare centers are still required, from when the antibody test is ordered until the confirmed diagnosis is delivered.

People who inject drugs (PWID) constitute the core of the HCV epidemics in most European countries, where injection drug use remains the main mode of transmission among incident HCV infections (European Monitoring Centre for Drugs & Drug Addiction, 2016). Despite its high HCV prevalence, PWID are a marginalized population with limited access to hepatitis C diagnosis (Grebely, Dore, Morin, Rockstroh & Klein, 2017), which is mainly performed in mainstream healthcare services in Catalonia. The identification of HCV viremic infections among PWID is additionally hampered by the use of the venous blood (they often have poor venous access) and the conventional two-step diagnosis algorithm, which requires several visits at a specific date and time to the healthcare center, leading to high rates of losses to follow-up (Grebely, Applegate, Cunningham & Feld, 2017). Thus, despite a high proportion of PWID reporting previous HCV antibody testing, they often do not receive confirmatory HCV-RNA testing and, consequently, are unaware of their infection, putting them at risk of transmitting this infection to others, and making them ineligible for antiviral treatment (Fernàndez-López, Folch, Majó, Gasulla & Casabona, 2016; Iakunchykova et al., 2018; Iversen et al., 2017; Kåberg, Hammarberg, Lidman & Weiland, 2017). Accordingly, PWID have been recently considered a key population by the WHO for targeted testing of HCV viremic infection and prioritized antiviral treatment (European Association for the Study of the Liver, 2018; World Health Organization, 2018). Simplified strategies for HCV-RNA testing that can be easily implemented at the community level are urgently needed to reach people who are injecting drugs; evidence suggests that patient-centered, decentralized models of care embedded within existing services caring for this collective are required to improve HCV diagnosis and treatment (Applegate, Fajardo & Sacks, 2018; Bajis et al., 2017).

As no single HCV elimination strategy is applicable to all countries and settings (Bruggmann & Litwin, 2013; Dore, Ward & Thursz, 2014), micro-elimination has emerged as a strategy in which diagnosis and treatment approaches are tailored to fit each specific population at-risk (such as PWID) or geographical area (Lazarus, Wiktor, Colombo & Thursz, 2017). In Catalonia, there is a comprehensive network of drug harm-reduction services (HRS), including 16 harm-reduction centers and six mobile units (12 of them with supervised consumption rooms), that altogether cared for about 6000 PWID in 2017 (personal communication ASPCAT). Through this network, programmatic data are collected on a single centralized database. This infrastructure constitutes a unique opportunity for a targeted HCV micro-elimination strategy (Lazarus, 2017). While HRS in Catalonia offer the HCV antibody point-of-care test (Ab-PoCT) and the seroprevalence in current PWID was estimated at 65.8% (Folch, 2018), the prevalence of viremic infection is unknown because seropositive individuals need referral to the healthcare system for diagnosis confirmation, which often leads to losses to follow up (Fernàndez-López et al., 2016). Local estimates of viremic HCV prevalence are necessary to estimate the burden of HCV infection, identify key gaps in the HCV cascade of care, design evidence-based policies and, monitor the progress towards HCV elimination (World Health Organization, 2016b). The use of minimally invasive dried blood spot (DBS) samples has been demonstrated to be a facilitating tool for increasing access to HCV testing among PWID attending community centers (Coats & Dillon, 2015; Martin et al., 2013). Furthermore, even though available commercial viral load assays have not yet been approved by major regulatory authorities for the use of DBS, meta-analyses have shown a high level of diagnostic performance of both in-house and commercial assays for the use of DBS for HCV-RNA detection (Lange et al., 2017; Vázquez-Morón et al., 2019). Thus, among current PWID attending HRS in Catalonia, we aimed (i) to assess the utility of an HCV-RNA detection assay on DBS as a one-step screening and confirmatory diagnosis strategy for hepatitis C, (ii) to estimate the prevalence of viremic HCV infection, and (iii) to identify factors associated with unawareness of viremic infection.

Material and methods

Study design and participants

The HepC*detect* II study (EMCDDA 2019) was designed as a community-based cross-sectional study in a convenience sample ($N = 410$) of PWID ≥18 years old who had injected drugs over the previous six months (current injectors), recruited at four HRS by their own staff from May 2016 to July 2017. These HRS are located in the following cities in Barcelona province: Sant Adrià del Besòs (hereafter, HRS-A); Terrassa (HRS-B); Gavà i El Prat de Llobregat (HRS-C); and L'Hospitalet de Llobregat (HRS-D). HRS-A cares for the largest number of users in Catalonia, as it is located near one of the most important drug-trafficking and consumption areas in Spain. Altogether, these four HRS cared for 2558 people in 2016, representing 45.1% of all current PWID that attend the HRS network in Catalonia (Agència de Salut Pública de Catalunya, 2017). An economic incentive of 6€ was offered to study participants in order to encourage their participation. Written informed consent was obtained in accordance with law 14/2007, of 3 July, on biomedical research and all other applicable legislation (study approved by the Ethics Committee at the Germans Trias i Pujol University Hospital).

The sample size was calculated in order to reliably estimate the prevalence of HCV viremic infection. We considered that at the time of study design, there were about 2200 individuals who attended the four participant HRS, and that the prevalence of anti-HCV antibodies from oral fluid samples in individuals who attended HRS in Catalonia was about 65.8% (REDAN study 2014–2015 (Folch, 2018); furthermore, we assumed that about 26% of those exposed had spontaneously cleared the infection (Micallef, Kaldor & Dore, 2006) and about 10% had been cured through antiviral treatment and not reinfected, so a minimum HCV-RNA prevalence of 45% was expected. Therefore, a minimum sample size of 400 individuals would allow us to estimate a viremic HCV prevalence of 45%, with a confidence of 95% and a precision of +/− 4.45 percentage units.

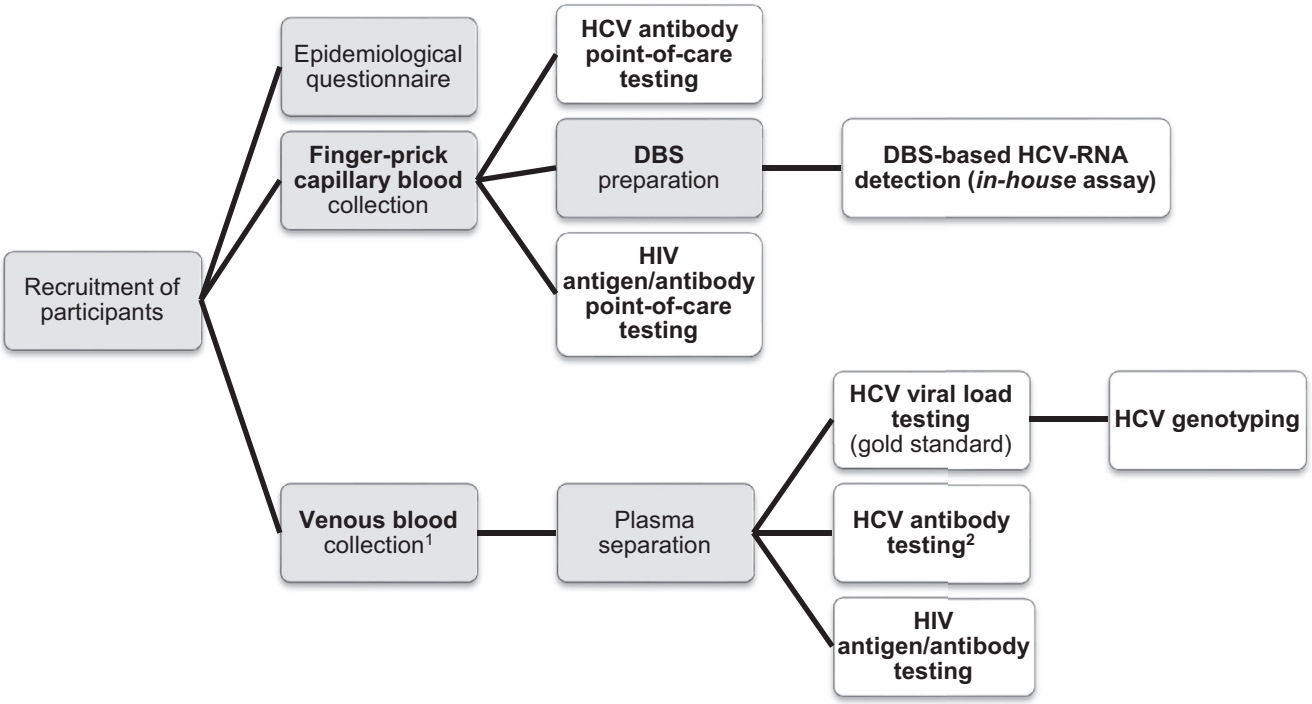

Fig. 1. *Procedures used for epidemiological and biological data collection.* Activities performed at the HRS and the laboratory are indicated by grey and white boxes, respectively; [1]Only performed at HRS-A; [2]Only performed in those cases in which a discordant result was observed between self-reported hepatitis C status and antibody point-of-care test results.

Data collection

An anonymous questionnaire administered by trained staff at the participating HRS was used for data collection. The questionnaire included questions related to socio-demographic characteristics, behavioral data regarding previous sexual practices, injected/non-injected drug use and types of drugs, imprisonment, self-reported HCV and HIV testing, previous diagnosis and treatment of HCV and HIV infection, and previous diagnosis of other unspecified sexually transmitted infections (STIs). Each participant was assigned an anonymous identifying number (ID) used to link the questionnaire to the tests results.

Clinical specimens and tests

The different clinical specimens and tests used are depicted in Fig. 1.

Point-of-care HIV and HCV testing

HRS in Catalonia offer HIV PoCT (HIV 1/2 Ag/Ab Combo, Alere Inc., Waltham MA, USA) from finger-prick capillary blood and HCV Ab-PoCT on oral fluid as part of their services when PWID report being unaware of their HIV or HCV status. For the purpose of this study, all participants underwent HCV Ab-PoCT testing from finger-prick capillary blood (LumiQuick Diagnostics, Inc., Santa Clara, CA, USA; 97.6% sensitivity and 99.5% specificity according to the manufacturer).

HCV-RNA testing in DBS

Finger-prick capillary blood was obtained from all participants using safety lancets Super (Sarstedt AG & Co. KG, Safety-Lancet Super, Nümbrecht, Germany). In order to ensure that two complete 50 µl spots were obtained per participant, capillary blood was collected with two EDTA capillaries marked to be filled with 50 µl of blood (Determine, EDTA Capillary tube, Chiba, Japan). Collected blood was then spotted (two 12 mm spots per participant) onto a Whatman card (903 protein saver card, GE Healthcare Europe GmbH, Barcelona, Spain). Spotted cards were air dried for at least one hour or until dry (uniform dark brown color), and then stored with desiccant with humidity indicator (GE Healthcare Europe GmbH, Barcelona, Spain) in individual pouches. DBS samples were shipped weekly at room temperature to a central laboratory for HCV-RNA testing. A previously developed *in-house* single-step reverse-transcription real-time PCR assay with a lower detection limit of 541 IU/mL of whole blood was used; this assay showed 100% sensitivity and specificity after a laboratory validation with samples from hospital outpatients from the Hepatology Unit, and HCV-RNA stability over a period of time up to two months was proven (HepC*detect* Study, Saludes et al., 2018). The number of days of storage of DBS at room temperature prior to HCV-RNA testing was recorded. An unrelated internal control RNA was added to each sample, and positive and negative controls were included in each run for quality control purposes as previously described (Saludes et al., 2018).

HIV and HCV testing in plasma

For the purpose of this study, as nursing staff were available at HRS-A, a venous blood sample was collected in EDTA tubes (BD Vacutainer® with EDTA K2, BD Switzerland Sarl, Vaud, Switzerland) in parallel to DBS collection. Plasma was separated by centrifugation and frozen at −30 °C until weekly shipment to the laboratory. The following serological and molecular markers were assessed in plasma, regardless of the results of the PoCT tests: HCV viral load (Abbott HCV RealTi*me*, Abbott Molecular Inc.; lower limit of detection of 12 IU/mL), and antigen and antibodies against HIV-1/2 (VITROS® HIV Combo, Ortho Clinical Diagnostics, Raritan, NJ, USA). Additionally, in those cases in which a discordant result was observed between self-reported hepatitis C status and HCV Ab-PoCT, HCV serology testing (VITROS® Anti-HCV, Ortho Clinical Diagnostics) was performed from plasma. Results were communicated to the HRS within 1–2 weeks. HRS professionals delivered HCV viral load and HIV serology results to participants and performed post-testing counseling on a return visit when possible; those cases in which it was not possible were considered lost to follow-up. Delivery of HCV viral load results and referral to care were recorded.

Table 1
Socio-demographic and bio-behavioral profile of study participants.

Characteristics	Total study population (N = 410) n (%)
Socio-demographic characteristics	
Age <30 years	43 (10.5)
Male gender	350 (85.4)
Foreign origin	115 (28.0)
Educational attainment > primary	142 (34.6)
Homeless[a]	53 (12.9)
Paid employment[a]	82 (21.4)
Ever been in prison	261 (63.8)
Bio-behavioral characteristics	
≤5 years of injected drug use	84 (20.5)
Injection while in prison	102 (27.0)
Daily injection[a]	193 (47.5)
Cocaine as the most frequently injected drug[a]	93 (22.9)
Sharing of syringes	180 (44.0)
Sharing of other injection equipment	214 (52.6)
Practicing front-backloading	121 (30.0)
Drug snorting	230 (58.1)
Sharing cocaine snorting straw	179 (79.2)
Drug smoking	187 (48.1)
Currently in treatment for drug addiction	240 (58.5)
Unprotected sex with penetration with sex worker, PWID, HIV-positive or MSM individuals[b]	94 (23.9)
Exchange of sex for drugs or money[b]	32 (7.9)
Past or current HCV infection (antibodies and/or RNA positive)	337 (83.8)
HIV infection	96 (23.4)
Self-reported STI[b]	29 (7.2)

[a] Previous 6 months;.
[b] Previous 12 months; STI, sexually transmitted infections.

HCV genotyping in plasma

HCV viremic cases recruited at HRS-A were genotyped to assess a potential effect of the HCV genotype on the HCV-RNA results obtained by the DBS assay. Thus, total nucleic acids extracted from plasma for HCV viral load testing, were subjected to a reverse-transcription (Saludes et al., 2013) and nested-PCR amplification as previously described (Martró et al., 2008). Purified amplification products were subjected to bidirectional Sanger sequencing with amplification primers. Sequence readings were edited and aligned together with reference sequences belonging to the different confirmed genotypes and subtypes, and maximum likelihood phylogenetic trees were obtained, as previously described (Saludes et al., 2019). For those participants with a viral load between 100 and 1000 IU/mL, the Abbott RealTime HCV Genotype II assay (Abbott Molecular Inc.) was used following manufacturer's recommendations.

Data analyses

Assessment of the utility of an HCV-RNA detection assay on DBS as an alternative one-step screening and confirmatory diagnosis strategy for hepatitis C

The following analyses were performed. Firstly, as DBS collection and storage conditions in real-life may differ from those used in the laboratory, the logistical feasibility of DBS sampling by HRS staff (including community workers and nurses) was evaluated as the proportion of samples collected that were adequate for HCV-RNA testing; DBS quality (color of the humidity indicator) and quantity (required number and diameter of the spots) were verified on their reception in the laboratory. Secondly, using paired DBS and plasma samples for those participants recruited at HRS-A, the clinical performance (sensitivity and specificity) of the *in-house* HCV-RNA assay on DBS was assessed in comparison with the reference viral load method (Abbott) at five thresholds in plasma: (i) detectable HCV-RNA (below or above 12 IU/mL; (ii) quantifiable HCV-RNA at ≥12 IU/mL; (iii) quantifiable HCV-RNA at ≥1000 IU/mL (European Association for the Study of the Liver, 2018); (iv) quantifiable HCV-RNA at >3000 IU/mL (World Health Organization, 2018); and (v) quantifiable HCV-RNA at >50,000 IU/mL (Terrault et al., 2005). DBS results were interpreted without knowledge of the results of the reference method in plasma. Besides, the putative effects of several relevant parameters on HCV-RNA results obtained from DBS samples were assessed (length of duration of storage at room temperature of DBS samples before processing, HIV co-infection, HCV genotype, gender and age of participants). Thirdly, as all participants from HRS-A were tested by the three HCV assays (Ab-PoCT, viral load in plasma and HCV-RNA in DBS), we assessed the performance of two different testing strategies when identifying viremic cases: the traditional two-step diagnostic strategy (Ab-PoCT screening followed by viral load confirmation in plasma if positive for antibody, provided that this service was available at the HRS), and the one-step RNA testing strategy from DBS. As we wished to assess the performance of the DBS-based assay for diagnosing HCV infection, participants reporting to be under antiviral treatment were excluded from the second and third analyses.

Statistical analyses

After validation of the HCV-RNA detection assay in DBS, the overall prevalence of viremic infection in PWID attending the four HRS was derived from DBS. The HCV seroprevalence was derived from the detection of antibodies by the Ab-PoCT in finger-prick blood. Self-reported HIV infection or a positive PoCT result was used to determine the prevalence of HIV infection. The proportion of viremic HCV participants unaware of their status was calculated as the number of participants who answered “no” or “I do not know” to the question “do you have hepatitis C?”, divided by those who were RNA positive on DBS. Proportions were compared using the Pearson's and the Fisher's exact test. For quantitative variables, means were compared using the Student's t-test after verification of the equality of variances using the Levene test. The 95% confidence intervals (CI) were calculated using the exact method. To identify factors associated with unawareness of viremic HCV infection, univariate and multivariate logistic regression models were performed. Variables with a significance level <0.10 in the univariate analysis were included in the multivariate analysis, after adjusting for age, years of injection and foreign origin, and the Odds Ratio (OR) with its respective 95% CI was calculated. Final multivariate models were derived using stepwise backward elimination process. Statistical significance was set at $P < 0.05$. The analyses were performed using SPSS version 17.

Results

Characteristics of the study population

A total of 410 individuals attending the four HRS were approached over the study period, and all accepted to participate (n = 300 in HRS-A, n = 44 in HRS-B, n = 27 in HRS-C, and n = 39 in HRS-D). The most relevant socio-demographic and behavioral characteristics of the study population are shown in Table 1. Participants had been injecting drugs for a mean of 17.7 years, while 20.5% reported ≤5 years of injected drug use. They were mostly male, with an average age of 40.5 ± 8.2 years. Foreign-born participants accounted for 28.0%, mainly coming from Central and Eastern European countries (CEE; 65.2%) and Italy (15.6%). The proportion of participants that reported being homeless over the past six months was 12.9%. Incarceration at least once in their life was reported by 63.8%, and 27.0% reported drug injection while in prison. Heroin was the most frequently used drug followed by cocaine. The lifetime prevalence of sharing syringes was 44.0%, and 52.6% reported sharing other injection equipment. Having been under drug addiction treatment at some time was reported by 88.5% of individuals.

Utility assessment of an HCV-RNA detection assay on DBS as an alternative one-step screening and confirmatory diagnosis strategy for hepatitis C

DBS sampling was logistically feasible at the four HRS, as only 0.98% (4/410) of collected DBS samples were rated as inadequate to be tested for HCV-RNA detection due to either quality or quantity. In these cases, a second sample was requested. DBS samples were shipped and stored at room temperature for a mean of 20.0 days (standard deviation, 11.0 days) before processing.

Paired plasma samples were obtained from the 300 participants recruited at Centre A in order to establish the clinical performance of the DBS-based assay in comparison with viral load results in plasma. Eleven cases were excluded, including those who reported to be under treatment ($n = 3$), and those for which an invalid HCV-RNA result was obtained from DBS (a run of nucleic acid extraction failed due to an error of the automated extractor and there was no sample left for re-testing, $n = 8$). Therefore, the comparison between DBS and plasma results was performed in 289 participants. Of these, 93 had an undetectable HCV viral load in plasma and all of them tested negative by the HCV-RNA assay from paired DBS samples; thus the DBS assay showed 100% specificity (95% CI, 96.0–100%). On the other hand, 196 participants had detectable viral loads in plasma (range, <12 to 33,096,148 IU/mL). As a considerable proportion of viremic PWID with low ($n = 20/196$, 10.2% below 3000 IU/mL; $n = 18/196$, 9.2% below 1000 IU/mL) and very low viral loads ($n = 10/196$, 5.1% below 100 IU/mL) was found, sensitivity of the DBS assay was assessed considering several relevant viral load thresholds (Table 2). When considering the requirement established by clinical guidelines for diagnostic viral load assays based on plasma (lower limit of detection ≤15 IU/mL (European Association for the Study of the Liver, 2018), i.e. considering all cases with detectable viral loads by Abbott) false-negative results in DBS samples were obtained in 11.7% (23 out of 196) of the cases. An HCV viral load <1000 IU/mL was the only factor significantly associated with obtaining false negative results ($p < 0.0001$); HIV infection, foreign origin, gender, age <30, or the mean number of days of DBS storage at room temperature before processing were not associated. Similarly, no evidence of a genotype/subtype-specific detection failure was found among the viremic patients successfully genotyped (94.9% of the 196 cases, as the other 10 cases had a viral load <100 IU/mL, which precluded its classification by any of the genotyping methods used). Circulating HCV genotypes and subtypes were 3a (38.7%), 1a (34.9%), 1b (17.7%), 4d (5.4%), 4a (2.7%), and 2a (0.5%). All these genotypes (except for 2a, which was only detected in one case) were represented among both true positive and false negative DBS samples. As the WHO guidelines support the use of tests that allow for increased access and affordability of HCV diagnosis (including DBS testing) that have a lower limit of detection of up to 3000 IU/mL (World Health Organization, 2018), we then used this threshold; when participants with viral loads below 3000 IU/mL were excluded, sensitivity was 97.2% (171 viremic participants were positive by DBS testing out of 176; 95% CI, 93.5–98.8%). HIV co-infection did not significantly influence sensitivity values of the DBS assay for any of the defined thresholds (Table 1), and specificity was 100% both in HIV positive (17/17; 95% CI, 81.6 − 100%) and negative participants (76/76; 95% CI, 95.2 − 100%).

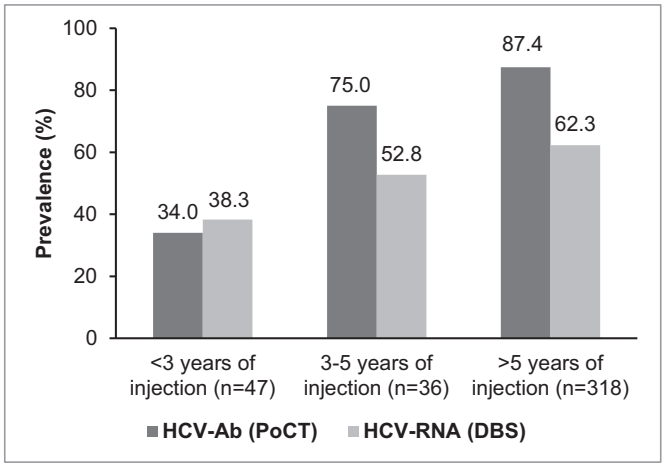

Fig. 2. *Relationship between HCV prevalence and the years of injected drug use.* The variable "years of injection" was missing for one individual. Antibody, Ab; PoCT, point-of-care test.

When the performance at detecting viremic cases was compared between the conventional two-step testing strategy vs. the alternative one-step strategy, no significant differences were found ($p = 0.230$): 64.7% (187/289; 95% CI, 59.0 − 70.0%) vs. 59.9% (173/289; 95% CI, 54.1 − 65.3%), respectively. Additionally, Fig. 2 shows the trend in the prevalence of HCV antibodies and RNA among the study population attending the four HRS by years of injection (<3, 3–5 and >5 years). As the DBS-based assay was being validated in real-life within this study, test results were not delivered to participants. However, we were able to assess the rate of delivery of HCV viral load results from plasma samples (reference method) to participants recruited at HRS-A on a return visit; results were successfully delivered to 76.0% viremic

Table 2
Sensitivity of the in-house HCV-RNA assay from DBS in comparison with the reference method of viral load in plasma considering several viral load thresholds.

HCV viral load threshold in plasma	Overall sensitivity		Sensitivity according to HIV status				
	Proportion	% (95% CI)	HIV positive		HIV negative		*p*-value
			Proportion	% (95% CI)	Proportion	% (95% CI)	
Detectable (below or above 12 IU/mL)[a]	173/196	88.3 (83.0–92.0)	41/48	85.4 (72.8–92.8)	132/148	89.2 (83.2–93.2)	0.480
Quantifiable (≥12 IU/mL)	172/191	90.1 (85.0–93.5)	41/47	87.2 (74.8–94.0)	131/144	91.0 (85.2–94.7)	0.623
≥1000 IU/mL[b]	171/178	96.1 (92.1–98.1)	41/42	97.6 (87.7–99.6)	130/136	95.6 (90.7–98.0)	1.000
>3000 IU/mL[c]	171/176	97.2 (93.5–98.8)	41/42	97.6 (87.7–99.6)	130/134	97.0 (92.6–98.8)	1.000
>50,000 IU/mL[d]	158/158	100 (97.6–100)	39/39	100 (91.0–100)	119/119	100 (96.9–100)	1.000

IU, International Units.
Clinical viral load thresholds used for the assessment of the sensitivity of the DBS-based assay.
[a] Lower limit of detection of the Abbott assay, in agreement with the lower limit of detection ≤15 IU/mL in serum or plasma recommended for HCV diagnosis (European Association for the Study of the Liver, 2018).
[b] In specific settings, a qualitative HCV-RNA assay with a lower limit of detection ≤1000 IU/ml can be used to provide broad affordable access to HCV diagnosis and care (European Association for the Study of the Liver, 2018).
[c] An HCV-RNA assay with a limit of detection of 3000 IU/mL or lower would be acceptable and identify 95% of those with viremic infection (World Health Organization, 2018).
[d] The vast majority of patients with an indication for anti-HCV therapy have an HCV-RNA level >50,000 IU/ml (Terrault et al., 2005).

individuals (n = 155) upon a return visit a median of 90 days later (IQR, 48–158 days), 84.5% (n = 131) of whom were newly referred to care (most of the remaining reported to be already linked to care).

Prevalence of viremic HCV infection

While the overall HCV seroprevalence was 79.8% (327/410; 95% CI, 75.6 − 83.4%), the prevalence of viremic infection was 58.5% (235/402; 95% CI, 53.6–63.2%, excluding the eight invalid HCV-RNA results representing 1.95% of all DBS). Past or current HCV infection (positive for HCV antibodies and/or HCV-RNA in DBS) was detected in 83.8% (337/402; 95% CI, 79.9–87.1%) of cases. Among those participants that self-reported a past or current HCV infection (72.0%, 295/410), 29% reported having received HCV antiviral treatment at some time. From all viremic participants, 15 cases (6.4%) were negative for HCV antibodies by the Ab-PoCT (six cases − 10.5% − among HIV co-infected). Among these 15 cases, four were classified as acute infections within the antibody window period (absence of antibodies was confirmed in paired plasma samples, and all cases were HIV negative). On the other hand, five were false negatives by this Ab-PoCT as antibodies were detected in plasma, while the rest could not be definitively classified as plasma was not available, although three of them reported having had a previous HCV diagnosis on the questionnaire.

In comparison, HIV infection was self-reported or detected by PoCT testing in 23.4% (95% CI, 19.6–27.7%) of participants, and among those who self-reported an HIV infection, 79.1% were under antiretroviral therapy. Co-infection with HIV was detected in 24.3% of viremic HCV cases. The prevalence of viremic HCV infection was not significantly different between HIV positive and negative PWID (60.0% vs. 58.0%, p = 0.727).

Estimation of the extent of unawareness of HCV viremic status

Results of self-reported HCV status were not recorded for 23 viremic participants. Among the remaining HCV viremic individuals, 35.8% (76/212) were unaware of their condition (new HCV diagnoses). In comparison, 8.3% (8/96) of HIV-positive participants were unaware of their HIV infection.

Results obtained in univariate and multivariate logistic regression analyses for the identification of factors associated with unawareness of viremic HCV infection are shown in Table 3. Univariate logistic regression analysis showed that foreign origin and ≤5 years of injected drug use were significantly associated with unawareness for viremic HCV infection, whereas reporting cocaine as the most frequently injected during the last six months, being currently in treatment for drug addiction, and HIV infection were significantly associated with awareness of viremic HCV infection. None of these variables were identified as independent predictors of unawareness of viremic infection according to the multivariate model adjusted by age, years of injection, and foreign origin.

Discussion

This study demonstrates the utility of a one-step screening and confirmatory diagnosis strategy based on the detection of HCV-RNA from DBS samples, as it provided reliable data on the prevalence of viremic infection among current PWID attending HRS in Catalonia for the first time. It also provided access for them to confirmatory diagnosis and raised their awareness of their infection in a community-based context. The high burden of viremic HCV infection observed, together with the fact that there is a low level of awareness of infection and little access to treatment, suggests that this strategy needs to be scaled up in the HRS network.

Although HRS in Catalonia only offered the Ab-PoCT in oral fluid before this study, it has been shown that implementation of DBS-based HCV-RNA testing at the four participating HRS is feasible. Additionally, the real-life validation of the in-house HCV-RNA detection assay in DBS showed an excellent clinical performance in individuals with viral loads above 3000 IU/mL (97.2% sensitivity and 100% specificity). Therefore, this assay fulfills the criteria for the most recent WHO recommendations for the identification of viremic infection, which include the use of HCV-RNA assays with a limit of detection of 3000 IU/mL or lower, with the aim of improving access to and affordability of HCV diagnostics, as they would identify 95% of all viremic infections (World Health Organization, 2018). This viral load threshold has been additionally supported by recent large studies showing that only 5% of viremic individuals show viral loads below 3000 (Bertisch et al., 2019) to 3311 IU/mL (Morgan Freiman et al., 2019). However, in this study it is remarkable that far more PWID were below this threshold (10.2% according to a highly sensitive commercial viral load assay in plasma). This fact led to false-negative results in DBS, especially among individuals with a viral load <1000 IU/mL in plasma, as would be expected because of the lower limit of sensitivity of the DBS-based in-house assay (541 IU/mL in whole blood) (Saludes et al., 2018). Several factors could have contributed to the proportion of low viral loads that we observed in our study population. Firstly, the above mentioned studies have found an association between young ages and low viral loads (Bertisch et al., 2019; Morgan Freiman et al., 2019). Morgan Freiman et al. have hypothesized that a low HCV viral load is a surrogate marker of a recently acquired infection. In fact, during acute infection frequent fluctuations in viremia may be observed; and even brief interludes of undetectable viral load (European Association for the Study of the Liver, 2018). Secondly, the same authors suggest an association of being from South East Asia with having low viral loads; interestingly, as many individuals from that region were active PWID and therefore at risk of early infection, the authors agreed there was a need to perform a sub-analysis of viral loads in PWID. On the other hand, Bertisch et al. did not find an association between a history of

Table 3
Univariate and multivariate logistic regression analyses of factors associated with unawareness of viremic HCV infection for the study population.

Characteristics	Proportions		Univariate analysis			Multivariate model		
	Aware (n = 136) n (%)	Unaware (n = 76) n (%)	OR	95% CI	p	AOR[a]	95% CI	p
Socio-demographic characteristics								
Age ≥30 years	126 (92.6)	67 (88.2)	1.69	0.66–4.37	0.277	0.86	0.31–2.64	0.860
Foreign origin	34 (25.0)	29 (38.2)	1.85	1.01–3.38	0.046	1.64	0.87–3.12	0.128
Bio-behavioral characteristics								
≤5 years of injected drug use	16 (11.8)	17 (22.4)	2.16	1.02–4.58	0.044	1.92	0.85–4.33	0.116
Cocaine (most frequently injected drug)[b]	43 (31.6)	14 (18.7)	0.50	0.25–0.98	0.045			
Currently in treatment for drug addiction	91 (66.9)	37 (48.7)	0.47	0.26–0.83	0.010	0.55	0.30–1.01	0.054
HIV infection	40 (29.4)	13 (17.1)	0.49	0.24–1.00	0.050			

[a] After adjusting for age, years of injection and foreign origin.
[b] Previous 6 months; OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

intravenous drug use and low viral load (Bertisch et al., 2019). Finally, the relatively high numbers of individuals with low viral loads observed in the present study could also be due to a high prevalence of cirrhosis, as heavy alcohol consumption is frequent among PWID (Irvin et al., 2019), and cirrhosis could hamper HCV replication in the liver (Duvoux et al., 1999; van Santen et al., 2018). In agreement with this, Morgan Freiman et al. observed that individuals with cirrhosis tended to have lower viral loads (Morgan Freiman et al., 2019). Unfortunately, in the present study we could not confirm the presence of an early HCV infection in those cases with detectable HCV antibody levels (the viral loads of the four participants with a seronegative acute infection were well above 3000 IU/mL) and we did not assess the stages of liver fibrosis. Further studies of the collective of current PWID are needed to determine the frequency and causes of low viral loads, as these cases might challenge simplified diagnostics assays in development (Bertisch et al., 2019; Peeling, Boeras, Marinucci & Easterbrook, 2017). However, even though alternative testing strategies might miss a small proportion of individuals with low viral loads, this has to be weighed against the benefits of identifying all those testing positive and who otherwise might not get tested through the conventional strategies. Besides, as viral loads may fluctuate over time, especially during acute infection, repeated testing of those with negative results due to low viral load would increase their chances of detection. It has been suggested that frequent screening of PWID in high-prevalence settings, as well as an improved care cascade would help to achieve the WHO HCV elimination target of an 80% reduction in incidence by 2030 (Scott et al., 2018).

The prevalence of viremic HCV infection among PWID who attend HRS in the province of Barcelona was 58.5%, which is the first estimation for Catalonia, and is within the upper range of prevalence observed among PWID in western European countries (18.9–65.8%) (Grebely et al., 2019). Additionally, the observed HCV seroprevalence was significantly higher than that reported by the latest cross-sectional sample of the REDAN biennial bio-behavioral study performed in 2014–2015 in PWID who attended the HRS network in Catalonia: 79.8% vs. 65.8% respectively ($p < 0.0001$) (Folch, 2018). This difference could be due to several causes: firstly, different characteristics of the study populations; secondly, an increase in the number of individuals exposed to HCV over the 2-year period between the two studies; and thirdly, the use of a more sensitive antibody assay in the present study (an HCV Ab-PoCT on finger-prick capillary blood with a sensitivity of 97.6% vs. an enzyme immunoassay in oral fluid with 86.7% sensitivity, Folch, Esteve, Zaragoza, Muñoz & Casabona, 2010). In Catalonia, on-site HCV testing with an Ab-PoCT for PWID attending HRS has been established since 2011 (Fernàndez-López et al., 2016), and those with a positive result are referred for viral load confirmation and disease evaluation. Nonetheless, it was estimated that 31% (11/35) of cases failed to attend for confirmatory RNA testing (Fernàndez-López et al., 2016) and non-attendance is also a main barrier to accessing treatment in other parts of the world (Blackburn, Patel & Zibbell, 2016; Iakunchykova et al., 2018; Iversen et al., 2017; Kåberg et al., 2017). Taking this into account and given the high prevalence of both HCV antibodies and RNA in this collective, direct testing of viremic HCV infection in a single step would be recommendable in the HRS setting in agreement with Scott et al. (2018). This modeling study suggested that carrying out annual HCV-RNA testing as a screening tool was justified for PWID in medium HCV-RNA prevalence settings (i.e. 50–74%) in order to achieve the WHO target for the reduction of new infections (Scott et al., 2018). In fact the DBS-based RNA screening strategy has proven reliable here, as evaluation in the field demonstrated good clinical performance and there was no statistically significant difference in the number of viremic cases identified between the one-step and the conventional two-step strategies. Another advantage of the one-step testing strategy is that it facilitates early diagnosis by detecting acute infections within the antibody window period as well as the viremic cases that give a false negative result for the Ab-PoCT. This allowed us to anticipate the diagnosis in 6.4% of viremic participants (10.5% among HIV-coinfected participants). In fact, it has been suggested previously that eliminating serological screening from the diagnostic algorithm could improve the diagnosis of viremic HCV infection among HIV-infected people, who are more prone to have false negative serological results (Grebely et al., 2017). Importantly, the DBS-based one-step strategy described here simplifies the testing process, although a study of the costs has not been performed. Given the high prevalence of both HCV antibodies and RNA observed, it would avoid the need for (i) using two different diagnostic tests in most of the cases (antibody and RNA), (ii) performing venepuncture by trained personnel (PWID may have a poor vein access, and personnel trained in venepuncture are not usually available in HRS), and (iii) maintaining the cold chain for shipment of samples to the central laboratory. Additionally, the limitations of the current HCV screening strategy implemented at HRS in Catalonia based on the Ab-PoCT are especially obvious among PWID with >5 years of injected drug use. This group represented 79.5% of the study population and showed a very high seroprevalence; with the current strategy, 87.4% of those tested would require referral to care for subsequent RNA confirmation. In contrast, direct screening for HCV-RNA in DBS would allow harm-reduction personnel to reserve referral to care for those PWID who are really in need of antiviral treatment (62.3%), while obviating referrals for those that have cleared the infection; this is especially important as the number of cured PWID will increase with the wider use of HCV antiviral therapies (Grebely et al., 2019; Iversen et al., 2019). Furthermore, those viremic individuals with a negative Ab-PoCT would not be missed with this strategy. Finally, HCV-RNA detection from DBS in the HRS setting could provide policy makers with useful information to monitor the local hepatitis C epidemic among PWID; data on prevalence of viremic infection is required to calculate many of the Core indicators to assess the progress towards elimination goals (Grebely et al., 2019; Iversen et al., 2019; World Health Organization, 2016b).

Another advantage is that this alternative on-site testing strategy would enhance PWID's awareness of their viremic HCV infection status. This would both improve awareness of viremic HCV infection, which is required to be linked to care, and decrease injection risk behavior (Bruneau et al., 2014). Remarkably, 35.8% of viremic participants were unaware of their status before participating in the study; assuming similar unawareness rates in PWID attending HRS in other geographical areas in Catalonia (6000 PWID in 2017), about 1257 persons could be unaware of their viremic infection, be at risk of transmitting this infection to others and not be considered for antiviral treatment. The proportion of unawareness was significantly higher for HCV than for HIV infection (8.3%) in the present study population. Regarding sociodemographic or bio-behavioral factors associated with unawareness of viremic infection, none of the variables significantly associated in the univariate analysis remained in the multivariate model. However, one of these factors (being in treatment for drug use) was significantly associated with awareness of previous exposure to HCV (based on antibody positivity) in a larger study performed in 2008–2012, which found that 18.9% of PWID attending the HRS network in Catalonia were unaware of it (Parés-Badell et al., 2017). According to the WHO elimination targets, with 90% of people infected aware of their status by 2030, a major increase in the diagnosis rate is still required (World Health Organization, 2017a). As centralized testing of DBS collected at HRS is still required, PWID have to attend a second visit to HRS to get their HCV-RNA results. However, the use of the DBS-based one-step diagnosis strategy in HRS could decrease the need of PWID to attend mainstream healthcare centers for phlebotomy and delivery of test results, thus reducing the number of individuals lost to follow up, and consequently, increasing the number of HCV-infected PWID that are aware of their status and that can be linked to care. Regarding delivery of HCV-RNA results at a return visit at HRS-A, when the same rate of delivery of viral load results was assumed for DBS results, it

proved to be feasible for up to 76.0% of viremic individuals, taking into consideration that it is a busy harm reduction center (the one with the largest number of different users per year in Catalonia). Ideally, high rates of results delivery and linkage to care should be guaranteed. While further simplification of HCV diagnostics with a newly available instrumented RNA PoCT has shown excellent clinical performance and provides results in one hour (Lamoury et al., 2018), the rate and time to delivery of results should be evaluated.

It is important to bear in mind that the 2018 international guidelines established that PWID should be treated without delay with direct acting antivirals (European Association for the Study of the Liver, 2018), that is to say, after the recruitment for this study had finished. Even so, a not inconsiderable proportion of those who reported past or current infection had received HCV antiviral treatment (29.0%). However, this proportion was remarkably lower than that for those who reported being HIV positive (79.1% were receiving antiretroviral treatment). Altogether, these findings indicate that, while universal HCV antibody testing has been available at the HRS network in Catalonia for the last eight years, there is a need to scale-up HCV-RNA testing and to facilitate access to treatment.

According to the literature, besides in-house assays, several commercial, highly sensitive, automated platforms for HCV viral load testing from serum/plasma have performed well in DBS (Catlett et al., 2019; Lange et al., 2017; Vázquez-Morón et al., 2019), and their use could facilitate scaling up DBS testing. However, CE marking or WHO prequalification that would enable these commercial assays to be used in clinical practice with delivery of results to patients have not been obtained yet, and together with costs this might limit their use at the present time. The need for regulatory approval of DBS-based assays has been called for (Easterbrook, Roberts, Sands & Peeling, 2017) and the Foundation for Innovative New Diagnostics (2019) is supporting manufacturers in this process (FIND. Hepatitis C & HIV). Until these approved assays are available, the use of in-house assays validated in real-life and complying with quality control measures according to local regulations (Genzen, 2019; Camaró, Catalá, Gimeno, Martínez & Olmos, 2013) will remain relevant.

This study presents limitations. Firstly, self-reported bio-behavioral information may not be completely reliable due to limited recall, stigmatization, or miscomprehension. However, we believe that this impact was minimized by the fact that the interviews were performed by trained staff within an environment of trust. Secondly, the in-house assay used for HCV-RNA testing from DBS is not as sensitive as commercial viral load assays, which could have led to somewhat underestimated overall prevalence of viremic infection. However, the results show that the estimation obtained should not significantly differ from that which would have been found if the conventional two-step algorithm including viral load in plasma with commercial assays had been used. Thirdly, the obtained prevalence data in the province of Barcelona might not be representative of all PWID attending HRS in Catalonia. Nevertheless, the most recent cross-sectional sample of the REDAN study (2014–2015) showed that there were no significant differences regarding seroprevalence data among the four Catalonia provinces. Fourthly, as PWID who attend HRS are those at higher risk of HCV infection (Wood et al., 2005), the observed prevalence is not representative of the whole PWID collective in Catalonia. Finally, cost-effectiveness studies are required to compare one-step vs. two-step diagnostic strategies in this high-risk population, where periodic RNA screening has been recommended (Scott et al., 2018).

In conclusion, this DBS-based one-step screening and diagnosis strategy represents a simple and reliable tool to increase the identification and self-knowledge of viremic HCV infections among PWID attending HRS in Catalonia. Furthermore, interventions to improve treatment access of HCV-infected PWID are urgently needed. In this sense, DBS testing could potentially enable assessment of treatment outcome and monitoring of reinfection in the HRS setting. Altogether, these data are crucial to guide efforts towards HCV micro-elimination among PWID in the context of the recently approved Plan for the Prevention and Control of Hepatitis C in Catalonia (Commission for the elaboration of the plan for the prevention & control of hepatitis C in Catalonia, 2018) in order to achieve the elimination goals set by the WHO.

CRediT authorship contribution statement

Verónica Saludes: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing. **Adrián Antuori:** Data curation, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Cinta Folch:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Noemí González:** Methodology, Resources, Visualization, Validation, Writing - review & editing. **Núria Ibáñez:** Methodology, Visualization, Writing - review & editing. **Xavier Majó:** Methodology, Visualization, Writing - review & editing. **Joan Colom:** Resources, Visualization, Validation, Writing - review & editing. **Lurdes Matas:** Resources, Visualization, Validation, Writing - review & editing. **Jordi Casabona:** Resources, Visualization, Validation, Writing - review & editing. **Elisa Martró:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

VS has received travel sponsorship from Gilead. EM is an advisory board member and has received research grants, as well as travel sponsorship and personal fees from Gilead as a speaker in meetings. EM also has received research grants and travel sponsorship for presentation of results at meetings by Abbott GmbH & Co. K.G. The other authors have no competing interests to declare.

Acknowledgments

The authors thank their participation in the study to all the other members of the HepC*detect* II Study Group: Laia Gasulla (ASPCAT), Sonia Cebrián (AIDE ONG, Terrassa, Barcelona, Spain), Jaume Minguell (Fundació AMBIT Prevenció, Gavà i El Prat de Llobregat, Barcelona, Spain), Aitor Remírez (AEC GRIS Fundació Privada, L'Hospitalet de Llobregat, Barcelona, Spain), Juliana Reyes-Urueña, Rafael Muñoz and Victoria González (CEEISCAT), Jordi Hernández and Sara González-Gómez (Microbiology Department, Laboratori Clínic Metropolitana Nord, Hospital Universitari Germans Trias i Pujol).

The authors also thank all the other professionals at HRS that have collaborated in this study: Dulce García, Vicente Cruz, Jennifer Hueltes, Judit Rebollo, Cristina Martínez, Marta Heras, Laura Quesada, Dídac Prat, and David Herrero.

Finally, the authors specially acknowledge and thank the people who inject drugs who have participated in this study.

The HepC*detect* II Study was partly funded by the competitive Fellowship Program from Gilead Spain (grant number GLD16-00135, 2017–2018), but Gilead had no role in study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to publish. This study was also funded by the Instituto de Salud Carlos III/FEDER, European Union (grant number PI15/000284, 2016–2018). EM holds a Miguel Servet position for researchers in the Spanish National Health System (Instituto de Salud Carlos III/FSE, European Union, grant number CPII15/00028, 2016–2019). Authors thank Harvey Evans for style corrections. Finally, authors thank the CERCA Programme/Generalitat de Catalunya for their support to the Germans Trias i Pujol Research Institute (IGTP).

References

Agència de Salut Pública de Catalunya. (2017). *Sistema d'Informació sobre Drogodependències a Catalunya.* Informe Annual Report of 2016.. Retrieved 1st October 2019 from drogues.gencat.cat/web/.content/minisite/drogues/professionals/epidemiologia/docs/SIDC-Informe-anual-2016-rev-09-05-2018-b.pdf.

Applegate, T. L., Fajardo, E., & Sacks, J. A. (2018). Hepatitis C virus diagnosis and the Holy Grail. *Infectious Disease Clinics of North America, 32*(2), 425–445. https://doi.org/10.1016/j.idc.2018.02.010.

Bajis, S., Dore, G. J., Hajarizadeh, B., Cunningham, E. B., Maher, L., & Grebely, J. (2017). Interventions to enhance testing, linkage to care and treatment uptake for hepatitis C virus infection among people who inject drugs: A systematic review. *International Journal of Drug Policy, 47*, 34–46. https://doi.org/10.1016/j.drugpo.2017.07.002.

Bertisch, B., Brezzi, M., Negro, F., Müllhaupt, B., Ottiger, C., & Künzler-Heule, P. Swiss Hepatitis C Cohort Study. (2019). Very low hepatitis C viral loads in treatment-naïve persons: Do they compromise hepatitis C virus antigen testing? *Clinical Infectious Diseases*. Epub ahead of print https://doi.org/10.1093/cid/ciz270.

Blackburn, N. A., Patel, R. C., & Zibbell, J. E. (2016). Improving screening methods for hepatitis C among people who inject drugs: Findings from the HepTLC initiative, 2012-2014. *Public Health Reports, 131*(Suppl_2), 91–97. https://doi.org/10.1177/00333549161310S214.

Bruggmann, P., & Litwin, A. H. (2013). Models of care for the management of hepatitis C virus among people who inject drugs: One size does not fit all. *Clinical Infectious Disease, 57*(Suppl_2), S56–S61. https://doi.org/10.1093/cid/cit271.

Bruneau, J., Zang, G., Abrahamowicz, M., Jutras-Aswad, D., Daniel, M., & Roy, E. (2014). Sustained drug use changes after hepatitis C screening and counseling among recently infected persons who inject drugs: A longitudinal study. *Clinical Infectious Diseases, 58*(6), 755–761. https://doi.org/10.1093/cid/cit938.

Camaró, M. L., Catalá, V., Gimeno, C., Martínez, R., & Olmos, P. (2013). Validación y verificación analítica de los métodos microbiológicos. Procedimientos en microbiología clínica. *Recomendaciones de la sociedad española de enfermedades infecciosas y microbiología clínica*.

Catlett, B., Carrera, A., Starr, M., Applegate, T. L., Lowe, P., Grebely, J., et al. (2019). Performance evaluation of the Hologic Aptima HCV Quant Dx assay for detection of HCV RNA from dried blood spots. *Journal of Clinical Virology, 112*, 40–44. https://doi.org/10.1016/j.jcv.2019.01.010.

Coats, J. T., & Dillon, J. F. (2015). The effect of introducing point-of-care or dried blood spot analysis on the uptake of hepatitis C virus testing in high-risk populations: A systematic review of the literature. *International Journal of Drug Policy, 26*(11), 1050–1055. https://doi.org/10.1016/j.drugpo.2015.05.001.

Commission for the elaboration of the plan for the prevention and control of hepatitis C in Catalonia. Generalitat de Catalunya. Departament de Salut. (2018). Pla de prevenció i control de l'hepatitis C a Catalunya. Retrieved 1st July 2019 fromhttp://salutpublica.gencat.cat/web/.content/minisite/aspcat/vigilancia_salut_publica/vih-sida-its/04_Hepatitis_viriques/Pla-Hepatitis-Definitiu_C_DEF.pdf.

Crespo, J., Lázaro de Mercado, P., Blasco Bravo, A. J., Aguilera Guirao, A., García-Samaniego Rey, J., Eiros Bouza, J. M., et al. (2019). El diagnóstico de la infección por el virus de la hepatitis C en España: una oportunidad para mejorar. *Enfermedades Infecciosas y Microbiología Clínica, 37*(4), 231–238. https://doi.org/10.1016/j.eimc.2018.05.016.

Dore, G. J., Ward, J., & Thursz, M. (2014). Hepatitis C disease burden and strategies to manage the burden (Guest editors Mark Thursz, Gregory Dore and John Ward). *Journal of Viral Hepatitis, 21*, 1–4. https://doi.org/10.1111/jvh.12253.

Duvoux, C., Pawlotsky, J. M., Bastie, A., Cherqui, D., Soussy, C. J., & Dhumeaux, D. (1999). Low HCV replication levels in end-stage hepatitis C virus-related liver disease. *Journal of Hepatology, 31*(4), 593–597.

Easterbrook, P. J., Roberts, T., Sands, A., & Peeling, R. (2017). Diagnosis of viral hepatitis. *Current Opinion in HIV and AIDS, 12*(3), 302–314. https://doi.org/10.1097/COH.0000000000000370.

European Association for the Study of the Liver. (2018). EASL recommendations on treatment of hepatitis C 2018. *Journal of Hepatology, 69*(2), 461–511.

European Monitoring Centre for Drugs and Drug Addiction. (2016). *Hepatitis C among drug users in Europe: Epidemiology, treatment and prevention*. (Vol. EMCDDA Ins). Retrieved 2nd June 2019 fromhttp://www.emcdda.europa.eu/system/files/publications/2953/TDXD16002ENN_final_web.pdf.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2019). *Hepatitis C: new models of care for drugs services*. Retrieved August 1st, 2019 from http://www.emcdda.europa.eu/publications/topic-overviews/hepatitis-c-models-of-care_en#panel7.

Fernàndez-López, L., Folch, C., Majó, X., Gasulla, L., & Casabona, J. (2016). Implementation of rapid HIV and HCV testing within harm reduction programmes for people who inject drugs: A pilot study. *AIDS Care, 28*(6), 712–716. https://doi.org/10.1080/09540121.2016.1164290.

Folch, C. (2018). *Acceso a servicios sanitarios entre las personas que se inyectan drogas inmigrantes que acuden a los centros de reducción de daños de Cataluña.* Toledo, Spain: XLV Jornadas Nacionales Socidrogalcohol.

Folch, C., Esteve, A., Zaragoza, K., Muñoz, R., & Casabona, J. (2010). Correlates of intensive alcohol and drug use in men who have sex with men in Catalonia, Spain. *European Journal of Public Health, 20*(2), 139–145. https://doi.org/10.1093/eurpub/ckp091.

Foundation for Innovative New Diagnostics (FIND). Hepatitis C & HIV. Retrieved 1st October (2019). fromhttps://www.finddx.org/hcv-hiv//.

Genzen, J. R. (2019). Regulation of laboratory-developed tests. *American Journal of Clinical Pathology, 152*(2), 122–131. https://doi.org/10.1093/ajcp/aqz096.

Grebely, J., Applegate, T. L., Cunningham, P., & Feld, J. J. (2017a). Hepatitis C point-of-care diagnostics: In search of a single visit diagnosis. *Expert Review of Molecular Diagnostics, 17*(12), 1109–1115. https://doi.org/10.1080/14737159.2017.1400385.

Grebely, J., Bruneau, J., Lazarus, J. V., Dalgard, O., Bruggmann, P., & Treloar, C. International Network on Hepatitis in Substance Users. (2017). Research priorities to achieve universal access to hepatitis C prevention, management and direct-acting antiviral treatment among people who inject drugs. *The International Journal on Drug Policy, 47*, 51–60. https://doi.org/10.1016/j.drugpo.2017.05.019.

Grebely, J., Dore, G. J., Morin, S., Rockstroh, J. K., & Klein, M. B. (2017c). Elimination of HCV as a public health concern among people who inject drugs by 2030 - What will it take to get there? *Journal of the International AIDS Society, 20*(1), 22146. https://doi.org/10.7448/IAS.20.1.22146.

Grebely, J., Larney, S., Peacock, A., Colledge, S., Leung, J., Hickman, M., et al. (2019). Global, regional, and country-level estimates of hepatitis C infection among people who have recently injected drugs. *Addiction, 114*(1), 150–166. https://doi.org/10.1111/add.14393.

Hill, A., Nath, S., & Simmons, B. (2017). PS16/5-The road to elimination of hepatitis C: Analysis of SVR versus new HCV infections in 91 countries. *Proceedinsg of the 6th European AIDS conference*.

Iakunchykova, O., Meteliuk, A., Zelenev, A., Mazhnaya, A., Tracy, M., & Altice, F. L. (2018). Hepatitis C virus status awareness and test results confirmation among people who inject drugs in Ukraine. *International Journal of Drug Policy, 57*, 11–17. https://doi.org/10.1016/j.drugpo.2018.03.022.

Irvin, R., Chander, G., Falade-Nwulia, O., Astemborski, J., Starbird, L., Kirk, G. D., et al. (2019). Overlapping epidemics of alcohol and illicit drug use among HCV-infected persons who inject drugs. *Addictive Behaviors, 96*, 56–61. https://doi.org/10.1016/j.addbeh.2019.04.023.

Iversen, J., Dore, G. J., Catlett, B., Cunningham, P., Grebely, J., & Maher, L. (2019). Association between rapid utilisation of direct hepatitis C antivirals and decline in the prevalence of viremia among people who inject drugs in Australia. *Journal of Hepatology, 70*(1), 33–39. https://doi.org/10.1016/j.jhep.2018.09.030.

Iversen, J., Grebely, J., Catlett, B., Cunningham, P., Dore, G. J., & Maher, L. (2017). Estimating the cascade of hepatitis C testing, care and treatment among people who inject drugs in Australia. *International Journal of Drug Policy, 47*, 77–85. https://doi.org/10.1016/j.drugpo.2017.05.022.

Kåberg, M., Hammarberg, A., Lidman, C., & Weiland, O. (2017). Prevalence of hepatitis C and pre-testing awareness of hepatitis C status in 1500 consecutive PWID participants at the Stockholm needle exchange program. *Infectious Diseases, 49*(10), 728–736. https://doi.org/10.1080/23744235.2017.1334263.

Lamoury, F. M. J., Bajis, S., Hajarizadeh, B., Marshall, A. D., Martinello, M., Ivanova, E., et al. (2018). Evaluation of the Xpert HCV viral load finger-stick point-of-care assay. *The Journal of Infectious Diseases, 217*(12), 1889–1896. https://doi.org/10.1093/infdis/jiy114.

Lange, B., Roberts, T., Cohn, J., Greenman, J., Camp, J., Ishizaki, A., et al. (2017). Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples – A systematic review and meta-analysis. *BMC Infectious Diseases, 17*(S1), 693. https://doi.org/10.1186/s12879-017-2776-z.

Lazarus, J. (2017). El papel de los sistemas de salud en la eliminación de las hepatitis víricas: ¿Estamos dando palos de ciego? *Les polítiques de Salut Pública sobre l'hepatitis C a Catalunya*. Retrieved 1st March 2019 from http://salutpublica.gencat.cat/web/.content/minisite/aspcat/publicacio_formacio_recerca/documentacio_jornades/2017_docu_jornades_aspcat/documentacio-de-la-jornada-politiques-de-salut-publica-en-hepatitis-c/1_Presentacio_Jeffrey_Lazarus_Jornada_HepatitisC_BCN_22-09-2017.pdf.

Lazarus, J., Wiktor, S., Colombo, M., & Thursz, M. EASL International Liver Foundation. (2017). Micro-elimination – A path to global elimination of hepatitis C. *Journal of Hepatology, 67*(4), 665–666. https://doi.org/10.1016/j.jhep.2017.06.033.

Martin, N. K., Hickman, M., Miners, A., Hutchinson, S. J., Taylor, A., & Vickerman, P. (2013). Cost-effectiveness of HCV case-finding for people who inject drugs via dried blood spot testing in specialist addiction services and prisons. *BMJ open, 3*(8), e003153. https://doi.org/10.1136/bmjopen-2013-003153.

Martró, E., González, V., Buckton, A. J., Saludes, V., Fernández, G., Matas, L., et al. (2008). Evaluation of a new assay in comparison with reverse hybridization and sequencing methods for hepatitis C virus genotyping targeting both 5' noncoding and nonstructural 5b genomic regions. *Journal of Clinical Microbiology, 46*(1), 192–197. https://doi.org/10.1128/JCM.01623-07.

Micallef, J. M., Kaldor, J. M., & Dore, G. J. (2006). Spontaneous viral clearance following acute hepatitis C infection: A systematic review of longitudinal studies. *Journal of Viral Hepatitis, 13*(1), 34–41. https://doi.org/10.1111/j.1365-2893.2005.00651.x.

Ministerio de Sanidad, Consumo y Bienestar Social. (2018). Plan estratégico para el abordaje de la hepatitis C en el sistema nacional de salud (PEAHC). Retrieved 1st July 2019 fromhttps://www.mscbs.gob.es/ciudadanos/enfLesiones/enfTransmisibles/hepatitisC/PlanEstrategicoHEPATITISC/docs/Plan_Estrategico_Abordaje_Hepatitis_C_(PEAHC).pdf.

Ministerio de Sanidad, Consumo y Bienestar Social. (2019). Prevalencia de la infección por hepatitis C en población general en España; 2017–2018. *Resultados del 2° Estudio de Seroprevalencia en España (2017–2018)*. Retrieved 10th July 2019 from https://www.mscbs.gob.es/ciudadanos/enfLesiones/enfTransmisibles/sida/docs/INFORME_INFECCION_VHC_ESPANA2019.pdf.

Morgan Freiman, J., Wang, J., Easterbrook, P. J., Robert Horsburgh, C., Marinucci, F., White, L. F., et al. (2019). Deriving the optimal limit of detection for an HCV point-of-care test for viraemic infection: Analysis of a global dataset. *Journal of Hepatology, 71*(1), 62–70. https://doi.org/10.1016/j.jhep.2019.02.011.

Parés-Badell, O., Espelt, A., Folch, C., Majó, X., González, V., Casabona, J., et al. (2017). Undiagnosed HIV and hepatitis C infection in people who inject drugs: From new evidence to better practice. *Journal of Substance Abuse Treatment, 77*, 13–20. https://doi.org/10.1016/j.jsat.2017.03.003.

Peeling, R. W., Boeras, D. I., Marinucci, F., & Easterbrook, P. (2017). The future of viral

hepatitis testing: Innovations in testing technologies and approaches. *BMC Infectious Diseases, 17*(Suppl 1), 699. https://doi.org/10.1186/s12879-017-2775-0.

Razavi, H., Sanchez, Y., Pangerl, A., & Cornberg, M. (2019). Global timing of hepatitis C virus elimination: Estimating the year countries will achieve the World Health Organization elimination targets. *Journal of Hepatology, 70*, e625–e854.

Saludes, V., Antuori, A., Reinhardt, B., Viciana, I., Clavijo, E., Schreiber, L., et al. (2019). Reliable resolution of ambiguous hepatitis C virus genotype 1 results with the Abbott HCV genotype plus RUO assay. *Scientific Reports, 9*(1), 3678. https://doi.org/10.1038/s41598-019-40099-3.

Saludes, V., Bascuñana, E., Jordana-Lluch, E., Casanovas, S., Ardèvol, M., Soler, E., et al. (2013). Relevance of baseline viral genetic heterogeneity and host factors for treatment outcome prediction in hepatitis C virus 1b-infected patients. *PloS One, 8*(8), e72600. https://doi.org/10.1371/journal.pone.0072600.

Saludes, V., Folch, C., Morales-Carmona, A., Ferrer, L., Fernàndez-López, L., Muñoz, R., et al. (2018). Community-based screening of hepatitis C with a one-step RNA detection algorithm from dried-blood spots: Analysis of key populations in Barcelona, Spain. *Journal of Viral Hepatitis, 25*(3), 236–244. https://doi.org/10.1111/jvh.12809.

Scott, N., Sacks-Davis, R., Pedrana, A., Doyle, J., Thompson, A., & Hellard, M. (2018). Eliminating hepatitis C: The importance of frequent testing of people who inject drugs in high-prevalence settings. *Journal of Viral Hepatitis, 25*(12), 1472–1480. https://doi.org/10.1111/jvh.12975.

Terrault, N. A., Pawlotsky, J.-. M., McHutchison, J., Anderson, F., Krajden, M., Gordon, S., et al. (2005). Clinical utility of viral load measurements in individuals with chronic hepatitis C infection on antiviral therapy. *Journal of Viral Hepatitis, 12*(5), 465–472. https://doi.org/10.1111/j.1365-2893.2005.00615.x.

van Santen, D. K., Schim van der Loeff, M. F., Cartier van Dissel, J., Martens, J. P. D., van der Valk, M., & Prins, M. (2018). High proportions of liver fibrosis and cirrhosis in an ageing population of people who use drugs in Amsterdam, the Netherlands. *European Journal of Gastroenterology & Hepatology, 30*(10), 1168–1176. https://doi.org/10.1097/MEG.0000000000001213.

Vázquez-Morón, S., Ardizone Jiménez, B., Jiménez-Sousa, M. A., Bellón, J. M., Ryan, P., & Resino, S. (2019). Evaluation of the diagnostic accuracy of laboratory-based screening for hepatitis C in dried blood spot samples: A systematic review and meta-analysis. *Scientific Reports, 9*(1), 7316. https://doi.org/10.1038/s41598-019-41139-8.

Wood, E., Tyndall, M. W., Li, K., Lloyd-Smith, E., Small, W., Montaner, J. S. G., et al. (2005). Do supervised injecting facilities attract higher-risk injection drug users? *American Journal of Preventive Medicine, 29*(2), 126–130. https://doi.org/10.1016/j.amepre.2005.04.011.

World Health Organization. (2016a). Global health sector strategy on viral hepatitis 2016–2021. Retrieved 1st May 2019 fromhttp://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/.

World Health Organization. (2016b). Monitoring and evaluation for viral hepatitis B and C: Recommended indicators and framework. Retrieved 15th May 2019 fromhttps://www.who.int/hepatitis/publications/hep-b-c-monitoring-evaluation/en/.

World Health Organization. (2017a). Action plan for the health sector response to viral hepatitis in the WHO European Region. Retrieved 2nd August 2018 fromhttp://www.euro.who.int/en/health-topics/communicable-diseases/hepatitis/publications/2017/action-plan-for-the-health-sector-response-to-viral-hepatitis-in-the-who-european-region-2017.

World Health Organization. (2017b). Guidelines on hepatitis B and C testing. Retrieved 3rd October 2018 from http://apps.who.int/iris/bitstream/10665/254621/1/9789241549981-eng.pdf.

World Health Organization. (2018). Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection. Retrieved 1st May 2019 fromhttps://www.who.int/hepatitis/publications/hepatitis-c-guidelines-2018/en/.