Hepatitis C virus : epidemiology and immunology

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Chapter 2

Epidemiology

Chapter 2.1

Major decline of hepatitis C virus incidence rate over two decades in a cohort of drug users

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Abstract

Injecting drug users (DU) are at high risk for hepatitis C virus (HCV) and HIV infections. To examine the prevalence and incidence of these infections over a 20-year period (1985-2005), the authors evaluated 1276 DU from the Amsterdam Cohort Studies who had been tested prospectively for HIV infection and retrospectively for HCV infection. To compare HCV and HIV incidences, a smooth trend was assumed for both curves over calendar time. Risk factors for HCV seroconversion were determined using Poisson regression. Among ever-injecting DU, the prevalence of HCV antibodies was 84.5% at study entry, and 30.9% were co-infected with HIV. Their yearly HCV incidence dropped from 27.5/100 person years (PY) in the 1980s to 2/100 PY in recent years. In multivariate analyses, ever-injecting DU who currently injected and borrowed needles were at increased risk of HCV seroconversion (incidence rate ratio 29.9, 95% CI 12.6-70.9) compared to ever-injecting DU who did not currently inject. The risk of HCV seroconversion decreased over calendar time. The HCV incidence in ever-injecting DU was on average 4.4 times the HIV incidence, a pattern seen over the entire study period. The simultaneous decline of both HCV and HIV incidence probably results from reduced risk behavior at the population level.

Introduction

The most important mode of hepatitis C virus (HCV) transmission is through exposure to infected blood.[1,2] Therefore injecting drug users (DU) are at high risk for HCV infection. Their main route of transmission is the sharing of needles or other injecting equipment.[3] In this population, the reported prevalences of HCV range from 40 to 85% in Europe and North America.[1,4-11]

Under the threat of AIDS, DU reduced their injecting risk behaviour and consequently their incidence of HIV infection in the mid-1980s.[12,13] However, their HCV incidence appears to be less affected by this decreased risk behavior, perhaps because HCV is more transmissible than HIV. This hypothesis is confirmed by several studies that show a high and stable prevalence of HCV antibodies in this population.[14-17] In recent years, we reported a high but declining HCV prevalence among young DU in Amsterdam,[18] whereas others still report high and stable HCV incidence among young DU who have recently started injecting.[15,17,19,20]

The open and ongoing Amsterdam Cohort Studies (ACS) among DU started in 1985, and stored serum was retrospectively tested for HCV antibodies. Therefore, the ACS has the unique potential to present HCV incidence data for DU over two decades. The objectives of our study were to measure the HCV incidence over this long period, to evaluate risk factors associated with HCV seroconversion, and to compare the HCV incidence to the HIV incidence in this cohort over the same period.

Materials and Methods

The ACS is an open, prospective cohort study initiated to investigate the prevalence, incidence, and risk factors of infections with HIV-1 and other blood-borne and/or sexually transmitted diseases, as well as the effects of intervention.[21] The DU cohort was initiated in 1985; recruitment is ongoing and in recent years has been directed in particular to young DU.

Participation in the ACS is voluntary, and informed consent is obtained for every participant at intake. ACS participants visit the Health Service of Amsterdam every 4-6 months. At every visit, they complete a standardized questionnaire about their health, risk behaviour, and socio-demographic situation. Questions about current behaviour refer to the period between the present and the preceding ACS visit. Questions at baseline refer to the period since 1980 or since the start of regular use of hard drugs. Blood is drawn for laboratory testing and storage.

Laboratory methods

To study HIV prevalence and incidence, all ACS participants since 1985 (n=1640) were prospectively tested for HIV antibodies by enzyme linked immunosorbent assays (ELISA), with confirmation by Western blot (since 1986: HIV Blot version 2.2, Genelab diagnostics).

To study the HCV prevalence and incidence, all participants with at least two visits between December 1985 and November 2005 (n=1276) were retrospectively tested for HCV antibodies, using the first sample available in each case. Third generation ELISA tests were used to detect HCV antibodies (AxSym HCV version 3.0; Abbott, Wiesbaden,

Germany). Individuals who were HCV negative at ACS entry were tested for HCV antibodies at their most recent ACS visit. On finding HCV seroconversion, samples taken in between these two visits were tested to identify the moment of seroconversion.

Statistical analyses

The date of HCV or HIV seroconversion was estimated as the midpoint between the last seronegative and the first seropositive ACS visit. The median duration of the HCV seroconversion interval between visits was 4.0 months, interquartile range (IQR) 3.7, 5.1 months. Using the Kaplan-Meier method, we examined the time elapsed from the start of injecting drugs to HCV seroconversion. Only HCV-negative DU were included and they were considered to be at risk from their start of injecting. Those who had started injecting before ACS enrolment entered the risk set at their date of ACS entry (i.e., left truncation). Those who did not seroconvert or who were lost to follow up were censored at their last ACS visit or ultimately 1 November 2005. We stratified the dates of starting injection into two decennia to investigate differences in HCV-free survival according to decade of starting injection.

Incidence rate curves were calculated by person-time methods. Poisson regression was used to test for the trend in HCV incidence over time and to determine risk factors for HCV seroconversion. All variables subject to change were treated as time-dependent variables. Due to the relatively long time-period between the point of infection and the appearance of HCV antibodies,[22] the most probable moment of infection was assumed to have occurred around the last seronegative visit. Therefore, we assigned the risk behaviour reported at that visit to the HCV seroconversion period. However, for nine participants who reported starting injection at the first HCV antibody-positive visit, we set back the report of injecting risk factors from this visit to the last HCV antibody-negative visit. Multivariate models were built using forward-stepwise techniques, and variables with a univariate p-value <0.20 were considered as potential independent determinants. A p-value <0.05 was considered statistically significant.[23,24] Interactions in the final model were checked.

Variables related to general characteristics, drug use, and sexual risk behaviour were examined as potential determinants of HCV seroconversion. General characteristics included sex, body mass index, calendar year of study visit, nationality, ethnicity, age, homelessness, hospitalization, and HIV status. The drug use variables included current injecting and the calendar period of starting injection. For current injectors, we also examined the frequency of injecting, the main type of drug injected, whether they injected mainly at home or borrowed needles, and needles obtained through a needle exchange program (NEP). Because there was a very strong association between current injecting and current borrowing of needles, we combined these two variables as follows: no current injecting; current injecting but no current borrowing of needles; current injecting and current borrowing of needles. Sexual behaviour included having a steady sexual partner, injecting drug use of the steady partner, having unprotected sex (with an injecting partner), and current prostitution (women only).

To compare the HCV and HIV incidence, we assumed that the observed data (i.e., the number of new infections per year) follows a Poisson distribution. We adopted a Bayesian approach. The logarithm of the incidence over calendar time was modelled using penalized splines. In this way, the incidence of both HCV and HIV was allowed to vary smoothly and nonlinearly over time.[25-27] If the trends have the same pattern, then the difference between the incidences on a logarithmic scale is a constant.

Results

General characteristics and HCV prevalence

In total, 1640 DU have been enrolled in the ACS since December 1985. Of these, 1259 DU met the follow up criteria of at least two visits before November 2005 and also had enough stored serum to allow HCV testing. Of these participants, 803/1259 (63.8%) were male and 937/1259 (74.5%) had a Dutch nationality. The median age at ACS entry was 30.5 years (IQR 26.5, 35.8) (Table 2.1.1).

Table 2.1.1 General characteristics of drug users in the Amsterdam Cohort Study (*=at entry).

	Total	Ever-injecting DU	Never-injecting DU
Total number of participants	1259	952	307
Median age * (IQR)	30.5 (26.5, 35.8)	29.84 (26.0, 36.0)	30.6 (26.8, 35.7)
% Male sex	63.8	61.3	71.3
% Dutch nationality	74.7	86.0	71.0
Median duration of follow up (IQR)	6.95 (3.56, 12.1)	7.33 (3.84, 12.6)	5.41 (2.60, 10.4)
Median age at start of injecting drugs (IQR)	-	21.7 (17.8, 26.0)	-
Main drugs injected (%) *			
cocktail, heroin/cocaine	-	40.0	-
heroin		12.2	
cocaine		8.9	
Main other drugs used (%) *	-		
cocktail, heroin/cocaine		4.4	41.0
heroin		31.5	3.0
cocaine		26.7	4.2
Frequency of injecting (%)			
no current injecting	-	28.5	-
daily		34.0	
weekly		30.7	
monthly		4.4	
Number of recently borrowed needles(%) *			
0	-	44.9	-
1-10		7.6	
>10		0.9	
unknown		46.4	
% HCV-antibody positive *	63.8	82.2	6.5
HCV seroconversions during follow up	59	58	1
% HIV-positive *	20.4	5.8	3.6
HIV seroconversions during follow up	95	90	5

Ever-injecting DU: DU who had injected before ACS entry (n=905) or started injecting during follow up (n=47). Current/recently: in previous six months.

Of the 1259, 952 participants were ever-injectors: DU who had ever injected drugs before entry (n=905) or who had started injecting drugs during follow up (n=47). The median age at start of injection was 21.7 years (IQR 17.8, 26.0).
The median ACS follow up time for ever-injectors was 7.3 years (IQR 3.8,12.6), whereas it was 5.4 years (IQR 2.6, 10.4) for never-injectors. In ever-injectors, the main drugs recorded at ACS entry were a cocktail of heroin and cocaine (40.0%), and most participants had injected daily or more frequently in the preceding 6 months (34.0%).
Of the 1259 DU, 803 (63.8%) had HCV antibodies at entry; of these, 30.6% (246/803) were HIV-co-infected. The prevalence at entry of HCV antibodies in ever-injectors varied from 92.9% in 1986 to 69.2% in 2001. The prevalence among never-injectors was 6.5% over the total study period and varied from 0 to 22.2% per calendar year.
When evaluating HCV prevalence at entry by the time elapsed since start of injection, such prevalence was 59/99 (59.6%) for participants who had injected for less than two years before entry vs. 137/164 (82.5%) for participants who had injected for three to five years before entry. Among participants with >10 years of injecting drug use before ACS entry, the HCV prevalence was 327/346 (94.5%).

HCV incidence

Of the 456 DU seronegative for HCV at ACS entry, 59 seroconverted during follow up, of whom 58 injected and 1 did not. Among ever-injectors, the incidence declined from 27.5/100 PY in the late 1980s to approximately 2/100 PY in recent years (Figure 2.1.1B). There was a significant downward trend in HCV incidence over calendar time (IRR 0.86 per calendar year; 95% CI 0.82-0.90, p<0.001) (Figure 2.1.1B).
In line with the decline of the HCV incidence, the time since starting injection until HCV seroconversion has lengthened in more recent calendar periods. In 1980-1989, the median interval was 2.27 years (IQR 1.2, 5.6 years), whereas in 1990-1999, the median was 9.10 years (IQR 2.1, ∞ years) (Figure 2.1.2).
When restricting our analysis to DU who reported injecting since the preceding visit, a higher incidence but similar pattern was observed. In 1985-1990, the incidence rate in this group was extremely high, between 50–80/100 PY, but it dropped to 5-10/100 PY in 1990-1999.

Comparison of HCV and HIV incidence

Of 1276 DU, those HIV-negative at entry numbered 1013, of whom 95 (including 90 ever-injectors) seroconverted for HIV during follow up. The HIV incidence rate among ever-injectors dropped from 8.52/100 PY in 1986 to approximately 0 since 2000, with a slight increase in 2005 (Figure 2.1.1A).
When the observed HCV and HIV incidence curves and their fitted smooth curves are plotted in one graph with two scales, the curves look similar in shape. When we plotted the differences between the logs of the fitted model, we found no convincing evidence for a difference in pattern. The mean value of the differences on a log-scale over the twenty years is 1.48; hence the scale factor is estimated to be 4.4 (data not shown). The observed and fitted incidence patterns for both HCV and HIV with 95% confidence intervals are shown in Figure 2.1.2C.

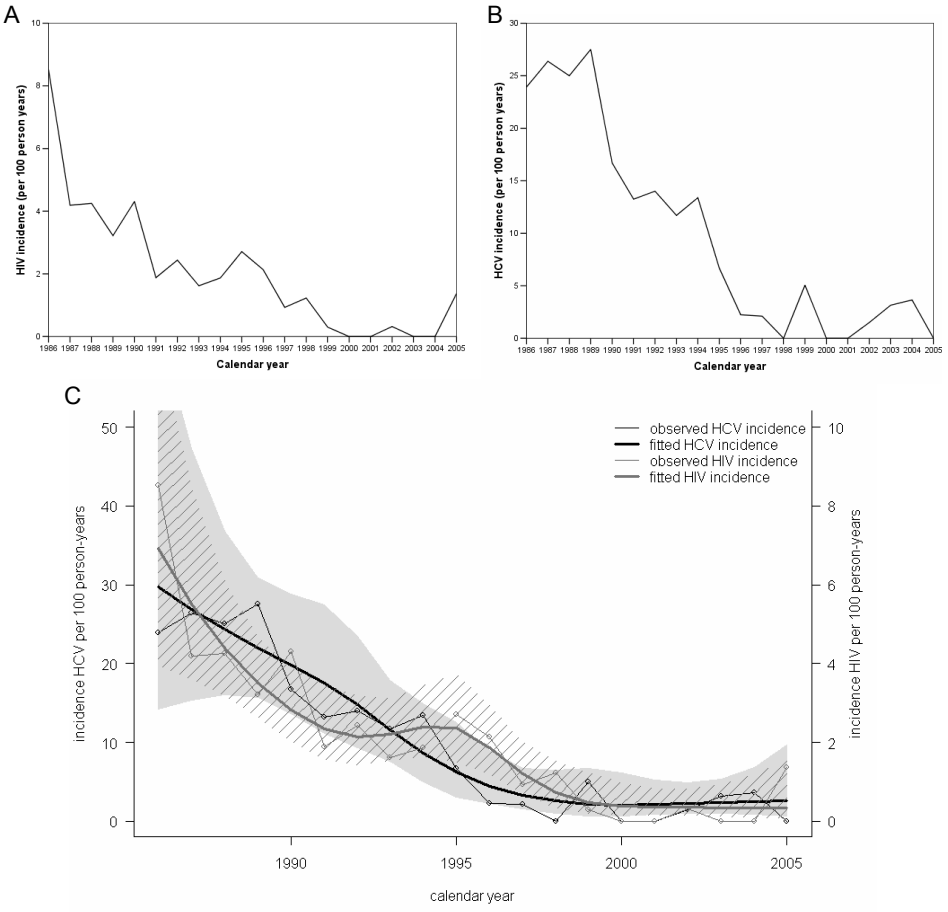

Figure 2.1.1 (A, B) Observed HIV and HCV incidence curves among ever injecting DU in the ACS (1985-2005); (C) observed and fitted HCV (left y-axis) and HIV (right y-axis) incidence curves among ever injecting DU in the ACS (1985-2005).

Risk factors for HCV seroconversion

Time since start injecting can be seen as a proxy for the duration of exposure time, and preliminary analysis showed a very strong association between time since start of injecting and the time point of HCV seroconversion (IRR 0.80 per year), 95% CI 0.74-0.86) (Table 2.1.2). Therefore, in bivariate analysis, to adjust for variation in time from start of injecting (and thus time of exposure), all other variables were adjusted for time from start of injecting as a time-updated variable.

After correction for time since starting injection, the following risk factors were found to be significantly associated with an increased risk of HCV seroconversion: the combined variable of current injecting and current borrowing of needles, earlier calendar year of visit, use of needle exchange programs (NEPs), type of drugs injected, frequency of injecting drug use, and earlier decennium of starting injection (Table 2.1.2).

Interestingly, in univariate analysis persons were more at risk for HCV if they had seroconverted for HIV (IRR 5.68; 95% CI 2.27-14.2) or were chronically infected with HIV (IRR 3.12; 95% CI 0.76-12.8) than if they were HIV-negative. The type of drugs injected, and frequency of injection were associated with an increased risk of HCV infection, their effect is attributable to current injecting drug use itself. In fact, when evaluating these variables among only DU injecting drugs within the past six months we found no association between NEP use, the type of drug injected, or injection frequency and HCV infection.

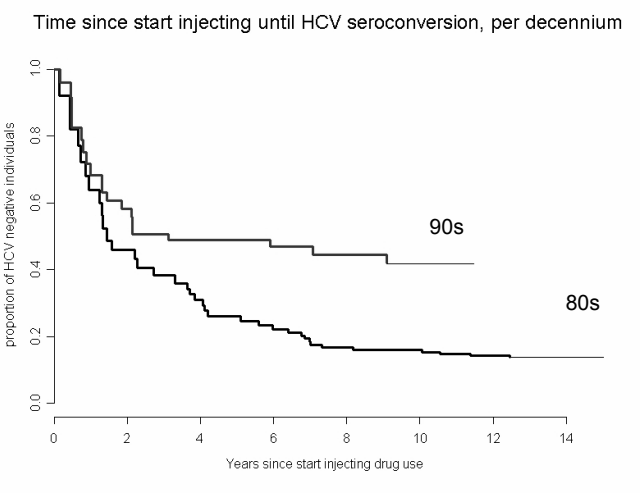

Figure 2.1.2 Kaplan-Meier estimates of the cumulative proportion of DU who remain without HCV infection since starting injection, grouped per decennium: the 1980s and 1990s. Curves were truncated when fewer than 10 persons remained at risk for HCV (thin line). Persons who started injecting before 1980 or after 2000 are not depicted in this figure, because at any moment in those periods, less than 10 persons were at risk for HCV.

In multivariate analysis, we found that current injecting combined with current borrowing of needles was a major risk for HCV seroconversion; the IRR was 29.9 (95% CI 12.6-70.9) for current injecting and borrowing compared to no injecting in the preceding period. The longer the time between start of injecting and study visit, the smaller the risk of HCV infection: IRR 0.89; 95% CI 0.83-0.96) Table 2.1.2. Calendar year remained significantly associated with a decreased risk of HCV infection when it was evaluated continuously in the model (IRR 0.87; 95% CI 0.82-0.93).

Table 2.1.2 Univariate, bivariate, and multivariate IRR of potential risk factors for HCV infection.

	UNIVARIATE ANALYSIS						BIVARIATE ANALYSIS *			MULTIVARIATE ANALYSIS		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Methadone dosage						0.18			0.92			
0 mg	34	408	8.33	2.23	(0.68, 7.26)		1.07	(0.33, 3.51)				
1-59 mg methadone	20	358	5.59	1.49	(0.44, 5.02)		0.95	(0.28, 3.21)				
>60 mg	3	80	3.75	1			1					
HIV status						0.006			0.25			
HIV-negative	51	831	6.14	1			1					
HIV primary infection	5	14	35.7	5.68	(2.27, 14.2)		2.18	(0.85, 5.57)				
HIV chronically infected	2	10	20.0	3.12	(0.76, 12.8)		1.95	(0.47, 8.07)				
Decennium of starting injection						0.002			0.13	**		
1970-79	1	146	0.68	1			1					
1980-89	37	443	8.35	12.2	(1.67, 88.9)		1.56	(0.19, 12.6)				
1990-99	19	239	7.95	11.6	(1.55, 86.6)		1.01	(0.12, 8.37)				
2000-present	1	23	4.35	6.26	(0.39, 100.0)		0.32	(0.02, 5.74)				
Use of NEPs						<0.001			<0.001			
No current injecting	10	623	1.61	1			1					
Current injecting, no NEP	18	92	19.6	12.3	(5.66, 26.6)		7.61	(3.43, 16.8)				
Current injecting, irregular NEP	11	36	30.6	19.1	(8.09, 44.9)		8.40	(3.39, 20.8)				
Current injecting, always NEP	19	99	19.2	11.9	(5.53, 25.6)		7.87	(3.58, 17.3)				
Age (per 10 years)	58	856	6.78	0.45	(0.31, 0.65)	<0.001	0.87	(0.59, 1.26)	0.45			
Type of drugs mainly injected						<0.001			<0.001			
No current injecting	10	623	1.61	1			1					
Heroin	9	46	19.8	12.2	(4.97, 30.1)		7.18	(2.86, 18.0)				
Cocaine	10	41	24.4	15.2	(6.31, 36.4)		8.69	(3.53, 21.4)				
Cocktail, heroin/cocaine	21	115	18.3	11.4	(5.37, 24.2)		6.51	(2.99, 14.2)				
Amphetamines	2	17	11.8	7.46	(1.64, 34.1)		3.81	(0.82, 17.6)				
Methadone	3	9	33.3	21.3	(5.87, 77.5)		35.5	(9.7, 129.5)				
Other/unknown	3	4	75.0	44.6	(12.3, 162.2)		25.6	(7.00, 93.5)				

			UNIVARIATE ANALYSIS				BIVARIATE ANALYSIS *			MULTIVARIATE ANALYSIS		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Frequency of injecting						<0.001			<0.001			
No current injecting	10	623	1.60	1			1					
More times per day	17	49	34.7	21.7	(9.92, 47.3)		10.4	(4.54, 23.9)				
Once daily	1	4	25.5	15.9	(2.03, 124.3)		12.2	(1.55, 95.9)				
More times per week	19	66	28.8	18.0	(8.36, 38.6)		10.5	(4.75, 23.3)				
Once weekly	1	12	8.23	5.13	(0.66, 40.1)		5.27	(0.67, 41.2)				
More times per month	3	37	8.13	5.07	(1.40, 18.4)		2.71	(0.72, 10.1)				
Once monthly	2	13	15.2	9.46	(2.07, 43.2)		8.18	(1.79, 37.4)				
Less than once monthly	4	48	8.36	5.21	(1.63, 16.6)		4.19	(1.31, 13.4)				
Frequency of non-injecting drug use						0.35			0.39			
More times per day	21	249	8.43	0.73	(0.25, 2.14)		0.60	(0.20, 1.74)				
Once daily	2	38	5.26	0.45	(0.08, 2.48)		0.45	(0.08, 2.47)				
More times per week	16	235	6.81	0.59	(0.20, 1.77)		0.60	(0.20, 1.80)				
Once weekly	2	68	2.94	0.25	(0.05, 1.39		0.20	(0.04, 1.09)				
More times per month	2	47	4.26	0.37	(0.07, 2.03)		0.47	(0.09, 2.58)				
Less than once monthly	0	17	0.00	1			1					
Type of drugs mainly used (non-injecting)						0.98			0.81			
Heroin	20	311	6.43	1			1					
Cocaine	24	332	7.23	1.12	(0.62, 2.03)		1.33	(0.73, 2.41)				
Cocktail, heroin/cocaine	2	32	6.25	0.96	(0.22, 4.11)		1.07	(0.25, 4.58)				
Amphetamines	1	13	7.69	1.16	(0.16, 8.66)		1.48	(0.20, 11.0)				
Having a steady partner						0.52			0.10			
No	36	496	7.26	1.19	(0.70, 2.02)		1.55	(0.91, 2.64)				
Yes	22	360	6.11	1			1					
Injecting drug use of the steady partner						0.10			0.99			
No	12	255	4.71	1			1					
Yes	10	105	9.52	2.04	(0.88, 4.71)		0.99	(0.42, 2.33)				

			UNIVARIATE ANALYSIS				BIVARIATE ANALYSIS *			MULTIVARIATE ANALYSIS		
	HCV sc	PY	Incidence rate (per 100 PY)	IRR	95% CI	p value	IRR	95% CI	p value	IRR	95% CI	p value
Homelessness						0.29			0.39			
No	52	800	6.50	0.61	(0.26, 1.43)		0.67	(0.29, 1.57)				
Yes	6	57	10.5	1			1					
Hospitalized in past 6 months						0.67			0.65			
No	56	817	6.85	1.34	(0.33, 5.50)		1.36	(0.33, 5.59)				
Yes	2	39	5.13	1			1					
Current prostitution (females only)						0.19			0.82			
No	24	243	9.88	1			1					
Yes	2	47	4.26	0.43	(0.10, 1.81)		1.19	(0.28, 5.07)				
Current injecting and borrowing needles						<0.001			<0.001			<0.001
No current injecting	10	623	1.61	1			1			1		
Current injecting, no current borrowing of needles	25	159	15.7	9.80	(4.71, 20.4)		6.26	(2.94, 13.3)		8.70	(4.03, 18.8)	
Current injecting and current borrowing of needles	12	23	52.2	32.7	(14.1, 75.7)		21.4	(9.17, 50.1)		29.9	(12.6, 70.9)	
Time since start of injecting	58	856	6.8	0.80	(0.74, 0.86)	<0.001	NA			0.89	(0.83, 0.96)	<0.001
Year of visit	58	856	6.8	0.86	(0.82, 0.90)	<0.001	0.94	(0.89, 0.99)	0.009	0.87	(0.82, 0.93)	<0.001
Sex						0.085			0.36			
Male	32	566	5.65	1			1					
Female	26	290	8.97	1.59	(0.95, 2.66)		1.28	(0.76, 2.16)				

NA = not applicable

* = adjusted for time since start of injection.

** = analyses were not adjusted for time since start of injection and decennium of start, because the decennium can be derived from the time since start of injection and calendar year of visit.

Discussion

This study describes the prevalence and incidence of HCV in a large group of DU in Amsterdam, the Netherlands, over two decades. Findings show that the HCV incidence dropped considerably in that period. Interestingly, when we compared the HCV incidence rate to the HIV incidence rate in the same group of DU that have ever injected the decrease was similar for the two infections. In line with the decline of the HCV incidence, the time from the start of injecting drugs until HCV seroconversion is longer at present than in the past.

To our knowledge, this is the first study to document among DU, over such a long period, a decline in HCV incidence that is not only strong but also comparable to the decline in HIV incidence. Our finding of a decline in HCV incidence contrasts with other studies that show a stable HCV incidence.[19,28] One explanation may be that those studies analyzed the HCV incidence over a shorter time interval, which might have been insufficient to show a significant decline. In Baltimore, USA, a significant decline of the HCV incidence was found in injecting DU followed between 1988 and 1996, but in contrast to our study with ongoing recruitment of participants, this decline was observed in a closed cohort study and a saturation effect probably has contributed to this decline.[29]

In addition, the risk behavior of the total group of DU included in the ACS has substantially declined over time in Amsterdam.[30] This finding suggests that a decline in risk behavior at the population level has contributed to the simultaneous decline of HCV and HIV incidence. The decreasing HCV incidence in Amsterdam DU, as opposed to high incidences in DU elsewhere, may likewise be partly explained by a larger reduction in injecting risk behavior in Amsterdam, compared to reductions elsewhere. The impact of methadone provision and NEP on this decline of risk behavior is very important and should be a focus of future studies. Methadone and NEP were readily available throughout the study period, and the median prescribed daily methadone dose increased during this period. Murray et al.[31] demonstrated by mathematical modeling that the level of risk behavior determines whether HCV incidence decreases. They calculated that if injecting risk behavior is sufficiently decreased (through intense needle exchange programs and/or harm reduction strategies), then HCV incidence will accordingly decline.

Mathematical models have additionally shown the natural course of an epidemic might bring a decline in the incidence of infection.[32] When a new infectious agent enters a population, the number of infected individuals and the incidence soon increase. Thereafter, as the number of susceptibles decreases, the chance for an infected individual to come into contact with an uninfected individual decreases as well. When the density of uninfected persons reaches a threshold below which the number of susceptibles cannot sustain an ongoing epidemic, incidence peaks and then starts to decline. In this light, the decrease in HIV incidence observed shortly after the introduction of HIV in Amsterdam in the early 1980s was due to the depletion of susceptibles, along with a reduction in risk behaviour. However, such depletion is less likely to be the case for HCV, which has existed among DU since the 1960s and possibly even before.[33,34] This implies that the decrease in injecting risk behaviour might have an even greater impact on HCV than on HIV.

The contrast in study findings may be explained in part by the HCV test used. We used third-generation ELISA tests to measure HCV antibodies, whereas studies from the late

1980s/early 1990s used first- or second-generation ELISA tests, which were more inclined to give false positive test results.[35]

The HCV prevalence among DU at ACS entry varies between 70-90%, with lower prevalence rates in recent years. This is consistent with what was described among DU in Amsterdam in the early 1990s[28] and among recently starting injectors in Amsterdam and elsewhere.[18,36] The HCV prevalence in never-injecting DU is much lower than in ever-injectors but still much higher than in low-risk populations (e.g., blood donors) or the general populations in Western countries,[1,37] household transmission, rare sexual transmission, and reliability/unreliability of answers given in interviews may contribute to this finding among never-injecting DU.

Among DU in Amsterdam who have injected in the past 6 months, incidence rates were extremely high in the 1980s (50–80/100 PY). Similarly high incidence rates have been described by Smyth et al. among young, DU who have recently started injecting in Ireland, in the 1990s.[10]

A possible limitation of our study is its lack of confirmatory testing for positive results of HCV antibody testing. However, such results in a high-risk population are likely to be true positives,[35] and 232/803 (28.9%) of the positive participants were tested at two study visits or more, all with consistent HCV-positive test results. Therefore we believe the lack of confirmatory testing did not influence our results. Furthermore, although the ACS is an open, prospective cohort study, the influx of new participants in recent years has been lower than in earlier years. Lower risk DU could be overrepresented due to the decease of high-risk DU. However, the most recent HCV seroconversions took place in young drug users who entered the cohort after 1994.

Our risk factor analysis showed that HCV seroconversion is associated not only with current injecting and borrowing needles, as expected, but also with calendar year and time since start of injecting. The majority (70%) of HCV infections could have been prevented by eliminating the borrowing of needles. This might partly reflect the effect of the use of NEP, which were always available during the study period, but individual factors also might play a role in the decision to use NEP.

In conclusion, HCV incidence in our cohort showed a sharp decline in the past two decades, similar to the decline in HIV incidence, most likely due to a decrease in injecting risk behavior. We found that those who started injecting in a recent calendar period are at lower risk of HCV infection, presumably due to prevention activities. Thus it is important to continue and enhance such activities among DU and others at risk of starting injection, especially because the HCV risk is highest just after the start of injecting, when probably injectors are inexperienced.

Although we did not find an independent effect from either participation in a methadone program or from the use of needle exchange programs, these prevention measures in combination are likely to have contributed to the decline in risk behavior related to drug use at the population level. Therefore, it is important to evaluate the possibilities for harm reduction worldwide. During the late 1980s many acute HCV infections occurred, so there might have been more DU with high HCV-RNA levels associated with acute HCV infection. Therefore, in that period there may have been more and/or easier transmission of HCV. Higher HCV-RNA levels have also been associated with HIV co-infection.[38] However, we believe that because the HCV prevalence remained relatively high and the pattern of the HIV and HCV incidence was comparable during the study period, on population level the HCV-RNA level varied only little over time, also because treatment prescription for HCV was very limited in our cohort.

Finally, it is important to decrease the prevalence of chronic HCV carriers and thus reduce the possibility for HCV transmission. DU should therefore be systematically screened for HCV infection, and those chronically infected should be treated.[39]

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Chapter 2.2

Full participation in harm reduction programs is associated with decreased risk for HIV and HCV: evidence from the Amsterdam Cohort Studies among drug users

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Abstract

Objectives
To investigate the impact of harm-reduction programs on HIV and hepatitis C (HCV) incidence among ever-injecting drug users (DU) from the Amsterdam Cohort Studies (ACS).

Methods
The association between use of harm reduction and seroconversion for HIV and/or HCV was evaluated using Poisson regression. 714 DU were at risk for HIV and/or HCV during follow up. Harm reduction was measured by combining its two most important components --methadone dose and needle exchange program (NEP) use-- and looking at 5 categories of participation, ranging from no participation (no methadone in the past 6 months, injecting drug use in the past 6 months, and no use of NEP) to full participation (≥60 mg methadone/day and no current injecting or ≥60 mg methadone/day and current injecting but all needles exchanged).

Results
Methadone dose or NEP use alone were not significantly associated with HIV or HCV seroconversion. However, with combination of these variables and after correction for possibly confounding variables, we found that, full participation in a harm reduction program (HRP) was associated with a lower risk of HIV and HCV infection in ever-injecting DU, compared to no participation (incidence rate ratio 0.43 (95% CI 0.21-0.87) and 0.36 (95% CI 0.13-1.03), respectively).

Conclusions
In conclusion, we found that full participation in HRP was associated with a lower incidence of HCV and HIV infection in ever-injecting DU, indicating that combined prevention measures --but not the use of NEP or methadone alone-- might contribute to the reduction of the spread of these infections.

Introduction

Injecting drug users (DU) are at high risk for blood-borne infections, including HIV and HCV, through the sharing of needles and injection equipment.[1] Various approaches to deal with the consequences of hard drugs have been taken; some countries aim to ban illicit drug use completely, whereas The Netherlands and others take a harm reduction approach. This harm reduction approach may have had a major impact on the HIV and HCV epidemic. The ultimate goal of harm reduction is to stop DU from using drugs, but until this is possible, the policy is to minimize the damage DU inflict on themselves and the society at large. Diverse programs (with a low, medium or high threshold) started in The Netherlands at the end of the 1970s, providing methadone in combination with social-medical care and needle-exchange facilities.[2] They have no waiting lists and are relatively easy to enter and re-enter. Ongoing drug use during participation is tolerated in low- and medium-threshold programs. Low-threshold programs have been operated since 1982 by the Amsterdam Health Service. For clients who have regulated their drug use, methadone can be prescribed in a medium-threshold program via their general practitioner. Clients who are willing to detoxify can receive methadone in a high-threshold program through an outpatient addiction clinic. Circulation between the different programs is permitted and 'promotion' to higher-threshold programs is encouraged. With the harm reduction approach, the Amsterdam methadone programs reached an estimated 2,700 of the 3,500 to 4,000 opiate users in Amsterdam.[3] All services are free of charge for residents of The Netherlands.

The effects of methadone provision or needle exchange programs (NEP) separately on HIV incidence have been examined, with conflicting results.[4,5] Very few studies describe the effect of either program on HCV incidence, although declining prevalence of HCV was reported after the introduction of NEP.[6]

The Amsterdam Cohort Study (ACS) among DU comprises a large group of DU who are prospectively tested for HIV. We tested their stored sera for HCV, retrospectively, and therefore had the unique opportunity to document the effect of harm reduction on the incidence of both HIV and HCV over a long time period.[7-9]

Materials and Methods

Study population and design

The Amsterdam Cohort Study (ACS) among DU is an open, prospective cohort study initiated to investigate the prevalence, incidence, and risk factors of infections with HIV and other blood-borne and/or sexually transmitted infections, as well as the effects of interventions.[10] It has collected detailed information on the participation in harm reduction programs (HRPs). The DU cohort was initiated in 1985; recruitment is ongoing and in recent years has been directed in particular toward young DU.

ACS participation is voluntary, and informed consent is obtained for every participant at intake. ACS participants visit the Amsterdam Health Service every 4-6 months. At intake and every visit, they give blood for HIV testing and storage; they also complete a standardized questionnaire about their health, drug use and sexual risk behaviour, and socio-demographic situation. At intake, questions about current behaviour refer to the preceding six months and/or to the period since 1980 or since the start of regular use of

hard drugs (i.e., heroin, cocaine, amphetamines and/or methadone at least three times per week). At follow up visits, questions refer to the time between the present and the preceding visit.

Laboratory methods

All ACS participants since 1985 (n=1640) were prospectively tested for HIV antibodies by enzyme linked immunosorbent assays (ELISA). All participants with at least two visits between December 1985 and November 2005 (n=1276) were retrospectively tested for HCV antibodies, using the first sample available in each case. Third generation ELISA tests were used to detect HCV antibodies (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). Individuals who were HCV-negative at ACS entry were tested for HCV antibodies at their most recent ACS visit. On finding HCV seroconversion (defined as the presence of HCV antibodies in a previously seronegative individual), we tested samples taken in between these two visits to indicate the seroconversion interval.

Statistical analyses

HIV and/or HCV-negative ever-injecting drug users entered the risk set at study entry or at their start of injecting drug use during follow up, and were followed up until seroconversion for respectively HIV or HCV, or until end of follow up, ultimately at 1 November 2005. The date of HCV or HIV seroconversion was estimated as the midpoint between the last seronegative and the first seropositive ACS visit. Poisson regression was used to determine the effect of harm reduction on HCV and HIV incidence. Incidence rates and incidence rate ratios (IRR) with their corresponding 95% confidence intervals (95% CI) were calculated. We evaluated the potential confounding effect of all variables listed below and evaluated interaction between variables included in the final model. Multivariate models were built using forward-stepwise techniques, and variables with a univariate p-value ≤0.10 were considered as potential independent determinants. All variables subject to change were treated as time-dependent variables, these variables refer to the six months prior to the visit. A p value ≤0.05 was considered statistically significant.

To study the impact of harm reduction on HIV and HCV seroconversion, we combined injecting drug use, use of NEP and methadone dosage into one variable with five categories (Table 2.2.1). Because higher doses of methadone are more effective than lower doses in lowering the prevalence of injecting drug use risk behaviour, we considered ≥60 mg methadone per day an adequate minimum dosage for opioid replacement therapy and used that dose as cut-off value for our definition of adequate harm reduction.[11-13]

General characteristics of persons evaluated included sex, nationality, age, HIV status in cases of HCV as outcome, HCV status in cases of HIV as outcome, HIV status of the steady partner, homelessness, and hospitalization. The drug use variables included current injecting (yes or no), frequency of injecting, the main type of drug injected, the time elapsed since start of injecting drug use, the frequency of non-injecting drug use, and the type of drug mainly used as non-injecting drug.

Table 2.2.1 Definition of five levels of harm reduction used to evaluate the effect of harm reduction on HIV and HCV incidence in the Amsterdam Cohort Studies.

No harm reduction	No methadone in the past six months, injecting drug use in the past six months, and no use of NEP
Incomplete harm reduction	Any dose of methadone daily in the past six months, injecting drug use in the past six months and irregular* or no use of NEP; OR 0-59 mg methadone daily in the past six months, injecting drug use in the past six months, and always use of NEP
Full harm reduction	≥60 mg methadone daily in the past six months and no injecting drug use in the past six months; OR ≥60 mg methadone daily, injecting drug use in the past six months, and always use of NEP
Limited dependence on harm reduction	1-59 mg methadone daily in the past six months and no injecting drug use in the past six months
No dependence on harm reduction	No methadone in the past six months and no injecting drug use in the past six months

* Irregular use of NEP=1-99% of needles used in the past six months obtained via NEP. Always use of NEP=100% of needles used in past six months obtained via NEP.

Results

General characteristics

In total 1640 DU were enrolled in the ACS, 1276 DU had at least 2 visits. DU with more than 1 visit were older (median 31.4 (interquartile range (IQR) 31.0-31.8) years vs. 28.7 (28.1-29.4) years), more often male (63.9% vs. 56.9%), more often of Dutch nationality (74.5% vs. 60.2%) and more often HIV positive (20.6% vs. 16.2%) when compared to DU with only 1 visit to the ACS.
952 DU were so called ever injecting DU: DU who had ever injected drugs before ACS entry (n=905) or who started injecting drugs during follow up (n=47). 714 of these ever-injecting DU were HIV and/or HCV negative at study entry and were at risk for HIV and/or HCV during follow up. 164 DU (22.9%) were negative for both infections at study entry, 546 DU (76.5%) were HIV-negative and HCV-positive, and 4 DU (0.6%) were HCV-negative and HIV positive. The HIV prevalence among HCV-negative DU was 2.4% at entry, while the HCV prevalence among HIV-negative DU was much higher (76.2%). The DU included were mainly of Dutch nationality and mainly male (Table 2.2.2).

HIV-negative DU had a longer median time since starting injection than HCV-negative DU (respectively, 7.4 and 2.4 years). Furthermore, the proportion of DU who had recently injected (i.e., in the past 6 months before ACS entry) was larger for the HIV-negative DU than for HCV-negative DU. HIV-negative DU injected more often than HCV-negative DU, and HCV-negative DU used non-injecting drugs more often than their HIV-negative counterparts (Table 2.2.2). The median follow up time was 3.56 years (IQR 1.15-7.91 years) for DU at risk for HCV and 8.13 years (IQR 4.25-13.0 years) for DU at risk for HIV.

Table 2.2.2 General characteristics at entry and during follow up of 710 HIV negative and 168 HCV negative ever-injecting DU included in HIV and HCV analysis respectively.

	HIV		HCV	
At entry				
HIV/HCV infection (n at risk)	710	%	168	%
Prevalence HIV infection at entry risk set	-		4	2.4
Prevalence HCV infection at entry risk set	541	76.2	-	
Overall HIV incidence (per 100 PY)	1.65			
Overall HCV incidence (per 100 PY)			6.78	
General characteristics				
Steady partner at entry	333	46.9	77	45.8
Median age at entry risk set (years (IQR))	30.0 (27.0-36.0)		29.0 (25.0-33.0)	
Female	274	38.6	56	33.3
Dutch nationality	526	74.1	147	87.5
Western European ethnicity	602	84.8	139	82.7
Injecting drug use				
Median time since start injecting (years (IQR))	7.21 (3.04-12.1)		2.43 (0.06-7.16)	
Injecting in the past 6 months	524	73.8	100	59.5
Among recent injectors				
Injecting more than 1 time a week	429	82.3	53	54.6
Main drug injected				
heroin				
cocaine	94	17.9	33	33.0
speedball (i.e., combination of heroin and cocaine)	77	14.7	14	14.0
other	271	51.7	37	37.0
	82	15.6	16	16.0
Non-injecting drug use				
Non-injecting drug use in the past 6 months	497	70.0	149	88.7
Frequency of non injecting drug use				
1 or more times daily	190	38.2	77	51.7
1 or more times weekly, but less than 1 or more times daily less than weekly	188 119	37.8 23.9	61 11	41.0 7.4
Main non injecting drug use at entry				
heroin	239	48.2	66	44.2
cocaine	215	43.3	73	49.0
other	42	8.5	10	6.7
Follow up				
Median number of visits at risk (IQR)	17 (8-29)		15 (8-28)	
Median number of PY (IQR)	8.13 (4.25-13.0)		3.56 (1.15-7.91)	
Median number of days between follow up visits (IQR)	128 (118-168)		128 (119-166)	

Under study, 90/710 DU at risk for HIV seroconverted and 58/168 at risk for HCV. The median duration of the HIV and HCV seroconversion interval between visits was 4.0

months (IQR 3.7-6.0 months) and 4.0 months (IQR 3.7-5.1 months), respectively. The HIV incidence ranged from 8.5/100 PY in the late 1980s to approximately 0 in the most recent years, whereas HCV incidence was very high in the late 1980s (27.5/100 PY) and declined to around 2/100 PY in more recent years.[14]

Effect of harm reduction participation on HIV and HCV incidence

When evaluating the separate effects on HIV and HCV seroconversion of methadone dose or NEP we found that having any prescribed dose of methadone was associated with lower incidence rates of HIV and HCV infection, but not to a statistically significant degree (p=0.084 and p=0.21, respectively). Use of NEP was associated with a higher risk of HIV and HCV seroconversion, but with restriction of this variable to injecting drug use in the preceding six months, the IRR changed towards one and no longer reached statistical significance (data not shown). However, when methadone dose and NEP were combined as described in Table 2.2.1, full participation in a HRP was associated with a two- to threefold reduction in the risk of HIV seroconversion and with a six- to sevenfold reduction in the risk of HCV seroconversion (Table 2.2.3).
In univariate analysis the following variables were also associated with a higher risk of HIV or HCV: injecting drug use in the past six months, borrowing needles in the past six months, more recent onset of injecting drug use, a higher frequency of injecting drugs, mainly injecting speedball, younger age, and having an HIV-positive steady partner. A change in methadone dosage in the past six months was associated with a higher risk for HCV seroconversion but not HIV seroconversion. DU who were chronically HIV-infected or had an acute HIV infection in the six months preceding the visit were at increased risk for HCV seroconversion (Table 2.2.3).
In multivariate analysis we found that after correcting for having an HIV-positive steady partner and a smaller number of years since starting injection (both factors being independently associated with HIV seroconversion), the combined harm reduction variable remained independently associated with HIV seroconversion (Table 2.2.4). That is, DU fully participating in HRPs were at a decreased risk of HIV seroconversion compared to DU not fully participating in a HRP (IRR 0.43, 95% CI 0.21-0.87).

In multivariate analysis for HCV, we found that with correction for time elapsed since start of injecting, DU fully participating in a HRP were at decreased risk of HCV seroconversion compared to DU not participating in a HRP (IRR 0.36, 95% CI 0.13-1.03). As with HIV, DU who recently started injecting drug use were at increased risk of HCV seroconversion. The effect of HIV status of the steady partner on HCV incidence had the same direction as its effect on HIV incidence (Table 2.2.4).

In sensitivity analyses, we found that the effects of harm reduction on HIV and HCV seroconversion did not substantially change when analysis was restricted to the years after 1989 (i.e., when a methadone dose of ≥60 mg daily was more readily available for DU). Also, when the lower limit of adequate methadone dosage was adjusted to ≥80 mg daily, the effects of harm reduction on HIV and HCV seroconversion did not substantially change.

Table 2.2.3 Univariate associations between general characteristics, drug use characteristics, sexual risk behaviour characteristics, and HIV and HCV seroconversion among DU in the ACS. Sc = seroconversion, PY = person years, IRR = incidence rate ratio, 95% CI = 95% confidence interval.

	HIV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value	HCV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value
Harm reduction												
Level of harm reduction (definitions described in table 2.2.1)						<0.001						<0.001
No harm reduction	3.80	18	473.6	1			23.16	11	47.5	1		
Incomplete harm reduction	2.80	46	1640.8	0.74	(0.43-1.27)		24.12	34	141.0	1.04	(0.53-2.05)	
Full harm reduction	1.22	18	1475.9	0.32	(0.17-0.62)		3.47	6	173.0	0.15	(0.056-0.40)	
Pre-ultimate goal	0.13	1	758.1	0.035	(0.005-0.26)		0.57	1	174.9	0.024	(0.003-0.19)	
Ultimate goal	0.57	6	1048.4	0.15	(0.060-0.38)		1.64	5	305.2	0.071	(0.025-0.20)	
Methadone dosage						0.084						0.21
0 mg	2.16	44	2036.9	1			8.34	34	407.7	1		
0-60 mg	1.37	21	1531.8	0.63	(0.38-1.07)		4.87	11	226.1	0.58	(0.30-1.15)	
≥60 mg	1.33	25	1880.6	0.62	(0.38-1.01)		5.67	12	211.8	0.68	(0.35-1.31)	
Needle exchange programme (% of needles obtained via)						<0.001						<0.001
No recent injecting	0.38	10	2633.8	1			1.60	10	623.4	1		
0 %	3.07	26	847.3	8.08	(3.90-16.8)		19.66	18	91.6	12.3	(5.66-26.6)	
1-99 %	2.30	8	347.8	6.05	(2.39-15.4)		30.56	11	36.0	19.0	(8.09-44.9)	
100 %	2.91	46	1578.8	7.67	(3.87-15.2)		19.10	19	99.5	11.9	(5.54-25.6)	
Change in methadone dosage compared to previous visit						0.11						<0.001
No change	1.34	39	2917.1	1			3.73	16	428.4	1		
Increase	1.82	18	991. 5	1.36	(0.78-2.37)		3.64	5	137.2	0.98	(0.36-2.66)	
Decrease	1.67	13	778.5	1.25	(0.67-2.34)		5.83	6	102.8	1.56	(0.61-3.99)	
Unknown	2.56	20	781.2	1.99	(1.12-3.83)		16.51	31	187.8	4.42	(2.42-8.08)	
General characteristics												
Sex	1.57	53	3384.1	1		0.62	5.65	32	566.0	1		0.085
Male												
Female	1.78	37	2084.3	1.13	(0.74-1.72)		8.96	26	290.2	1.58	(0.94-2.66)	
Age (per 10 years increase)				0.52	(0.39-0.69)	<0.001				0.46	(0.32-0.65)	<0.001

	HIV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value	HCV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value
Homelessness						0.18						0.29
No	1.58	82	5176.5	1			6.5	52	799.6	1		
Yes	2.74	8	291.9	1.71	(0.83-3.54)		10.6	6	56.7	1.63	(0.70-3.79)	
Hospitalization in preceding 6 months						0.09						0.67
No	1.56	80	5124.1	1.86	(0.96-3.59)		6.85	56	817.1	0.75	(0.18-3.05)	
Yes	2.90	10	344.3	1			5.11	2	39.2	1		
HCV/HIV status at visit						0.41						0.0055
Negative	1.13	10	888.5	1			6.13	51	831.5	1		
Positive	1.73	79	4553.6	1.54	(0.80-2.98)		34.82	5	14.4	5.68	(2.27-14.2)	
Acute infection in previous 6 months	4.64	1	21.6	4.12	(0.53-32.2)		19.18	2	10.4	3.12	(0.76-12.8)	
Drug use variables												
Injecting in past 6 months						<0.001						<0.001
Yes	2.83	80	2831.1	7.45	(3.86-14.4)		20.74	48	231.5	12.9	(6.54-25.6)	
No	0.38	10	2633.8	1			1.60	10	623.4	1		
Borrowing of needles						<0.001						<0.001
No recent injecting	0.38	10	2633.8	1			1.60	10	623.4	1		
Recent injecting, no borrowing	2.71	54	1996.3	7.12	(3.63-14.0)		14.51	23	158.5	9.05	(4.31-19.0)	
Recent injecting, borrowing 1-9 times	3.30	12	363.6	8.69	(3.76-20.1)		48.11	14	29.1	30.0	(13.3-67.5)	
Recent injecting, borrowing ≥10 times	2.17	1	46.2	5.71	(0.73-44.6)		23.78	2	8.41	14.8	(3.25-67.7)	
Frequency of injecting drug use in previous 6 months						<0.001						<0.001
No injecting drug use in previous 6 months	0.38	10	2633.8	1			1.60	10	623.4	1		
≥2 times/day	3.66	32	874.6	9.63	(4.74-19.6)		34.72	17	49.0	21.6	(9.91-47.3)	
1 time/day	2.71	3	110. 6	7.15	(1.97-26.0)		25.45	1	3.93	15.8	(2.03-123.8)	
≥2 times/week	3.05	29	949.6	8.04	(3.92-16.5)		28.83	19	65.9	18.0	(8.36-38.7)	
1 times a week	0.00	0	147.5	0			8.23	1	12.2	5.13	(0.66-40.1)	
≥2 times/month	2.15	5	232.1	5.67	(1.94-16.6)		8.13	3	36.9	5.07	(1.40-18.4)	
1 time/month	3.92	4	102.0	10.33	(3.24-33.0)		15.19	2	13.2	9.47	(2.07-43.2)	
Less frequent	1.53	6	393.2	4.02	(1.46-11.1)		8.36	4	47.9	5.21	(1.63-16.6)	

	HIV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value	HCV Incidence (/100 PY)	sc (n)	PY	IRR	95% CI	p value
Main drug injected in previous 6 months						<0.001						<0.001
No injecting drug use in previous 6 months	0.38	10	2633.8	1			1.60	10	623.4	1		
Heroin	2.26	13	574.2	5.96	(2.61-13.6)		19.62	9	45.9	12.2	(4.97-30.1)	
Cocaine	1.81	8	442.7	4.76	(1.88-12.1)		24.31	10	41.1	15.2	(6.31-36.4)	
Speedball	3.41	48	1408.6	8.97	(4.54-17.7)		18.30	21	114.7	11.4	(5.37-24.2)	
Amphetamines	1.87	4	213.9	4.92	(1.54-15.7)					7.45	(1.63-34.0)	
Methadone	3.02	4	132.6	7.95	(2.49-25.3)					21.3	(5.87-77.5)	
Other	4.94	3	60.8	13.0	(3.58-47.2)		26.92	8	29.7	44.5	(12.2-161.6)	
Time since start injection drug use (years)				0.93	(0.91-0.96)	<0.001				0.80	(0.74-0.86)	<0.001
Sexual risk behaviour												
Heterosexual risk behaviour in previous 6 months						0.87						0.59
No	1.59	52	3270.9	1			7.57	36	475.9	1		
Yes	1.73	38	2192.5	1.09	(0.72-1.66)		5.79	22	380.0	0.77	(0.45-1.30)	
HIV status of steady partner						0.0013						0.020
No steady partner	1.63	67	4122.5	1			7.83	53	676.7	1		
HIV positive	6.90	10	145.0	4.24	(2.18-8.25)		10.71	2	18.7	1.37	(0.33-5.61)	
HIV negative	1.14	11	963.7	0.70	(0.37-1.33)		2.62	3	114.6	0.33	(0.10-1.07)	
Unknown HIV status	0.98	2	203.8	0.60	(0.15-2.46)		0.00	0	34.0			

Table 2.2.4 Multivariate analysis of the effect of participation in harm reduction programs on HIV and HCV seroconversion.

	HIV			HCV		
	IRR	95% CI	p value	IRR	95% CI	p value
No harm reduction	1		<0.001	1		<0.001
Incomplete harm reduction	0.87	0.50-1.52		1.17	0.59-2.31	
Full harm reduction	0.43	0.21-0.87		0.36	0.13-1.03	
Limited dependence on harm reduction	0.046	0.006-0.35		0.044	0.006-0.35	
No dependence on harm reduction	0.20	0.078-0.50		0.13	0.044-0.40	
Time since start injection drug use (per year)	0.95	0.92-0.98	<0.001	0.87	0.81-0.93	<0.001
No steady partner	1		0.004	1		0.026
HIV positive steady partner	4.53	2.23-9.21		3.49	0.84-14.5	
HIV negative steady partner	0.82	0.43-1.57		0.42	0.13-1.37	
Steady partner with unknown HIV status	0.75	0.18-3.06				

IRR=incidence rate ratio, 95% CI=95% confidence interval.

Discussion

Our data suggest that the combination of adequate methadone therapy and full participation in NEP substantially contributed to the reduction of the incidence of HIV and HCV in DU in Amsterdam, although a statistically significant effect was not seen when methadone dose or NEP were considered separately. It is likely that Amsterdam's comprehensive program, in which methadone treatment and NEP are combined, explains the reported decline of HIV and HCV incidence.

We found no evidence that the effect of harm reduction was larger on HCV incidence than on HIV incidence, since our risk estimates for the different levels of harm reduction participation were comparable. One explanation might be that the Amsterdam harm reduction approach, which maintains contact with as many DU as possible, has an effect not only on injecting but also on sexual risk behaviour due to counselling and condom distribution. Our findings are in line with the reduction of sexual and drug-related risk behaviour seen in the ACS since the mid 1990s. Having an HIV-positive steady partner was associated with a higher risk of HIV infection, showing that HIV is more effectively transmitted sexually than HCV.[7]

The evaluation of HRPs is complicated, because it is hard to link participation in HRPs to outcome variables such as the incidence of blood borne infections. In some observational studies, methadone programs and NEP have been shown to reduce the incidence of HIV but not HCV.[5,6,15,16] Ecological studies have shown a declining HCV prevalence after the introduction of NEP, while HCV incidence remained high.[17-20] To our best knowledge, our study describes the combined effect of methadone therapy and NEP on HCV incidence, and over the longest period of time. The ACS among DU is a well-defined open cohort study with ongoing recruitment, that has been followed over the past 20 years. On average, 90% of participants that visited the ACS a given calendar year returned the next year as well. Despite its great strengths, ACS is not a randomized controlled trial and therefore a causal association between harm reduction participation and risk for HIV or HCV infection can not be proven. However, we could

not think of any unmeasured confounder both affecting harm reduction participation and HIV or HCV infection.

Although NEP and methadone prescription were not available at the study setting, we cannot exclude that a cohort effect might partially explain the observed decrease in HIV and HCV incidence and injecting behaviour we observed in our cohort. Furthermore, risk behaviour was self-reported, and bias toward socially desirable answers could cause underestimation of the proportion engaged in risk behaviour. Although the data on HRP participation were also self-reported, Langendam *et al*. studied the harm reduction measures in the ACS and matched the self-reported methadone doses to the central methadone registry (CMR) and they found no clear difference in the self-reported dose and the dose at the CMR.[21]

As expected, DU not injecting drugs in the past 6 months and taking a low dose of methadone daily (i.e., with limited dependence on harm reduction) and DU not injecting drugs in the past 6 months and not receiving any methadone (i.e., with no dependence on harm reduction) were at lower risk for HIV and HCV seroconversion than were DU fully participating in a HRP. Interestingly, the limited-dependence group were at lower risk for HIV and HCV seroconversion than the no-dependence group, although the difference was not statistically significant. It could be that, because DU receiving a low dose of methadone are still surrounded by the social-medical care network associated with the methadone therapy, they might return more easily to a higher dose of methadone or call for other help in case of problems than DU who have completely stopped methadone and are out of the network.

The most important implication of our study is that only when methadone is combined with provision of needles and syringes through exchange programs is there a significant reduction of HIV and HCV incidence. Our finding is most important for countries with recent and sometimes explosive outbreaks of HIV and/or HCV among DU, like in the former Soviet Union and Asia.[22,23] To provide only needles and syringes or only methadone will not be sufficient to curb the rapid spread of these and other blood borne infections among DU. It is essential to offer a comprehensive program in which both measures are combined, preferably also with social-medical care and counselling.

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Chapter 2.3

Never injected, but hepatitis C virus-infected:

A study among self declared never-injecting drug users from the Amsterdam Cohort Studies

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Abstract

The aim of this study was to gain insight in transmission routes of hepatitis C virus (HCV) infection among never-injecting drug users (DU), by studying incidence, prevalence, determinants, and molecular epidemiology of HCV infection. From the Amsterdam Cohort Studies among DU, 352 never-injecting DU were longitudinally tested for HCV antibodies. Logistic regression was used to identify factors associated with antibody prevalence. Part of HCV NS5B was sequenced to determine HCV genotype and for phylogenetic analyses, in which sequences were compared with those from injecting DU. HCV antibody prevalence was 6.3% and HCV incidence was 0.49/1,000 person years. HIV-positive status, female sex, and starting injection drug use during follow up (a putative marker of past injection drug use), were independently associated with HCV prevalence. The main genotypes found were genotype 3a (50%) and 1a (30%). Phylogenetic analysis revealed that HCV strains in never-injecting DU did not cluster together and did not differ from HCV strains circulating in injecting DU. We found a higher HCV prevalence in never-injecting DU than in the general population. Phylogenetic analysis shows a strong link with the injecting DU population. The increased risk could be related to underreporting of injecting drug use or to household or sexual transmission from injectors to non-injectors. Our findings stress the need for HCV testing of DU who report never injecting, especially given the potential to treat HCV infection effectively.

Introduction

Acute hepatitis C virus (HCV) infection is usually asymptomatic, and leads to chronic infection in 50-80% of patients.[1] Decades of chronic HCV infection can lead to liver cirrhosis and, in 1-5% of these patients, eventually to hepatocellular carcinoma as well.[2] In recent years, treatment success rates have substantially improved.[3] The most important mode of HCV transmission is through exposure to infected blood,[1,4] and although sexual and household transmission have been described, they appear to happen only occasionally.[5-7]

While never-injecting drug users (DU) do not share needles and/or syringes, their HCV prevalence is still higher than in the general population. Some studies suggest that HCV infection in never-injecting DU is associated with the sharing of drug-use paraphernalia, especially utilities used for consumption of crack, but others could not confirm these findings (reviewed in ref. 8). Alternatively, never-injecting DU might become infected with HCV through needle-stick accidents, household transmission, or sexual exposure. Recent review of research describing HCV among non-injecting DU points to a substantial gap in our knowledge of HCV in never-injecting DU, as no uniform risk factors could be identified.[8]

The Amsterdam Cohort Study (ACS) among DU comprises a large group of never-injecting DU. It was designed to evaluate the sexual and blood borne transmission of HIV, other blood borne pathogens, and sexually transmitted diseases, as well as the determinants of transition to injecting drug use. This design has the potential to determine prevalence, incidence, and risk factors for HCV infection among never-injecting DU. Additionally, we used phylogenetic analysis to investigate whether HCV strains isolated from never-injecting DU were closely related to strains circulating among injecting DU, or whether separate introductions had occurred through unrelated modes of transmission.[9]

Methods

The ACS among DU is an open, prospective cohort study initiated in 1985.[9] Participation in the ACS is voluntary, and informed consent is obtained for every participant at intake. Recruitment is ongoing and in recent years has been directed in particular towards young DU. Both injecting and non-injecting DU are included and visit the Amsterdam Health Service every 4-6 months. Each study visit standardized questionnaires on (injecting) drug use and sexual risk behaviour are administered by trained research nurses and blood is drawn for prospective HIV testing and storage of serum. To study HCV prevalence and incidence we retrospectively tested stored serum from all participants having at least two visits between December 1985 and November 2005 (n=1276), using the first available sample in each case. Individuals who were HCV negative at ACS entry were tested for HCV antibodies at their last ACS visit before November 2005. On finding HCV seroconversion (defined as the presence of HCV antibodies in a previously seronegative individual), we tested samples taken between these two visits to determine the moment of seroconversion (defined as the midpoint between the last HCV seronegative sample and the first seropositive visit).[10] Third generation commercial microparticle EIA system tests were used to detect HCV antibodies (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). 28.9% of the

seropositive participants were tested at two study visits or more, all with consistent positive HCV-antibody test results. Presence of HCV antibodies in all never-injecting DU was confirmed with Western blot (Deciscan HCV Plus immunoblot; BioRad). All ACS samples were stored at -80˚C.
All ACS participants since 1985 (n=1640) were tested for HIV antibodies by enzyme linked immunosorbent assays (ELISA), since 2003 AxSym HIV Ag/Ab Combo (Abbott) at each study visit. Results were confirmed by Western blot, since 1986, by HIV Blot version 2.2 (Genelab diagnostics).

Statistical analyses

Anti-HCV antibody prevalence and incidence were calculated. Follow-up time was calculated from HCV-negative study entry through HCV seroconversion, the moment of starting injecting drug use, or November 2005, whichever occurred first.
Risk factors for the presence of HCV antibodies at study entry were examined using logistic regression. All risk factors refer to the past 6 months, unless stated otherwise. They included: general and demographic factors (sex, nationality, ethnicity, calendar year of visit); drug use-related risk factors (ever-injecting drug use, years of regular heroin/cocaine/amphetamines use, start of injecting drug use during ACS follow up, alcohol use) and specifically cocaine-use-related factors (years of regular cocaine use/cocaine snorting/basing of cocaine); sexual risk behaviour (having sex with injecting DU/commercial sex workers/men who have sex with men since 1980, main sexual preference since 1980, number of commercial sexual contacts since 1980, having a steady sexual partner, having an injecting steady sexual partner, HIV status of the steady sexual partner, condom use (with steady sexual partner/casual partner/commercial contacts) and other clinically relevant variables (subjects' history of HIV, jaundice, blood transfusion, tattoo, piercing).
Multivariate logistic regression models were built using forward stepwise techniques. All variables with a p-value ≤0.10 in univariate analysis were considered for entry into the model. Statistical analysis was performed by use of STATA (version 9.2; StataCorp) and SPSS (version 15.0; SPSS Inc.) software. All statistical tests were two-sided; a p-value ≤0.05 was considered to be statistically significant. Interaction and confounding were checked between the variables in the final models and all variables with a univariate p-value ≤0.20.

Reverse-transcription polymerase chain reaction (RT-PCR) methods

After HCV antibody screening, HCV-seropositive samples were additionally tested for the presence of HCV RNA. RNA isolation was performed on 100 μl of serum using the TriPure method (Roche Diagnostics). Each RNA isolate was used as input for two nested multiplex RT-PCRs. The first PCR, which targets the conserved HCV core region, was devised as a genotyping system to differentiate genotypes 1a, 1b, 2a, 2b, 3a, 4, 5a and 6a. The second RT-PCR, which targets the NS5B region, was used for phylogenetic analysis. Conditions and primers for both PCRs have been described elsewhere.[11]

Sequencing and phylogenetic analysis

The sequencing reaction and analysis were performed as described earlier.[11] Briefly, NS5B PCR products were ethanol precipitated. Sense and antisense strands were

separately cycle-sequenced using the BigDye Terminator system (version 1.1; Perkin Elmer). Sequence products were purified using DyeEx spin kits (Qiagen) and analyzed on an ABI-310 automated sequencer (Applied Biosystems). Sequence alignment of the 436-bp NS5B fragment was performed using the BioEdit software package.[12] Viral genotype was confirmed after phylogenetic analysis of the NS5B sequences obtained from subjects (GenBank accession numbers EU410492 to EU410507) along with established GenBank reference sequences.[13] Mega software (version 3.1; available at: http://www.megasoftware.net) was used to construct a phylogenetic tree by the neighbour-joining method, using the Tamura-Nei substitution model with γ-distribution (α=0.40). Bootstrap values (n=1,000) were calculated to analyze the stability of tree topology. HCV sequences obtained from DU who reported never injecting were compared to all known HCV sequences from injecting DU participating in the ACS (un-published data).[11,14]

Results

General characteristics

Among the 1276 DU who participated in the ACS and had two or more visits between December 1985 and November 2005, 364 DU reported never having injected drugs before study entry. Of these 364, 352 (96.7%) had serum available for HCV testing. They were mainly male (69.3%) and of Dutch nationality (305/352, 86.6%); of the 305 Dutch participants 101 (33.1%) were of Surinamese ethnicity. Of 352 never-injecting DU, 154 preferred cocaine as their main type of non-injected drugs (43.8%). Of the 352, 22 (6.3%, 95% CI 3.9–9.4%) were HCV antibody-positive at study entry and 14/352 (4.0%, 95% CI 2.2-6.6%) DU were HIV-positive (Table 2.3.1). The total HCV-negative and never-injecting follow up time was 2005 person years (PY); the median follow up time per participant was 6.4 years (interquartile range (IQR) 3.01-11.3 years). Only one never-injecting DU seroconverted for HCV during follow up; the HCV incidence was 0.049 per 100 PY (95% CI 0.01–0.35 per 100 PY). However, 47 never-injecting DU started injecting during follow up, of whom 7 were HCV-positive at study entry and 23 seroconverted for HCV after starting injection.

In addition to the observed HCV incidence, we calculated an estimated incidence using prevalence data, assuming that the duration of regular hard-drug use before study entry equals the time of exposure to HCV. Information on the number of years of regular cocaine/regular heroin use was available for 285/352 individuals (81.0%), including 20/22 HCV positive never-injecting DU. The duration of regular use of heroin or cocaine was used as the time of exposure. These 285 individuals had a total of 2,539 person years of regular drug use. The estimated time of HCV infection was defined as the midpoint of years of duration of regular use of hard drugs, yielding an estimated incidence of 0.79 per 100 PY. Assigning the estimated time of HCV infection to either the start of regular hard drug use before study entry (maximum estimated HCV incidence) or at study entry (minimum estimated HCV incidence), changed the estimated HCV incidence only slightly to 0.82 or 0.76 per 100 PY, respectively.

Table 2.3.1 General characteristics of never-injecting drug users (DU) at entry in the Amsterdam Cohort Studies among DU.

	HCV + n=22	HCV - n=330
General drug use and HCV related characteristics		
Median age (IQR)	30 (26-37)	30 (26-36)
Female sex	12/22 (54.4%)	96/330 (29.1%)
Dutch nationality	19/22 (86.4%)	286/330 (86.7%)
Homeless in the past 6 months	0/14 (0%)	45/262 (17.2%)
Main type of drug used in the past 6 months		
Heroin	6/20 (30%)	137/300 (45.7%)
Cocaine	13/20 (65%)	141/300 (47%)
Heroin and cocaine together	1/20 (5%)	15/300 (5%)
Other	-	7/300 (2.3%)
HIV-positive (%)	3/22 (13.6%)	11/330 (3.33%)
Ever tattoo	6/14 (43%)	91/194 (47%)
Ever piercing	2/14 (14%)	20/194 (10%)
Jaundice (ever)	2/8 (25%)	4/68 (6%)
Blood transfusion (ever)	2/8 (25%)	5/67 (7.5%)
Follow up characteristics		
Median number of visits to ACS (IQR)	15 (6-25)	12 (5-22)
Median years follow up in ACS (IQR)	7.58 (4.58-14.1)	6.13 (2.99-11.1)
Number of HCV seroconversions	-	1
HCV viral characteristics		
HCV RNA positive	15 (68%)	NA
Genotypes mainly related to injecting drug use		
1a	4 (26.7%)*	
3a	8 (53.3%)*	
Genotypes mainly related to other risks		
1b	2 (13.3%)*	
2a	1 (6.7%)*	

NA = not applicable; * % among all HCV RNA positive individuals.

Associations with the presence of HCV antibodies

In univariate logistic regression (Table 2.3.2), the following variables were significantly associated with the presence of HCV antibodies at entry in the ACS: female sex (OR 2.93, 95% CI 1.22-7.00) and starting injection during follow up, a putative marker of past injection drug use (OR 3.38, 95% CI 1.30-8.80). Although the association had only borderline significance, HIV-positive participants had a higher risk of being HCV-positive (OR 4.58, 95% CI 1.18-17.8, p=0.053) (Table 2.3.2). No significant association of HCV with crack use was found, although the OR for cocaine compared to heroin as the main type of drug used was 2.11 (95% CI 0.78-5.70), and the OR for one or more times daily cocaine use was higher compared to less frequent cocaine use in the six months preceding ACS entry.

In multivariate logistic regression, HIV-positive status (OR 5.07, 95% CI 1.21-21.3), female sex (OR 2.85, 95% CI 1.15-7.05) and starting injection during follow up in ACS (OR 2.78, 95% CI 1.03-7.47), were independently associated with the presence of HCV antibodies.

Table 2.3.2 Univariate and multivariate logistic regression. Determinants of HCV in never-injecting drug users (DU) at entry in the Amsterdam Cohort Studies among DU.

		Univariate			Multivariate		
	Proportion HCV+	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Demographic variables							
Age (per 10 years of increase)		1.29	0.75-2.23	0.36			
Sex				0.017			0.023
Male	10/244	1			1		
Female	12/108	2.93	1.22-7.00		2.85	1.15-7.05	
Year of visit				0.063			
1985-1992	13/125	1					
1993-1998	4/114	0.31	0.10-1.00				
1999-2005	5/113	0.40	0.14-1.16				
Nationality				0.97			
Dutch	19/305	1					
Non-Dutch	3/47	0.97	0.28-3.43				
Years of education after primary school				0.36			
<3	3/31	1					
3	4/35	1.20	0.25-5.86				
4-5	3/78	0.37	0.071-1.96				
>5	3/69	0.42	0.081-2.23				
Alcohol use in the past 6 months				0.38			
No	12/139	1					
Yes	9/151	0.67	0.27-1.64				
Drug use related risk factors							
Main type of non-injecting drug used in past 6 months				0.32			
Heroin	6/143	1					
Cocaine	13/154	2.11	0.78-5.70				
Cocktail of heroin/cocaine (i.e., speedball)	1/15	1.52	0.17-13.5				
Frequency of non-injecting drug use (main drug used) in past 6 months				0.70			
Multiple times daily	11/137	1					
Once daily	1/20	0.60	0.07-4.94				
Several times weekly, but less than daily	5/113	0.53	0.18-1.57				
Several times monthly, but less than weekly	1/20	0.60	0.07-4.94				
Once monthly	1/4	3.81	0.37-39.9				
Less frequent	1/11	1.14	0.13-9.79				
Non-injecting drug use of steady partner				0.35			
Not applicable, no steady partner	13/169	1					
No, never	4/35	1.55	0.47-5.06				
Yes, now or ever	4/91	0.55	0.17-1.74				
Start of injecting drug use during follow up				0.02			0.043
No	15/305	1			1		
Yes	7/47	3.38	1.30-8.80		2.78	1.03-7.47	
Years of regular heroin use				0.10			
Less than 6 months (or never start)	1/49	1					
6 months-5 years	3/66	2.29	0.23-22.6				
≥5 years	16/170	4.99	0.64-38.6				

		Univariate			Multivariate		
	Proportion HCV+	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Years of regular amphetamines use				0.59			
Less than 6 months (or never start)	18/242	1					
6 months or more	2/43	0.67	0.15-3.02				
Cocaine related risk factors							
Years of regular cocaine use				0.61			
Less than 6 months (or never start)	3/45	1					
6 months-5 years	6/112	0.79	0.19-3.32				
≥5 years	11/128	1.31	0.35-4.95				
Frequency of cocaine use in 6 months before ACS entry				0.45			
No cocaine use	1/38	1					
Once or more times monthly	1/28	1.37	0.082-22.9				
Once or more times weekly	5/87	2.26	0.25-20.0				
Once or more times daily	6/61	4.04	0.47-34.9				
Sexual risk behaviour							
Sex with injecting DU since 1980				0.59			
No	8/149	1					
Yes	4/54	1.41	0.41-4.89				
Sex with commercial sex workers since 1980				0.80			
No	5/94	1					
Yes	7/114	1.16	0.36-3.80				
Sex with MSM since 1980				0.16			
No	8/172	1					
Yes	4/36	2.56	0.73-9.02				
Main sexual preference since 1980 (excluding contacts with commercial sex workers)				0.18			
Exclusively heterosexual	15/285	1					
Not exclusively heterosexual	5/47	2.14	0.74-6.20				
Number of prostitution contacts in the 6 months preceding ACS entry (males and females)				0.76			
No prostitution contacts	1/20	1					
1-9	10/159	1.27	0.15-10.5				
≥10	8/95	1.75	0.21-14.8				
Prostitution contacts in the 6 months preceding ACS entry (males and females)				0.49			
No	8/155	1					
Yes	4/51	1.56	0.45-5.43				
Steady partner in the 6 months preceding ACS entry				0.96			
No	13/202	1					
Yes	9/137	1.02	0.42-2.46				
Steady partner that injects/injected drugs in the 6 months preceding ACS entry				0.98			
Steady partner injects/injected drugs	2/35	1					
Steady partner does/did not inject drugs	7/105	1.18	0.23-5.96				
Not applicable, no steady partner	13/202	1.13	0.24-5.26				

		Univariate			Multivariate		
	Proportion HCV+	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Last HIV test result of steady partner				0.88			
Not applicable, no steady partner in the 6 months preceding ACS entry	20/283	1					
Positive	1/10	1.46	0.18-12.1				
Negative	0/37	-	-				
Unknown	1/20	0.69	0.088-5.44				
Always use of condoms with steady partner				0.33			
Not applicable, no steady partner in the 6 months preceding ACS entry	2/15	1					
No	17/254	0.47	0.097-2.24				
Yes	3/83	0.24	0.037-1.60				
Always use of condoms with casual partners				0.07			
Not applicable, no casual partners in the 6 months preceding ACS entry	1/51	1					
No	18/212	4.64	0.60-35.6				
Yes	3/89	1.74	0.18-17.2				
Use of condoms with prostitution partners				0.47			
Always use of condoms	3/36	1					
Not always use of condoms	3/26	1.43	0.27-7.75				
Not applicable, no prostitution partners	16/289	0.64	0.18-2.33				
Other risk factors							
HIV status				0.053			0.026
Negative	19/338	1			1		
Positive	3/14	4.58	1.18-17.8		5.07	1.21-21.3	
Tattoo (ever)				0.77			
No	6/97	1					
Yes	8/111	1.18	0.39-3.52				
Piercing (ever)				0.65			
No	12/186	1					
Yes	2/22	1.45	0.30-6.95				
Jaundice (ever)				0.11			
No	6/70	1					
Yes	2/6	5.33	0.80-35.4				
Blood transfusion (ever)				0.16			
No	6/68	1					
Yes	2/7	4.13	0.66-26.1				

OR=odds ratio, 95% CI=95% confidence interval.

HCV RNA and phylogenetic analysis

Of 22 HCV-antibody positive never-injecting DU at ACS entry, 15 (68.2%) had detectable HCV RNA. The most frequent HCV genotype found was 3a (53.3%), followed by genotype 1a (26.7%) (Table 2.3.1). HCV genotypes 1a and 3a are generally associated with injecting drug use, and in injecting DU in the ACS they account for 252/317 (79%) of HCV infections for which genotyping was performed. Hence, the proportion of injection-related HCV genotypes was comparable among injecting DU and never-injecting DU.[11,14,unpublished data] Figure 2.3.1 shows a phylogenetic tree of HCV

genotype 3a, comprising the eight NS5B sequences obtained from never-injecting DU together with all available genotype 3a NS5B sequences (n=65) from injecting DU.[11,14,unpublished data] Comparable to a pedigree, a phylogenetic tree illustrates the evolutionary relationships between genes or organisms or, in our case, the relationship among aligned NS5B sequences of several HCV genotype 3a viral variants. The more related two sequences are, the smaller the horizontal distance between those sequences in the tree. Based on phylogenetic analysis, sequences from never-injecting DU could not be distinguished from those of injecting DU. Sequences derived from never-injecting DU were interspersed with those of injecting DU, and they were not distinct phylogenetic isolates, nor did they form separate never-injecting DU clusters. This was observed also in HCV genotype 1a sequences (data not shown). The three never-injecting DU not infected with HCV genotype 1a or 3a harboured distinct strains of genotype 1b and 2a, which in the Netherlands and Belgium are linked to blood transfusion and nosocomial transmission rather than injecting drug use.[15,16] The proportion of never-injecting DU infected with these types (20%) was somewhat larger than the proportion observed among injecting DU (9%) in the ACS, but the difference was not statistically significant (p=0.26, Pearson Chi square).

Interestingly, only one never-injecting (male) DU seroconverted during follow up despite denying injecting drug use. He has regularly reported a steady sexual relationship with an injecting (female) DU who also participates in the ACS. She is a known injecting DU and became chronically infected with HCV genotype 2b at least 2.7 years before her male never-injecting DU sexual partner seroconverted for HCV. When comparing their two HCV sequences, the sequences were 100% identical (data not shown), making accidental exposure during household contacts or sexual transmission the likely route of transmission in this couple.

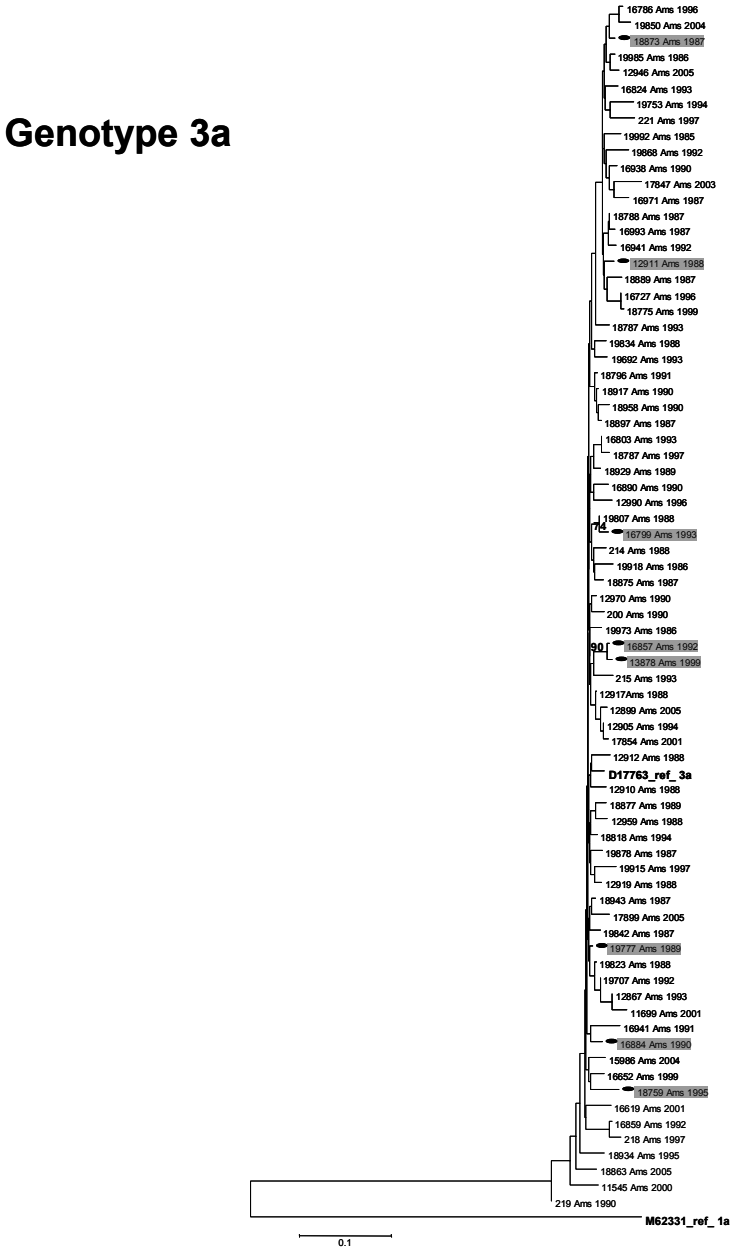

Figure 2.3.1 NS5B Phylogenetic tree of prevalent HCV genotype 3a infections among never-injecting drug users (DU) (shaded) and ever-injecting DU in Amsterdam, using the neighbour-joining method based on Tamura-Nei substitution with γ-distribution (α=0.40). Each isolate code contains the year of sampling.

Discussion

In this cohort of never-injecting DU, the HCV prevalence was 6.3% (95% CI 3.7-8.8%). Although much lower than the prevalence in injecting DU in the same cohort (83.5%),[10] this is substantially higher than in the general population in The Netherlands (estimated to be 0.1-0.4%).[17] In literature, the HCV prevalence in never-injecting DU ranges from 2.3 to 35.3%.[8] However many studies were not specifically designed to measure HCV prevalence in never-injecting DU and often did not include questions on non-injection drug use risk factors for HCV.

The observed HCV incidence was very low at 0.049/100 PY, sixteen-fold lower than the HCV incidence estimated from the prevalent cases at study entry (0.79/100 PY). This suggests underreporting of past injecting drug use, which may have led to misclassification of injecting DU as never-injecting DU. However, this estimated HCV incidence has limitations: it does not take into account losses to follow up in the unknown cohort that the prevalence sample is supposed to represent. Nor does it take differential recruitment of rates of healthy and infected subjects into account. However, when interpreted with caution, it could support our hypothesis of underreporting of injection drug use. Especially when injecting was incidental or stopped before entry in the ACS, participants may deny past risk behaviour, as has been described for HCV-positive blood donors in the Netherlands.[15]

Starting injection later during follow up was independently associated with a higher prevalence of HCV antibodies at entry. Of 352 never-injecting DU, 47 switched to injecting drug use after a median of 56 months (IQR 20-58 months). Of the 47, 7 were among the 22 found to be HCV seroprevalent at entry. Again, this finding could suggest that some injecting DU were misclassified as never-injecting DU. They might have given socially desirable answers and denied injecting, since it is perceived among DU as damaging to their appearance and as overstepping a limit in the drug-using scene in Amsterdam.[18] Alternatively, the DU who started injecting during follow up were already actively participating in the scene of injecting DU and were therefore more likely to become exposed to HCV through routes other than injecting drug use, such as needle stick accidents. Since HIV and HCV share transmission routes, the finding that HIV-positive never-injecting DU had a higher HCV prevalence at entry compared to HIV-negative participants, could imply that HIV-positive status is an indicator of unreported injecting drug use. On the other hand, HIV is transmitted sexually much more efficiently than HCV, and HCV might be transmitted more easily to and/or from HIV-positive individuals, compared to HIV-negative individuals, since HIV co-infection is associated with higher HCV RNA viral load.[19]

Phylogenetic analysis revealed that the HCV sequences of never-injecting DU did not cluster together, suggesting that they were not a uniform group that became infected through sharing of non-injection drug use paraphernalia. In contrast, the non-injecting DU clustered together with the sequences found in injecting DU in the ACS (Figure 2.3.1), indicating that they have close links with injecting DU and possibly underreport injection drug use. So although these DU did not report injecting drug use, they were infected from the pool of injecting DU. Although self-reported data on methadone prescription in this cohort have been investigated and shown to be consistent with data from the Dutch Central Methadone Registration, self-reported data on sexually transmitted diseases (STD) were shown to be less consistent with diagnosis of such diseases.[20,21] In this study, based on the findings from logistic regression and

phylogenetic analysis, some misclassification of ever-injecting DU seems likely in this never-injecting DU population.

Female sex was also associated with a higher HCV prevalence at entry, possibly indicating that women having sex with an HCV-positive partner are at higher risk for sexual transmission than men, as has been shown for HIV.[22,23] However, this gender difference has not yet been described for HCV.[24] We did not find an association between the presence of HCV antibodies and sexual behaviour. Furthermore, we observed only one HCV seroconversion during >2,000 person years of follow up, indicating that the risk of sexual transmission --and also household transmission-- is very small as has been demonstrated in partner studies among discordant heterosexual couples.[25,26] Unfortunately we were not able to perform risk factor analysis based on just one HCV seroconversion, but such analysis of incident cases in a longitudinal study would be more robust than a cross-sectional analysis of prevalent cases.

HCV has been detected on drug-use paraphernalia, and it has been hypothesized that HCV can be transmitted via these utilities (e.g., straws used for cocaine snorting).[27] In line with our phylogenetic finding of non-clustering of never-injecting DU, we did not find statistically significant associations between cocaine use and the presence of HCV antibodies. However, questions on snorting paraphernalia were not included in the ACS questionnaires used in our study period. Some questions (e.g., having a tattoo, having a piercing) were added to the questionnaires in 2001 and thus yield data for only a portion of participants included in this study. A similar limitation holds true for the data on having received a blood transfusion, a question not asked after 1989, shortly before HCV screening of donor blood was introduced in developed countries. Moreover, never-injecting DU might potentially have received a blood transfusion when travelling to countries where transfusion is not yet safe. Although the direction of the effect of having received a blood transfusion was as expected (i.e., higher risk for those who have received a blood transfusion compared to those who did not), the main HCV genotype related to transmission by blood transfusion is genotype 1b, whereas the main genotypes circulating among never-injecting and injecting DU are 1a and 3a. Remarkably, in The Netherlands between 1997-2002, genotypes 1a and 3a, were found in 9/18 (50%) of HCV RNA-positive new donor candidates who most likely acquired HCV through a contaminated blood transfusion in the past.[15]

In conclusion, although the incidence of HCV was very low in this study among never-injecting DU, the prevalence was much higher than in the general population. In the methadone outposts of the Amsterdam Health Service, HCV screening is offered every year irrespective of recent injecting drug use. Although, we could not distinguish whether the increased risk of HCV infection in never-injecting DU was related to underreporting of injection or to household or sexual transmission, HCV strains of never-injecting DU cluster with those found among injecting DU. HCV treatment has improved substantially since 2000 and is effective in up to 80-90% of patients.[3] Therefore, whatever the route of transmission, it is clear that routine HCV testing and treatment should be extended to both never-injecting and injecting DU.

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