

Reactive markers of HIV, hepatitis B, hepatitis C and syphilis among people who use drugs in Sofia, Bulgaria: a cross-sectional bio-behavioural survey

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Abstract

Background

In eastern Europe and central Asia, HIV incidence among people who inject drugs has continued to rise, yet contemporary bio-behavioural data from Bulgaria are scarce. We measured the prevalence of reactive screening markers for HIV, hepatitis B, hepatitis C and syphilis among people who use drugs in Sofia and examined their correlates.

Methods

Cross-sectional survey of 480 adults reporting illicit drug use in the previous month, recruited through mobile outreach in June–August 2024. Capillary blood was screened on site with rapid tests. Because the outcomes are common, associations are expressed as adjusted prevalence ratios from modified Poisson regression with robust variance, with covariates chosen from a directed acyclic graph rather than by significance screening.

Results

Of 480 participants (78.8% men, median age 40 years), 320 (66.7%, 95% confidence interval 62.3–70.9) had at least one reactive marker. Anti-HCV was reactive in 305 (63.5%, 59.1–67.9), anti-HIV in 61 (12.7%, 9.9–16.0), treponemal antibody in 15 (3.1%, 1.8–5.1) and HBsAg in 11 (2.3%, 1.1–4.1). Reactivity was concentrated among the 304 participants who had ever injected (anti-HCV 89.1% vs 19.3% among those who had not; anti-HIV 17.1% vs 5.1%), whereas treponemal antibody and HBsAg did not differ by injecting history. Anti-HCV reactivity rose from 25.0% at 20–29 years to 82.0% at 40–49 years (test for trend $p < 0.001$); anti-HIV showed no age trend ($p = 0.37$). Ever injecting was the strongest correlate of both anti-HCV (adjusted prevalence ratio 4.13, 2.99–5.70) and anti-HIV reactivity (4.29, 2.01–9.16). Current unstable housing was associated with anti-HIV reactivity (1.88, 1.14–3.11) but not with anti-HCV. Of 61 participants with reactive HIV tests, 53 (87%) were anti-HCV reactive.

Conclusions

Two thirds of people who use drugs reached by outreach in Sofia carry a reactive marker of blood-borne infection, and anti-HCV reactivity is close to universal among people with long injecting histories. The age pattern points to exposure accumulated over decades rather than a recently introduced epidemic. Because antibody testing cannot separate current from cleared hepatitis C, RNA testing is needed before treatment need can be estimated, and HIV and hepatitis services for this population should be integrated.

Background

People who inject drugs carry a burden of blood-borne infection out of all proportion to their number. Global syntheses estimate that 15–18% of people who inject drugs are living with HIV, that 52% are anti-HCV positive and 9% HBsAg positive [1, 2], and that about 39% have current, viraemic HCV infection [2, 3]. In 37 countries more than 60% are anti-HCV positive [4]. A meta-analysis of studies published between 2008 and 2018 estimated 15% for HIV, 60% for anti-HCV and 6% for HBsAg among people who inject drugs, far above the corresponding figures among female sex workers (5%, 1% and 3%) [5]. Injecting accounts for an estimated 79% of new HCV infections in high-income countries and 43% globally [6], and pooled HCV incidence among people who inject drugs, about 12 per 100 person-years, is roughly seven times the pooled HIV incidence of 1.7 per 100 person-years [7]. Part of that difference lies in the higher per-exposure infectivity of HCV through receptive needle sharing, estimated at about 1.7 times that of HIV [8]. It is compounded by the far higher prevalence of HCV among injecting partners, so that a level of sharing low enough to keep HIV incidence modest can still produce near-universal HCV exposure over an injecting career.

Sexually transmitted infection has been documented less systematically in this group. The first dedicated meta-analysis, published in 2025, estimated active syphilis at 3.2% overall but 8.1% among women who inject drugs [9]. This asymmetry matters in a population in which parenteral and sexual risk are often treated as interchangeable markers, and it is one of the contrasts this study was able to examine directly.

The epidemiology also varies more between settings than global pooled estimates suggest. In the Eastern Mediterranean region, pooled HIV prevalence among people who inject drugs is 19%, higher than the global figure, while HCV prevalence (45%) is lower, with wide variation between countries [10]. In Germany, pilot monitoring of people who had injected in the past year found HIV in 2.7% alongside past or current HCV infection in about 72% [11]. Country-level data are therefore not interchangeable, and regional averages can conceal both unusually severe and unusually well-controlled epidemics.

Eastern Europe and central Asia, as defined for HIV surveillance, is the only region in which HIV incidence and mortality continued to rise through the late 2010s, and injecting drug use compounded by structural barriers to prevention is the principal reason [12, 13]. Across central Asia and the Caucasus, HIV prevalence among people who inject drugs ranges from 0% to 30% and HCV prevalence from under 1% to 92% [14]; in Ukraine, anti-HCV prevalence in this population rose from 50% to 73% between 2007 and 2020 [15]. The region has experienced large, overlapping epidemics of syphilis, hepatitis C and HIV, and hepatitis C is among the ten leading causes of death there [16]. Globally, needle and syringe provision and opioid agonist treatment fall short of World Health Organization coverage benchmarks in most countries, and only 47% of people who inject drugs have ever been tested for HCV antibody [17–19].

This shortfall matters because the effectiveness of those interventions is well established, although the underlying evidence is largely observational. Opioid agonist treatment reduces HIV acquisition by about half and HCV acquisition by a comparable margin, and combined with high-coverage needle and syringe

provision the reduction in HCV acquisition approaches three quarters [20–23]. An overview of systematic reviews reached the same conclusion for needle and syringe programmes, while noting that the evidence is stronger for HIV than for HCV and that effect sizes depend on the coverage actually achieved [24]. Opioid agonist treatment also reduces all-cause and overdose mortality and improves engagement with HCV care [25, 26]. Modelling for settings in eastern Europe and central Asia suggests that the resources released by moving from a criminalising to a public health approach to drug use would pay for scaling up antiretroviral therapy and opioid agonist treatment enough to prevent the majority of new HIV infections among people who inject drugs [27]. The obstacle in this region is less the absence of effective tools than the policy environment governing their delivery.

Structural disadvantage shapes who acquires these infections, independently of individual injecting practice. In a systematic review and meta-analysis, recent homelessness or unstable housing was associated with a 39% increase in HIV acquisition and a 64% increase in HCV acquisition among people who inject drugs after adjustment [28], and modelling attributes 8% of new HIV and 11% of new HCV infections in this population globally to unstable housing [29]. Incarceration, policing practice and barriers to sterile equipment operate in a similar way, both directly and by displacing people from services [30–32].

The drugs being used also bear on transmission. Stimulants are widely injected, and stimulant injection carries its own excess of injecting and sexual risk, over and above opioid-related harm [33, 34]. Having injected methamphetamine has been independently associated with HCV seropositivity among people who inject drugs [35], and amphetamine use has been associated with raised HIV incidence in populations in which sexual rather than parenteral transmission predominates [36]. A population in which stimulants are widely used cannot be assumed to share the risk profile of the opioid-injecting populations from which most European estimates derive.

Bulgarian data are sparse and mostly a decade or more old. A survey of people who inject drugs in Sofia in the early 2000s found anti-HCV in 73.9%, HBV markers in 6% and HIV in 0.5% [37]. Molecular analyses dated the introduction of HIV-1 into Bulgarian injecting networks to the early 2000s, with 359 diagnoses among people who inject drugs by 2011 and HCV co-infection in 92.6% of those tested [38]; HCV itself had spread among people who inject drugs in Bulgaria for decades before reaching a plateau in the early 2000s [39]. National series of people living with HIV have reported HBsAg in 9–10% and anti-HCV in 23–26%, concentrated in key populations including people who inject drugs, men who have sex with men, prisoners and Roma communities [40, 41]. Neighbouring countries show how quickly this can change. Romania recorded an outbreak of multiple blood-borne infections among people who inject drugs from 2011 [42], and the 2011 Athens outbreak arose in a population in which one in eight people were receiving opioid substitution and the median number of free syringes received in the preceding month was zero [43]; transmission there continued for years afterwards [44].

Against that background, we conducted a bio-behavioural survey among people who use drugs reached by outreach services in Sofia. Our aims were to measure the prevalence of reactive screening markers

for HIV, hepatitis B, hepatitis C and syphilis, to describe how that burden is distributed by injecting history, age and social circumstances, and to identify the correlates most relevant to the design of harm reduction services. Because HCV antibody records lifetime exposure rather than current infection, we also consider what these data can and cannot establish about ongoing transmission. We report associations rather than causal effects, for reasons set out in the Methods.

Methods

Study design and setting

We conducted a cross-sectional bio-behavioural survey among people who use drugs, both those who inject and those who do not, in Sofia, Bulgaria, from late June to August 2024. The survey was carried out together with the Center for Humane Policy, a Sofia-based non-governmental harm reduction organisation, and participants were reached through its community-based mobile outreach. Reporting follows the STROBE recommendations for cross-sectional studies, and the completed checklist is provided in Additional file 2.

Participants

Eligible participants were adults aged 18 years or over who reported illicit drug use, injecting or non-injecting, during the month before interview and who were able and willing to give written informed consent. No further exclusions were applied, because the intention was to characterise the whole population reachable through outreach. No formal sample size calculation was performed: every client reached by the outreach service during the fieldwork period who met these criteria was invited, none declined, and all 480 were enrolled. All 480 contributed both questionnaire and specimen data. Each participant received a voucher worth EUR 50 for food products.

Recruitment and sampling

Recruitment took place through community-based field outreach, using a mobile outreach office stationed at locations across Sofia known to be frequented by people who use drugs. Fieldwork was carried out by the Center's outreach workers, who provide harm reduction and support services to this population. Because these workers were already known and trusted at the recruitment sites, they could approach potential participants, explain the survey, establish eligibility and invite those who qualified. This was a practical necessity when working with a group that has good reason to be wary of research and of officialdom more generally.

Sampling was by convenience rather than by probability or respondent-driven methods. Respondent-driven sampling would have permitted weighted population estimates, but it requires seed recruitment, coupon management and measurement of network size that were beyond the resources available, and evidence that its assumptions hold in practice remains limited [45, 46]. The consequences for generalisability are considered in the Discussion.

Each participant was assigned a code built from the first letter of their given name, the month and day of birth, and the first letter of their mother's given name. The code linked questionnaire and specimen without recording names or other personal identifiers on either.

Data collection

Two sources of data were collected from each participant. The first was a structured face-to-face interview administered by trained interviewers. The questionnaire had thirteen sections: demographic characteristics; drug use; injecting behaviour; marriage; sexual history; sexual partners in the past 12 months; female partners; male partners; condom use; care for sexually transmitted infections; knowledge of HIV, HIV testing and pre-exposure prophylaxis; hepatitis B and C; and contact with outreach services. Interviewers were trained in questionnaire administration, informed consent, research ethics and non-stigmatising interaction before fieldwork began.

The second was capillary blood, collected by trained healthcare personnel and screened on site with commercially available rapid diagnostic tests. HIV screening used the ONE STEP Anti-HIV (1&2) test (InTec Products, Xiamen, China). HCV antibody, hepatitis B surface antigen and treponemal antibody were screened with the corresponding RightSign rapid test cassettes (Hangzhou Biotest Biotech, Hangzhou, China). All tests were run according to the manufacturers' instructions, and specimens and materials were labelled, stored and disposed of under standard biosafety procedures.

In outreach settings, rapid tests of this class have shown sensitivity and specificity above 95% for HCV antibody [47, 48] and HIV antibody [47] against laboratory reference assays, although the specific cassettes used here were not among the brands evaluated. No confirmatory laboratory testing was performed. We therefore describe all outcomes as reactive screening markers rather than as confirmed infections, and the distinction has substantive consequences. Anti-HCV reactivity indicates past or present exposure. It does not distinguish current viraemic infection from infection that has cleared spontaneously, as happens in about a quarter of people who inject drugs who acquire HCV [49, 50], or that has been cured by treatment, after which antibody usually persists. Treponemal antibody generally indicates lifetime rather than active syphilis [51]. Unconfirmed reactive HIV and HBsAg results carry a false-positive probability that is not negligible at the prevalences observed here.

Statistical analysis

All analyses were carried out in R version 4.5.3 using the tidyverse and the kkstatfun package, version 0.2.7 [52]. Package versions are listed in Additional file 1, Table S9.

Categorical variables are summarised as counts and percentages and continuous variables as medians with interquartile ranges (IQR), because age, age at first injection and duration of use were all skewed. Prevalences of reactive markers are reported with Clopper-Pearson exact binomial confidence intervals, which behave better than the normal approximation for the rarer markers; with eleven HBsAg-reactive

participants, a Wald interval would have been too narrow. Groups were compared with the chi-square test, with Fisher's exact test where expected cell counts fell below five, and with the Mann-Whitney U test for continuous variables. Trend across ordered age groups was assessed with the chi-square test for trend in proportions and summarised as an odds ratio per age stratum.

Because the four markers were measured in the same individuals, co-occurrence was not treated as a simple tabulation. For each pair of markers, the observed number of participants reactive for both was compared with the number expected under independence given the two marginal prevalences, and the departure was tested with Fisher's exact test. This separates co-reactivity that merely reflects two common markers in the same population from genuine clustering within individuals.

Covariates were selected from an explicit directed acyclic graph rather than by stepwise or significance-driven screening, following current guidance on causal diagrams in observational epidemiology [53–55]. The assumed structure is shown in Additional file 1 (Section S1, Figure S1), and minimal sufficient adjustment sets were derived with the dagitty package. Edges were oriented by temporal order wherever the exposure is a lifetime one. Because the injecting variable records whether a participant has ever injected, opioid agonist treatment can only follow it, and the graph carries an edge from injecting to opioid agonist treatment. Needle sharing and opioid agonist treatment lie on paths from structural disadvantage to reactivity. They are mediators, not confounders, and conditioning on them would remove the part of the association a harm reduction programme would seek to act on, so both were excluded from all models.

The two exposures of interest were current unstable housing (no stable place to live at interview) and ever having injected drugs. On the assumed graph, {sex, education} and {age, injecting, sex} are each sufficient adjustment sets for housing, and {age, housing, sex} is sufficient for injecting. The main model for each outcome included age (per 10 years), sex, education, housing and injecting. This single covariate set contains a sufficient adjustment set for both exposures and no mediator, so each exposure's coefficient can be read as an adjusted association under the assumed structure. The coefficients of the remaining covariates are not interpreted as effects. Estimates from the minimal adjustment sets are reported alongside in Additional file 1, Table S5.

We describe the resulting estimates as adjusted associations and not as total causal effects. The design is cross-sectional, and the temporal order of housing instability, injecting and seroconversion cannot be established from these data. The graph is used to choose a defensible covariate set and to identify what must not be conditioned on, not to license a causal reading of the output. For the same reason we do not report population attributable fractions: "ever injected" is a lifetime history rather than a modifiable exposure, and an attributable fraction computed from it would invite an interventional interpretation the design cannot support.

Associations are reported as adjusted prevalence ratios (aPR) from modified Poisson regression with robust sandwich variance, reported alongside adjusted odds ratios (aOR) from logistic regression for comparability with earlier studies. The prevalence ratio is the primary measure. With anti-HCV reactivity

at 63.5%, the outcome is far from rare and the odds ratio greatly overstates the prevalence ratio: an aOR of 25.5 for injecting corresponds to an aPR of 4.13, and reporting only the former would invite readers to read a fourfold difference in prevalence as a twenty-five-fold one [56–58]. Modified Poisson regression was preferred to log-binomial regression, which is more efficient where it converges but often fails to converge with common outcomes and several covariates, as it did here [59, 60]. Educational attainment was collapsed from five categories to three (none or primary, lower secondary, upper secondary or higher) because the original categories left zero to two HIV-reactive participants in the upper strata and produced complete separation. For HBsAg and treponemal antibody, with eleven and fifteen reactive participants, ordinary maximum likelihood is unreliable, and Firth's penalised likelihood was used with age, sex, housing and injecting as covariates.

Effect modification of the housing–HIV association by injecting was examined by stratified analysis with Mantel-Haenszel pooled estimates, and homogeneity of stratum-specific odds ratios was tested with Woolf's test. Departure from additivity was quantified by the relative excess risk due to interaction, the attributable proportion and the synergy index [61]. These measures are defined on the risk scale and were estimated from a modified Poisson model including the product term and adjusted for age and sex. With HIV reactivity at 12.7% the rare-disease approximation does not hold, and computing these measures from odds ratios would overstate departure from additivity. Confidence intervals come from 2000 case-resampling bootstrap replicates.

Robustness to unmeasured confounding was assessed with E-values, which express the minimum association an unmeasured confounder would need with both exposure and outcome, conditional on the measured covariates, to explain away an observed estimate [62]. E-values are reported as a descriptive property of the observed associations and not as evidence of causation, a distinction that has drawn justified criticism when the two are conflated [63]. Discrimination and calibration of the logistic versions of the main models were described with the area under the receiver operating characteristic curve (AUC) with a DeLong interval, the Brier score and the Hosmer-Lemeshow test across deciles of predicted risk. These describe how much of the variation in reactivity the measured variables account for; neither model is proposed as a screening instrument.

Missing data were minimal for all model covariates (education 2.3%, all others below 1%). Models were fitted on complete cases, and the analytic denominator is stated for every model. Injecting duration and needle sharing were asked only of participants who reported having injected; these are structural skips, not missing data, and are denominated on that group. A full accounting is given in Additional file 1, Table S1.

Four sensitivity analyses were carried out (Additional file 1, Table S8): substituting homelessness of more than a month in the past 12 months for current unstable housing; restricting the analysis to participants who had ever injected; refitting with log-binomial regression; and multiple imputation of missing education by chained equations (20 imputations), pooled by Rubin's rules over robust variances.

Two-sided p values below 0.05 were considered statistically significant. No adjustment was made for multiple comparisons. Secondary analyses are interpreted as estimation, not as formal hypothesis testing, and the argument rests on confidence intervals more than on p values.

Use of large language models

Claude (Anthropic) was used to edit the English of the manuscript and to check that reported numbers and cited claims matched the analysis output and the cited sources. The authors reviewed every change and take full responsibility for the content.

Results

Characteristics of the sample

Of the 480 participants, 378 (78.8%) were men. The sample was middle-aged: the median age was 40 years (IQR 33–44), and only 39 participants (8.1%) were under 25. We return to this age structure below. Characteristics are set out in Table 1.

Socioeconomic disadvantage was pervasive. Eighty-four participants (17.5%) had no stable place to live at interview, and 142 (29.6%) reported at least a month of homelessness during the preceding year. Educational attainment was low: 33 (6.9%) had never attended school and 2 had attended without completing any level, 71 (14.8%) had completed only primary education and 154 (32.1%) lower secondary education. A further 190 (39.6%) had completed upper secondary education and 19 (4.0%) held a higher degree; education was not recorded for 11 (2.3%). Roma participants made up 22.1% (n = 106) of the sample, several times their share of the national population. This over-representation is consistent with the concentration of Roma people among those with HIV and viral hepatitis co-infection in national series [41].

The median age at first sexual intercourse was 15 years (IQR 13–16). Seventy-eight participants (16.3%) had ever married. Sexual intercourse in the preceding 12 months was reported by 373 (77.7%) and in the preceding month by 270 (56.3%). Of the 372 who answered the question on condom use, 124 (33.3%) had used a condom at last intercourse, although the median number of partners in the preceding six months was one. At least one same-sex partner in the preceding six months was reported by 16 of 96 women (16.7%) and 20 of 346 men (5.8%) who answered.

Table 1
Demographic and social characteristics of participants (n = 480)

Characteristic	n (%) or median (IQR)
Age, years	40 (33–44)
Age group	
Under 20 years	5 (1.0)
20–29 years	88 (18.3)
30–39 years	132 (27.5)
40–49 years	222 (46.3)
50 years or over	33 (6.9)
Men	378 (78.8)
Roma ethnicity	106 (22.1)
Education	
Never attended school	33 (6.9)
Attended, no level completed	2 (0.4)
Primary	71 (14.8)
Lower secondary	154 (32.1)
Upper secondary	190 (39.6)
Higher	19 (4.0)
Not recorded	11 (2.3)
No stable place to live at interview	84 (17.5)
Homeless for more than a month in the past 12 months	
Yes	142 (29.6)
No	335 (69.8)
Not recorded	3 (0.6)
Age at first sexual intercourse, years	15 (13–16)
Sexual intercourse in the past 12 months	373 (77.7)
Sexual intercourse in the past month	270 (56.3)
Condom used at last intercourse ^a	124/372 (33.3)
Ever married	78 (16.3)

Characteristic	n (%) or median (IQR)
Age at marriage, years ^b	24 (19.8–30.0)

IQR, interquartile range. ^a Among participants who answered the question. ^b Among 72 ever-married participants with a recorded age at marriage.

Drug use and injecting behaviour

Drug use and injecting behaviour are summarised in Table 2. A history of injecting was reported by 304 participants (63.3%). The median age at first illicit drug use was 17 years (IQR 15–20) and at first injection 20 years (IQR 17–24), and the median duration of drug use at interview was 20 years (IQR 10–26).

Methadone was the substance most often reported (53.5%), followed by methamphetamine (32.1%), heroin (25.6%), clonazepam (22.9%), synthetic cannabinoids (21.2%) and cannabis (20.6%). Use was intensive: 370 participants (77.1%) used drugs daily or more often, and only 28 (5.8%) less than weekly. Two hundred and seventy-seven (57.7%) had received treatment for a substance use disorder at some point, and 132 (27.5%) were in treatment at the time of the survey. Opioid agonist treatment had ever been received by 228 participants, 47.5% of the whole sample; among the 277 ever treated for a substance use disorder, 226 (81.6%) had received it. The first of these figures is the one that describes coverage in this population.

Of the 298 participants who had injected and answered the question, 38 (12.8%) reported that the needle or syringe used at their last injection had previously been used by someone else, most often a friend (21) or a regular sexual partner (6). Over the preceding month, 4.3% had used previously used equipment every time or at least half the time, and 6.9% had used pre-filled syringes of unknown origin. Yet 385 of the 408 participants who answered (94.4%), including 293 of 297 who had injected (98.7%), said they could obtain new needles and syringes whenever they needed them. Reported access to sterile equipment is almost universal, while sharing persists at a level sufficient to sustain transmission, which points less to scarcity of equipment than to the circumstances in which sharing occurs [32].

Table 2
Drug use, treatment history and injecting behaviour (n = 480)

Characteristic	n (%) or median (IQR)
Age at first illicit drug use, years	17 (15–20)
Duration of drug use, years ^a	20 (10–26)
Ever injected drugs	304 (63.3)
Age at first injection, years ^b	20 (17–24)
Duration of injecting, years ^b	20 (10.5–25)
Substances used ^c	
Methadone	257 (53.5)
Methamphetamine	154 (32.1)
Heroin	123 (25.6)
Clonazepam	110 (22.9)
Synthetic cannabinoids	102 (21.2)
Cannabis	99 (20.6)
Cocaine	53 (11.0)
Amphetamine	37 (7.7)
Frequency of drug use	
Less than weekly	28 (5.8)
Weekly	77 (16.0)
Daily or more often	370 (77.1)
Not recorded	5 (1.0)
Ever treated for a substance use disorder	277 (57.7)
In treatment for a substance use disorder at interview	132 (27.5)
Ever received opioid agonist treatment	228 (47.5)
Used a previously used needle or syringe at last injection ^d	38/298 (12.8)
Can obtain new needles and syringes when needed ^e	385/408 (94.4)

IQR, interquartile range. ^a n = 468. ^b Among participants who had ever injected (n = 303 for duration). ^c Not mutually exclusive. ^d Among participants who had ever injected and answered. ^e Among all

participants who answered.

Prevalence of reactive markers

Three hundred and twenty participants (66.7%, 95% CI 62.3–70.9) had at least one reactive rapid test (Table 3). HCV antibody was by far the most common marker, found in 305 participants (63.5%, 59.1–67.9), followed by HIV antibody in 61 (12.7%, 9.9–16.0), treponemal antibody in 15 (3.1%, 1.8–5.1) and hepatitis B surface antigen in 11 (2.3%, 1.1–4.1).

Table 3
Prevalence of reactive markers of blood-borne infection (n = 480)

Marker	Reactive/tested	Prevalence, % (95% CI)
HCV antibody	305/480	63.5 (59.1–67.9)
HIV antibody	61/480	12.7 (9.9–16.0)
Treponemal antibody	15/480	3.1 (1.8–5.1)
Hepatitis B surface antigen	11/480	2.3 (1.1–4.1)
Any reactive marker	320/480	66.7 (62.3–70.9)

CI, Clopper-Pearson exact binomial confidence interval.

The burden was concentrated almost entirely among those who had injected (Fig. 1). Anti-HCV reactivity was 89.1% (95% CI 85.1–92.4) among the 304 participants who had ever injected, against 19.3% (13.8–25.9) among the 176 who had not. For HIV the figures were 17.1% (13.0–21.8) and 5.1% (2.4–9.5). Ninety-one per cent of those who had injected carried at least one marker, against a quarter of those who had not. The two markers that are transmitted sexually as well as parenterally behaved differently: treponemal antibody was reactive in 3.0% of those who had injected and 3.4% of those who had not, and HBsAg in 2.6% against 1.7%, with no meaningful gradient for either. In crude terms, ever having injected was associated with 4.6 times the prevalence of anti-HCV reactivity (PR 4.61, 95% CI 3.40–6.26) and 3.3 times the prevalence of HIV reactivity (PR 3.35, 1.69–6.62).

Age structure

Anti-HCV reactivity rose steeply with age, from 25.0% (95% CI 16.4–35.4) at 20–29 years to 59.1% (50.2–67.6) at 30–39 and 82.0% (76.3–86.8) at 40–49 (chi-square for trend 81.1, df = 1, $p < 0.001$; odds ratio 2.87 per age stratum, 95% CI 2.25–3.68), before falling to 69.7% (51.3–84.4) among the 33 participants aged 50 or over. HIV showed no such gradient: 3.4% at 20–29, 19.7% at 30–39, 14.0% at 40–49 and 3.0% at 50 or over, with no evidence of a linear trend (chi-square 0.80, df = 1, $p = 0.37$). The contrast is shown in Fig. 2.

Injecting duration showed a similar pattern. Among participants who had injected, anti-HCV reactivity was 62.5% (10/16) among those injecting for less than a year, 90.0% (18/20) at one to five years and

90.7% (243/268) beyond five years. The first two groups are small and their intervals wide, so the early part of this curve should not be over-read. What the data do support is that reactivity is already high among those with the shortest injecting histories and is effectively at a plateau thereafter.

Exposure of this kind accumulates over time, and cross-sectional prevalence cannot by itself show how much transmission is occurring now; we return to this in the Discussion.

Co-reactivity

Sixty-six participants carried two reactive markers and three carried three; a further 251 carried exactly one and 160 carried none. The only pairing that occurred appreciably more often than chance was HIV with HCV: 53 participants (11.0%) were reactive for both, against 38.8 expected under independence, an observed-to-expected ratio of 1.37 and an odds ratio of 4.38 (95% CI 2.00–10.95, $p < 0.001$). No other combination departed from independence (Table 4). HBsAg with HCV was observed nine times against seven expected, and treponemal antibody with HCV ten times against 9.5; neither is distinguishable from chance at this sample size.

Table 4
Co-occurrence of reactive markers compared with the frequency expected under independence

Combination	Observed, n (%)	Expected	Obs/exp	OR (95% CI)	p
HIV and HCV	53 (11.0)	38.8	1.37	4.38 (2.00–10.95)	< 0.001
HBsAg and HCV	9 (1.9)	7.0	1.29	2.63 (0.53–25.25)	0.342
Treponemal antibody and HCV	10 (2.1)	9.5	1.05	1.15 (0.35–4.37)	1.000
HIV and treponemal antibody	3 (0.6)	1.9	1.57	1.75 (0.31–6.76)	0.421
HIV and HBsAg	0 (0.0)	1.4	0.00	0.00 (0.00–2.74)	0.374

Expected counts assume independence given the two marginal prevalences. Obs/exp, observed-to-expected ratio; OR, odds ratio; p, Fisher's exact test.

Only the HIV–HCV pairing clustered within individuals, as a shared parenteral route would predict [64, 65]. Of the 61 participants with a reactive HIV test, 53 (87%) were also anti-HCV reactive.

Correlates of HIV and HCV reactivity

Adjusted estimates are given in Table 5 and Fig. 3, based on 469 participants with complete data on education.

Ever having injected was the strongest correlate of both markers: the aPR was 4.13 (95% CI 2.99–5.70, $p < 0.001$) for HCV and 4.29 (2.01–9.16, $p < 0.001$) for HIV. The corresponding aORs were 25.50 and 5.54. Estimates from the minimal adjustment set for injecting (age, sex and housing; $n = 480$) were almost identical, at 4.18 for HCV and 4.22 for HIV (Additional file 1, Table S5).

Current unstable housing was associated with HIV reactivity (aPR 1.88, 95% CI 1.14–3.11, $p = 0.013$), and the estimate was similar with the minimal adjustment set of sex and education (1.77, 1.07–2.94, $p = 0.027$). For HCV the association ran in the opposite direction and was imprecise (aPR 0.88, 0.77–1.02, $p = 0.081$; 0.76, 0.61–0.95 with the minimal set). We take the HIV association to be the substantive one; the inverse HCV association is examined in the sensitivity analyses below.

Age remained associated with HCV after adjustment (aPR 1.10 per 10 years, 1.01–1.20, $p = 0.026$) but not with HIV (0.85, 0.64–1.13, $p = 0.267$). Education showed a graded association with HIV: relative to participants with no schooling or primary education only, those with upper secondary education or above had an aPR of 0.43 (0.24–0.76, $p = 0.004$), with lower secondary education intermediate at 0.66 (0.39–1.14). No comparable gradient appeared for HCV. Men and women did not differ once injecting and education were accounted for (HIV aPR 1.32, 0.71–2.45; HCV aPR 1.08, 0.94–1.26), which suggests that the crude male excess in HCV reflects the sex composition of those who inject rather than sex itself.

Table 5
Adjusted prevalence ratios and odds ratios for HIV and HCV reactivity ($n = 469$)

Variable	HIV aPR (95% CI)	HIV aOR (95% CI)	HCV aPR (95% CI)	HCV aOR (95% CI)
Age, per 10 years	0.85 (0.64–1.13)	0.82 (0.54–1.23)	1.10 (1.01–1.20)	1.45 (1.05–1.99)
Men (vs women)	1.32 (0.71–2.45)	1.40 (0.68–3.14)	1.08 (0.94–1.26)	1.61 (0.85–3.03)
Lower secondary education	0.66 (0.39–1.14)	0.59 (0.29–1.21)	1.01 (0.86–1.18)	1.09 (0.53–2.21)
Upper secondary or higher	0.43 (0.24–0.76)	0.35 (0.17–0.73)	1.03 (0.90–1.20)	1.28 (0.64–2.55)
No stable place to live	1.88 (1.14–3.11)	2.25 (1.13–4.35)	0.88 (0.77–1.02)	0.56 (0.28–1.10)
Ever injected drugs	4.29 (2.01–9.16)	5.54 (2.49–13.63)	4.13 (2.99–5.70)	25.50 (14.74–45.55)

Each column is from one model containing all listed variables, fitted to the 469 participants with complete data on education. aPR, adjusted prevalence ratio from modified Poisson regression with robust variance; aOR, adjusted odds ratio from logistic regression. Reference categories: women, no schooling or primary education only, stable place to live, never injected.

HBsAg and treponemal antibody were modelled with Firth's penalised likelihood because of the small numbers (Additional file 1, Table S4). Nothing was associated with HBsAg, and all intervals were uninformative. For treponemal antibody the only association was with sex, and in the opposite direction to the parenteral markers: men had lower odds of reactivity than women (OR 0.29, 95% CI 0.11–0.77, $p = 0.013$), while injecting was unrelated (OR 1.25, 0.41–3.81). Syphilis in this population follows sexual rather than parenteral exposure and falls disproportionately on women, in line with the meta-analysis of sexually transmitted infection among people who inject drugs [9].

Stratified analysis, interaction and sensitivity analyses

The association between unstable housing and HIV was similar among participants who had and had not injected (stratum-specific odds ratios 2.73 and 1.76; Woolf $p = 0.591$), with a Mantel-Haenszel pooled odds ratio of 2.48 (95% CI 1.31–4.72). On the additive scale, relative to participants who had neither injected nor lacked stable housing, the prevalence ratio was 3.94 for injecting alone, 1.68 for unstable housing alone and 8.03 for both. The relative excess risk due to interaction was 3.41 (95% CI – 1.01 to 13.94), the attributable proportion 0.42 (– 0.16 to 0.74) and the synergy index 1.94 (0.79 to 7.55). All three intervals include the null, so we report no evidence of departure from additivity. The point estimates lie in the direction of synergy, but with 14 HIV-reactive participants in the doubly exposed group the analysis has little power to distinguish synergy from additivity (Additional file 1, Table S6).

Two of the four sensitivity analyses (Additional file 1, Table S8) qualify the findings. First, substituting homelessness of more than a month in the past year for current unstable housing attenuated the HIV association to the null (aPR 1.25, 95% CI 0.77–2.02, $p = 0.375$). The housing–HIV association therefore attaches to current housing instability, not to any experience of homelessness in the preceding year. Second, restricting the analysis to participants who had ever injected removed the inverse HCV–housing association entirely (aPR 1.02, 0.92–1.13, $p = 0.758$) while leaving the HIV association intact (aPR 1.90, 1.08–3.32, $p = 0.025$). The apparent protective association between unstable housing and HCV in the full sample therefore reflects confounding by injecting history: housed participants were older, had injected for longer and were almost uniformly anti-HCV reactive.

Multiple imputation of missing education produced estimates indistinguishable from the complete-case analysis (HIV: housing aPR 1.84 vs 1.88, injecting 4.56 vs 4.29), so the complete-case restriction is not consequential. Log-binomial models failed to converge for either outcome, as expected with common outcomes and several covariates [59, 60].

E-values are given in Additional file 1, Table S7. The injecting associations are not readily explained away: an unmeasured confounder would need to be associated with both exposure and outcome by a risk ratio of at least 8.05 for HIV and 7.73 for HCV, conditional on the measured covariates, to reduce them to the null. The housing–HIV association is more fragile, with an E-value of 3.17 for the point estimate and 1.54 for the confidence limit, so a moderately strong unmeasured confounder could account for it.

The HCV model discriminated well (AUC 0.877, 95% CI 0.841–0.914; Brier score 0.114; Hosmer-Lemeshow $p = 0.565$) and the HIV model considerably less so (AUC 0.714, 0.645–0.784; Brier score 0.102; $p = 0.411$) (Additional file 1, Section S7). Injecting history, age, sex, education and housing account for most of the variation in HCV reactivity, as expected for a marker acquired almost entirely parenterally that approaches saturation with cumulative exposure. The same variables account for much less of the variation in HIV reactivity, which suggests that HIV in this population is shaped by factors the survey did not measure, such as incarceration history and the size and structure of injecting networks [30, 44].

Discussion

Two thirds of the people reached by outreach services in Sofia in 2024 carried a reactive marker of blood-borne infection. Anti-HCV reactivity was 63.5% overall and 89.1% among those who had ever injected. The overall figure is similar to the 60% pooled estimate for people who inject drugs [5], and the figure among those who had injected is well above the global estimate of 52% [1]. It also exceeds the 73.9% recorded among people who inject drugs in Sofia two decades ago [37]. The two surveys differ in sampling and assays, so the comparison is approximate, but it gives no sign that lifetime HCV exposure has fallen in this population over that period. Pilot monitoring in Germany found a comparable level of past or current HCV infection, about 72%, among people who had recently injected, but HIV prevalence of only 2.7% [11]. By that standard, HIV reactivity of 17.1% among those who had injected in Sofia is high, and it represents a substantial rise from the 0.5% recorded in the early 2000s [37]. That rise is consistent with molecular evidence that HIV was introduced into Bulgarian injecting networks in the early 2000s and spread during the intervening period [38].

The age structure of reactivity says more about this epidemic than the overall prevalence does. Anti-HCV reactivity climbed from a quarter of participants in their twenties to more than four fifths of those in their forties, while HIV showed no trend across the same range. A steep age gradient in a lifetime marker, in a population with a median twenty-year drug-using career, is what exposure accumulating over time would produce; recent intense transmission would look different, with high prevalence already among the young. The injecting-duration pattern supports the same reading, since reactivity is already high in the shortest-duration group and plateaus thereafter. Studies elsewhere have likewise found a large share of people infected within the first years of injecting [35, 65, 66]. This is an inference about the shape of accumulated exposure, not a measurement of incidence. Cross-sectional antibody prevalence cannot establish current transmission intensity, and the small numbers in the shortest-duration group make the early part of the curve imprecise. The lower prevalence at 50 years or over rests on 33 participants and may reflect survivorship or a different drug-using history in this group.

Anti-HCV reactivity records exposure, not current infection, and this matters for planning. About a quarter of people who acquire HCV clear it spontaneously [49, 50], and anyone successfully treated with direct-acting antivirals usually remains antibody-reactive. The number of participants who currently need HCV treatment is therefore smaller than 305, and it cannot be determined without RNA testing. Settings that have measured both have found large gaps between antibody and viraemic prevalence, particularly

where direct-acting antivirals have been widely available [67, 68]. Establishing the viraemic fraction in Sofia is the most useful next step, because it defines treatment need and because antibody prevalence alone cannot show whether Bulgaria is progressing towards elimination targets [69, 70]. Elimination among people who inject drugs is achievable in principle, since direct-acting antivirals cure more than 95% in this group, but it requires testing, linkage and treatment at a scale that health-system, provider and patient barriers currently prevent in most settings [71]. A survey that stops at antibody testing, as ours did, cannot tell a programme how far it has to go.

The concentration of burden among those who had ever injected was near-total, with adjusted prevalence ratios above four for both HIV and HCV, and it did not extend to treponemal antibody or HBsAg. This internal contrast supports the specificity of the injecting association: if reactivity were simply tracking a general propensity to risk or general social disadvantage, the sexually transmitted marker would have followed it. Treponemal antibody was instead associated with female sex, with an odds ratio of 0.29 for men. This matches the meta-analysis in which active syphilis was over three times as common among women who inject drugs as among men [9], although that review measured active infection whereas treponemal antibody here records lifetime exposure. Syphilis prevalence also remains high among key populations in the region [16]. Transactional sex is the obvious candidate pathway. Syphilis has risen among people who inject drugs, and among women with syphilis who use drugs, in the United States and Australia [72–74], so syphilis among women who use drugs in Sofia warrants active monitoring. Our data cannot demonstrate the pathway, and fifteen reactive results support little more than the observation itself.

The substance profile of this sample differs from that of the opioid-injecting populations from which most European estimates derive. Methamphetamine was the second most commonly reported substance, used by a third of participants. Injecting methamphetamine has been associated with HCV seropositivity independently of other injecting risk [35], stimulant use with raised HIV incidence where sexual transmission predominates [36], and stimulant injection with a distinct risk profile involving more frequent injecting and different sexual risk [33, 34]. We did not model individual substances. The unadjusted comparisons in Additional file 1 (Tables S2 and S3) largely separate a long-standing opioid-injecting group from a younger, mostly non-injecting group using cannabinoids, and we read them as markers of which drug scene a participant belongs to rather than as independent correlates. A population with this much stimulant use should not be assumed to behave like the heroin-injecting cohorts on which European harm reduction models were built, and how stimulant use interacts with sexual transmission of HIV in this setting remains unknown.

Current unstable housing was associated with HIV reactivity, with an aPR of 1.88, and the association persisted on restriction to those who had injected. Its direction and approximate magnitude are consistent with the meta-analysis of homelessness and blood-borne virus acquisition among people who inject drugs (adjusted relative risk 1.39 for HIV; crude relative risk 1.82 for unstable housing specifically) [28], although those estimates concern incident rather than prevalent infection. Two qualifications matter. First, the association attached to current lack of stable accommodation and not to

homelessness of more than a month during the previous year, which was null. Second, its E-value of 3.17, with 1.54 for the confidence limit, means that a moderately strong unmeasured confounder could account for it. This is a finding to act on cautiously and to test in stronger designs, not a demonstrated causal effect. The apparent inverse association between unstable housing and HCV disappeared on restriction to those who had injected and should be read as confounding by injecting history. We report it because it is exactly the kind of artefact that a model reporting a single “homelessness” coefficient across both outcomes would propagate unnoticed.

Several service implications follow. Because 87% of participants with a reactive HIV test were also anti-HCV reactive, a figure close to the 82% pooled HCV prevalence among people with HIV who inject drugs [75], HIV care here is co-infection care, and separate HIV and hepatitis services will not serve this population well [76]. Opioid agonist treatment had ever been received by 47.5% of participants. The survey did not record current receipt, but only 27.5% were in any treatment for a substance use disorder at interview, which places current coverage at most at that level and below the 40 per 100 threshold that defines high coverage [17]. Shortfalls of this kind are common across the region [18]. Meanwhile, almost all participants who had injected said they could obtain sterile equipment whenever needed, yet 12.8% had used a previously used needle or syringe at their last injection. The constraint is therefore unlikely to be availability as such. More plausibly it lies in the situations in which sharing happens, such as police presence, incarceration, withdrawal, and the impracticality of carrying equipment while street homeless [30–32]. Reviews of needle and syringe provision find that effect size depends on the coverage actually achieved, not on the mere existence of a programme [24], and a service that distributes adequate volumes of equipment can still leave transmission untouched if people cannot carry or use it safely.

These findings should also be read against the regional picture. In eastern Europe and central Asia, HIV incidence and mortality among people who inject drugs have continued to rise [12, 13], and harm reduction coverage and testing remain well below need in most countries [18, 19]. The Athens outbreak arose where opioid substitution and syringe provision had barely reached the people at risk [43], and transmission continued for years [44]. Bulgaria combines high lifetime HCV exposure, established HIV among people who inject drugs, partial opioid agonist treatment coverage and no routine RNA confirmation, a position from which the epidemic could move in either direction. Effective interventions exist; the question is whether the policy environment allows them to be delivered at scale. Modelling for the region indicates that the resources released by shifting from a criminalising to a public health approach would themselves be enough to fund antiretroviral therapy and opioid agonist treatment at levels that would prevent most new HIV infections among people who inject drugs [27]. Our data cannot test that projection for Bulgaria, but they leave little doubt about the scale of need among people who use drugs in Sofia.

Limitations

The design is cross-sectional, so every association reported is with prevalent reactivity, not incident infection. This bears most heavily on the age and duration gradients, which describe exposure

accumulated over an injecting career rather than the current force of infection. It also means the direction of behavioural associations cannot be settled from these data. For example, condom use at last intercourse was higher among participants with a reactive HIV test than among those without (58% vs 31%; Additional file 1, Table S2), which most likely reflects counselling after diagnosis rather than any protective effect, and we did not model condom use as a correlate for that reason.

Sampling was by convenience through outreach services. People reached by a harm reduction organisation are likely to differ systematically from those who are not, probably towards greater service contact and higher testing and treatment coverage, so the estimates apply to the population reachable through outreach in Sofia rather than to all people who use drugs in the city. No population size estimate exists against which to weight the sample, and neither respondent-driven nor time-location sampling was feasible within the resources available.

No confirmatory laboratory testing was performed. All outcomes are unconfirmed reactive rapid test results, and although tests of this class perform well in outreach settings [47, 48], some reactive HIV and HBsAg results at these prevalences will be false positives. Rapid HBsAg tests can also miss infections with low antigen levels [47], so the 2.3% HBsAg prevalence may be an underestimate. More importantly, anti-HCV reactivity measures lifetime exposure, not current infection. Viral RNA was not measured, so the proportion with active, transmissible infection is unknown and will be appreciably lower than 63.5%. Treponemal antibody likewise indicates lifetime rather than active syphilis [51]. The survey also did not record whether participants with reactive results already knew their status, which limits what can be said about case-finding and testing coverage.

The injecting variable records lifetime history rather than recent injecting, and the questionnaire did not allow current and former injecting to be separated reliably. A lifetime exposure cannot be given an interventional reading, which is why we report no attributable fractions, and associations with recent injecting, the quantity most relevant to programme design, cannot be estimated. Future survey rounds should record injecting within a defined recent period.

All behavioural data were self-reported and subject to recall and social desirability bias, with sharing of injecting equipment the item most likely to be under-reported. Interviews were conducted with outreach workers already known to participants, and confidentiality was assured, which should reduce but cannot eliminate this bias.

Finally, the numbers of HBsAg- and treponemal-antibody-reactive participants were small, so those models have little power and wide intervals. Several variables that the causal diagram identifies as relevant were not measured, among them incarceration history, injecting network structure and the circumstances in which sharing occurs. Their absence is the most likely explanation for the modest discrimination of the HIV model.

Conclusions

Two thirds of people who use drugs reached by outreach in Sofia carry a reactive marker of blood-borne infection, and anti-HCV reactivity approaches saturation among those with long injecting histories. The age structure of that reactivity points to exposure accumulated over decades, not to a recently introduced epidemic. The burden is concentrated among people who have injected, while treponemal antibody and HBsAg show no such pattern. Current unstable housing was associated with HIV reactivity independently of injecting, though not robustly enough to support a causal reading. Because antibody reactivity cannot distinguish current from resolved HCV infection, the immediate priority is RNA testing to establish treatment need. Alongside it, opioid agonist treatment should be expanded well beyond its current reach, and HIV and hepatitis services should be integrated for a group in which the two infections so often occur together.

Abbreviations

aOR

adjusted odds ratio

aPR

adjusted prevalence ratio

AUC

area under the receiver operating characteristic curve

CI

confidence interval

HBsAg

hepatitis B surface antigen

HBV

hepatitis B virus

HCV

hepatitis C virus

HIV

human immunodeficiency virus

IQR

interquartile range

OR

odds ratio

PR

prevalence ratio

RNA

ribonucleic acid

STROBE

Strengthening the Reporting of Observational Studies in Epidemiology.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Medical University of Plovdiv (decision R 1442/10.06.2024) before data collection began. All participants gave written informed consent before enrolment. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the current study are not publicly available because they contain sensitive health and behavioural information on a criminalised and stigmatised population, but are available from the corresponding author on reasonable request. The analysis code is available from the corresponding author on request.

Competing interests

YG, KZ and EN are affiliated with the Center for Humane Policy, which carried out recruitment and fieldwork for this study. The authors declare that they have no other competing interests.

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Authors' contributions

Contributions are described using the CRediT taxonomy. VR: conceptualisation, methodology, investigation, data curation, project administration, writing – original draft, writing – review and editing. KK: methodology, formal analysis, software, validation, visualisation, data curation, writing – original draft, writing – review and editing. YG, KZ and EN: investigation, resources, writing – review and editing. AK: conceptualisation, supervision, writing – review and editing. All authors read and approved the final manuscript.

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Figures

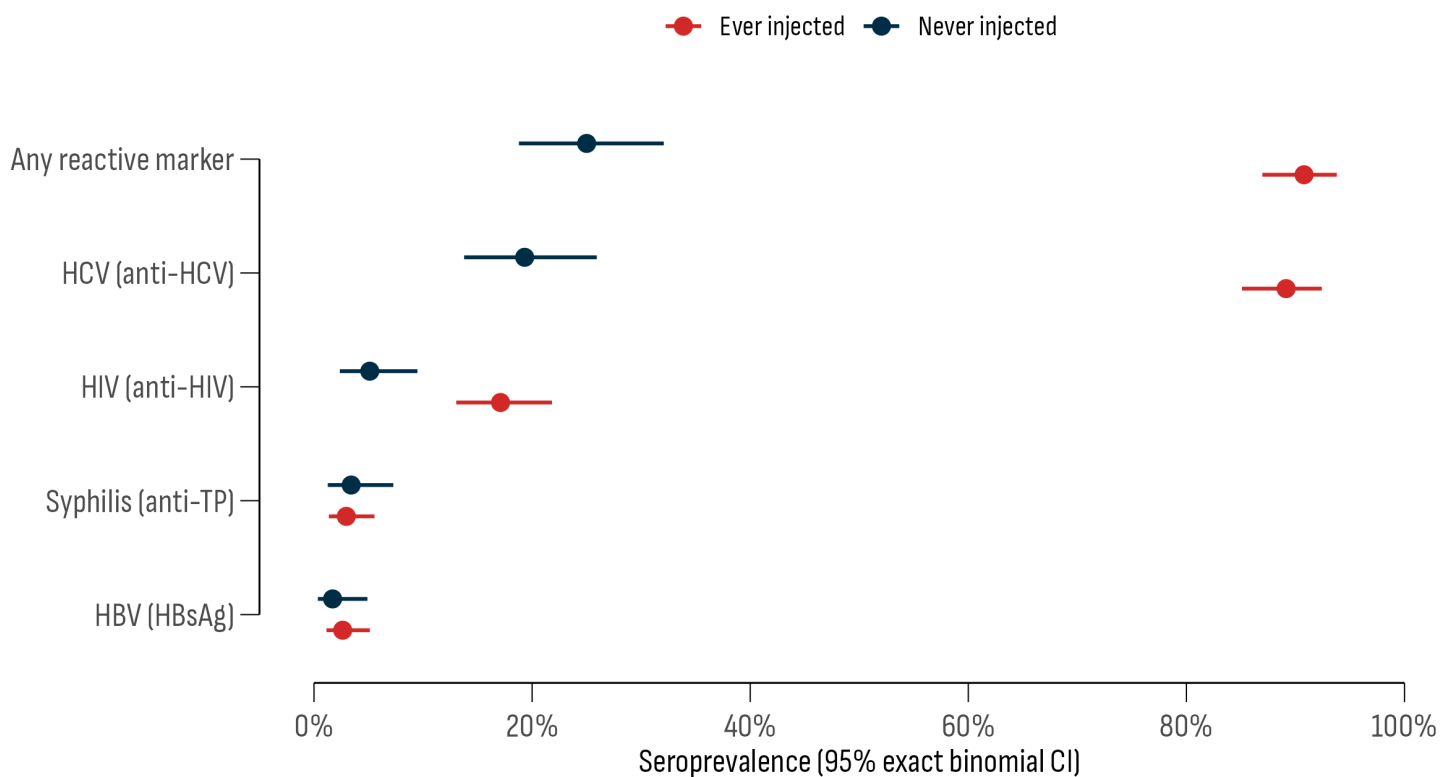


Figure 1

Prevalence of reactive markers by injecting history. Points are prevalences and bars 95% Clopper-Pearson exact binomial confidence intervals among people who use drugs in Sofia, June–August 2024 (n = 480; 304 had ever injected, 176 had not). HIV and HCV antibody reactivity differ sharply by injecting history; HBsAg and treponemal antibody do not.

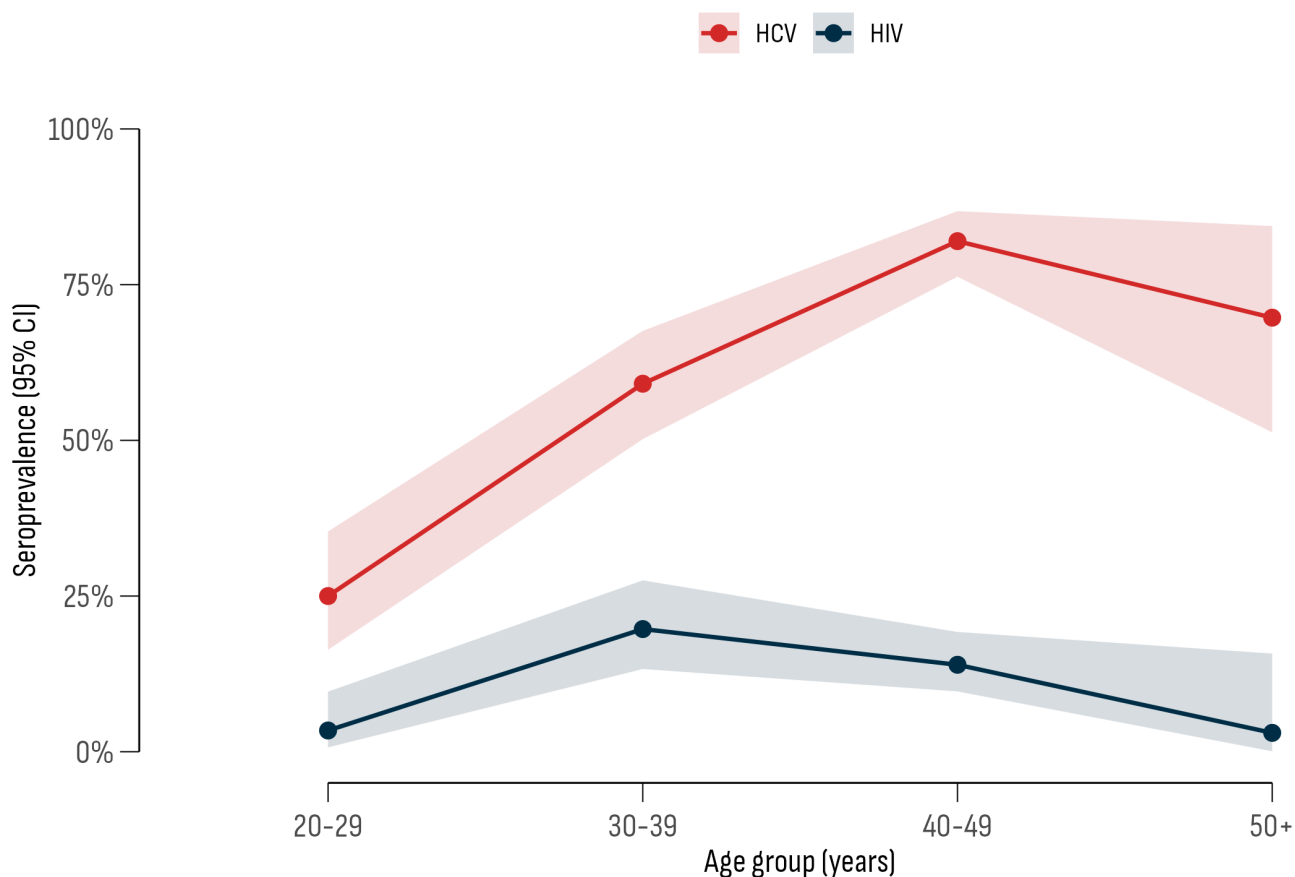


Figure 2

Prevalence of anti-HCV and anti-HIV reactivity by age group. Shaded bands are 95% confidence intervals. Anti-HCV reactivity rises steeply up to 40–49 years, whereas HIV shows no trend. The group under 20 years ($n = 5$) is omitted because its confidence interval spans almost the entire range.

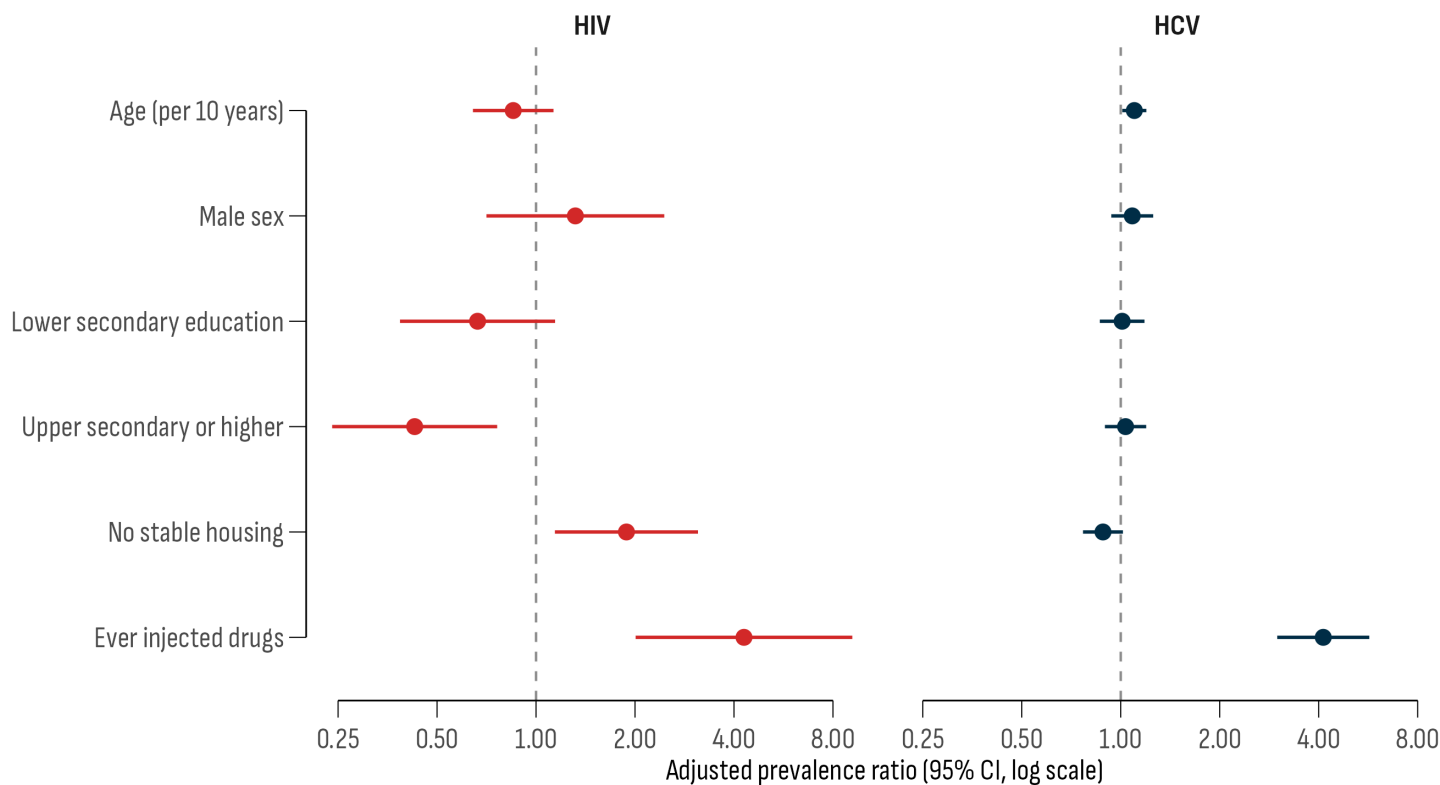


Figure 3

Adjusted prevalence ratios for HIV and HCV reactivity. Estimates from modified Poisson regression with robust variance, each model containing all variables shown (n = 469), plotted on a log scale with 95% confidence intervals. The dashed line marks a prevalence ratio of 1. Reference categories: women, no schooling or primary education only, stable place to live, never injected.

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