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Liver fibrosis progression at autopsy in injecting drug users infected by hepatitis C: A longitudinal long-term cohort study

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Conflicts of interest

Knut Boe Kielland, Gerd J. M. Delaveris, Sidsel Rogde, Ellen J. Amundsen and Tor Jacob Eide declare no conflict of interest. Olav Dalgard received research grants from Schering Plough and Roche Norway, has been on advisory boards for Janssen Cilag, MSD, and Hoffman la Roche, and has given sponsored lectures for Janssen Cilag, MSD, and Hoffman la Roche.

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Abstract

Background & aims: There is a paucity of unbiased data on the natural history of hepatitis C virus (HCV) infection in injecting drug users (IDUs). The purpose of this study was to assess the risk of developing advanced fibrosis associated with chronic hepatitis C (CHC) infection among injecting drug users (IDUs) who underwent an autopsy.

Methods: A longitudinal cohort design was applied, in which the stage of liver fibrosis in anti-HCV positive IDUs with or without chronic HCV infection was assessed in liver tissue from autopsies performed up to 35 years after HCV exposure. The cohort originated from 864 IDUs consecutively admitted for drug abuse treatment 1970-1984. Stored sera, mostly drawn at the time of admission for drug treatment, were available in 635 subjects. 220 out of 523 anti-HCV positive subjects had died before 2009. Liver tissue from autopsies was available from 102/220 subjects, of which 61 were HCV RNA positive. Liver sections were classified according to METAVIR scores for fibrosis. Two pathologists, both blinded for serologic results, scored sections of liver tissue.

Results: Among HCV RNA positive subjects 16.4% (10/61) had septal fibrosis (F3) or cirrhosis (F4) compared to 2.4% (1/41) among anti HCV positive/HCV RNA negative subjects ($p=0.026$). Of 18 HCV RNA positive subjects autopsied <15 years after HCV exposure none had F3 or F4. Among subjects autopsied > 25 years after exposure 35% (6/17) had F3-F4.

Conclusions: Among IDUs chronically infected by HCV, 1/3 developed septal fibrosis or cirrhosis 25 years or more after exposure.

Keywords: Hepatitis C; HCV; Liver fibrosis; Cirrhosis; METAVIR; Steatosis; Longitudinal, Drug abuse; IDU; PWID.

Abbreviations: HCV, hepatitis C virus; IDU, injecting drug user; RNA, ribonucleic acid; anti-HCV; hepatitis C antibody; CHC, chronic hepatitis C; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; HCC, hepatocellular carcinoma; CI, confidence interval; IQR, interquartile range; ALT, alanine transaminase

Introduction

Hepatitis C virus (HCV) infection has been endemic among injecting drug users (IDUs) in Norway for four decades [1]. The risk of serious illness from liver disease increases with age [2]. There are few longitudinal studies on HCV-induced liver fibrosis with established time-point of HCV exposure, and the great majority have been performed among patients infected through medical procedures such as transfusions and medical injections with infected syringes or sera containing HCV [3-7]. In Western countries the main cause of HCV-infection has been the use of contaminated syringes and needles among IDUs. Still, to the best of our knowledge few longitudinal long-term studies have been published with IDUs as the main subjects, and most have used a limited observation time [8-10]. There is a number of crossectional studies of HCV related fibrosis [11]. However, these studies are intrinsically hampered by serious biases.

IDUs are different in several aspects from other groups of patients chronically infected by HCV. They have an increased burden of disease, are usually young at the time of exposure, and in most cohorts about 2/3 are males. Some studies also indicate increased use of alcohol among IDUs [12, 13]. There is a need for unbiased long-term studies which follow IDUs with all degrees of chronic HCV-infections for several decades.

The aim of the present study was to assess the risk of developing advanced fibrosis in IDUs with chronic HCV infection.

Patients and methods

Study design

The study had a longitudinal cohort design. It was based on a cohort of IDUs admitted to Statens klinikk for narkomane (National Clinic for Drug Abuse) during the years 1970 – 1984[14].

Participants

Consecutive subjects admitted for drug abuse treatment at Statens klinikk for narkomane 1970-1984 were included if they had sera drawn at the time of admission or later with detectable anti-HCV and with autopsy material available.

Among the 864 patients admitted, 523 were anti-HCV positive and could be tested for HCV RNA. By December 31, 2008, 220 of the 523 had died, and liver sections from autopsies were available for 102, the study group. These were divided into two groups, the anti-HCV positive/HCV RNA positive who had chronic hepatitis C (CHC) and the anti-HCV positive/HCV RNA negative subjects who had been exposed to HCV with later spontaneous clearance. The 118 who died and were anti-HCV positive, without liver tissue available for analysis, constituted a comparison group for analysis of representativity. Details on the selection of subjects are shown in Fig.1.

The patients were followed up through register linkage to the Causes of Death Registry (Statistics Norway), which includes information on whether an autopsy had been performed. Data on HCV antiviral medical treatment prior to 2004 were obtained by linkage to Scandinavian research trials on antiviral HCV treatment and from 2004 through the Norwegian Prescription Database (Norwegian Institute of Public Health), which began

operation that year and included all treated cases in Norway from 2004 onward. Norwegian 11-digit identification numbers were used for register linkage. The cohort was also linked to The Norwegian Cancer Registry for information on hepatocellular carcinoma (HCC) and to the Nordic Liver Transplant Registry.

Serum examination and time point of HCV transmission

As acute HCV infection was not registered in any of the patients, the time point of HCV transmission had to be estimated. All patient case records at Statens klinikk for narkomane were studied by one of the authors (KBK) to establish age at first drug injection. Medical records for most patients contained this information directly, and for most of the others it was possible to establish the age of first injection with reasonable certainty through circumstantial information, This included information about heroin or morphine misuse, or finding of anti-HBs antibodies, both of which in this population with high degree of certainty would imply drug injections. The time point of HCV transmission in most cases was estimated as two years after the first injection of drugs. Details on these procedures have been described elsewhere [14].

At the time of admission for treatment of drug abuse or later, serum from most of the patients was drawn for analysis and stored at the Norwegian Institute of Public Health (NIPH) at -20° C[14]. The sera were examined for anti-HCV (Ortho-Clinical Diagnostics HCV 3.0 ELISA), hepatitis B surface antigen (HBsAg), and hepatitis B core antibody (anti-HBc) (Bio-Rad Monolisa HBsAg Ultra and anti-HBc PLUS). HCV RNA was detected by an “in house” polymerase chain reaction (PCR) to detect viral RNA (detection limit 500 virus copies per ml corresponding to 100 IU per ml) [15].

Evaluation of liver tissue

When autopsy is performed in Norway, liver tissue is regularly stored. Eighty-one of the included samples of liver tissue were from autopsies performed at the Institute of Forensic Medicine at the University of Oslo, which is now part of the Norwegian Institute of Public Health. The remaining 21 originated from autopsies performed at other departments of pathology or forensic pathology. Liver tissue was available from all 102 autopsies as tissue blocks fixated in standard 10% neutral formalin and paraffin.

New histological sections were processed at the Department of Forensic Pathology and Clinical Forensic Medicine at the Norwegian Institute of Public Health. Three histological sections from each sample were cut to a 4 μm thickness and stained with hematoxylin eosin (HE), acid fuchsin orange G-stain (AFOG) and Perls' iron stain. The area of sections varied between 0.5 cm^2 and 3.0 $cm^{2.}$

All three sections from each case were examined by two pathologists working independently and blinded for serologic results and anamnestic information. The liver pathology was scored according to the METAVIR scales for fibrosis stage (F0 - F4) where F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = septal (or bridging) fibrosis and F4 = cirrhosis [16]. Inflammatory activity, steatosis and autolysis were also graded on four point scales from 0 to 3.

Statistics

Rates of advanced fibrosis (F3-F4) were calculated as cases of F3-F4/total cases. In the Kaplan- Meier plot, the end point was death accompanied with advanced fibrosis (F3-F4). Subjects dying without advanced fibrosis were censored. Log rank tests were used for testing significance in Kaplan-Meier analysis. The rate of progression of fibrosis was calculated as

METAVIR stage/years since HCV-exposure. Pearson's Chi-squared test was used for comparison of frequencies of ordinal variables. Significance level was set at 5% and confidence interval (CI) at 95%. The Statistical Package for the Social Sciences (SPSS) version 18.0 was employed for statistical analysis.

Ethics

The study was approved by the National Committee for Research Ethics. No consent was collected from the participants at the time of admission to treatment, and in accordance with the National Committee for Research Ethics no later attempt was made to collect consent from relatives. The Director of Public Prosecutions approved the use of tissue material collected at medicolegal autopsies.

Results

Patient description

The study group consisted of 102 anti-HCV positive individuals. Of this group 76 (74.5%) were males, 61 (59.8%) were HCV RNA positive. Among males 43/76 (56.6%) and among females 18/26 (69.2%) were HCV RNA positive. The mean time from the first drug injection to admittance for drug abuse treatment was 5.4 years (SD 3.0) and from HCV exposure to admittance 2.7 years (SD 3.6). The mean age at autopsy was 37.3 years (SD 9.4), and mean observation time from HCV exposure to autopsy was 16.9 years (SD 9.3).

Causes of death

The main cause of mortality was drug-related deaths (mainly opioid poisoning). A total of five individuals had committed suicide, while seven died from other external causes (Table 1).

Death by liver disease occurred in 3/61 (4.9%) of HCV RNA positive patients versus none of the 41 HCV RNA negative patients. One of the liver deaths was due to HCC. No other case of HCC was registered, nor any liver transplantation.

Fibrosis score and rate of fibrosis progression

We observed cirrhosis (F4) in 8/102, (8%, CI 3% – 13%) of autopsied anti-HCV positive IDUs. In the group with CHC 7/61, (11%, CI 3% – 19%) had cirrhosis compared to 1/41, (2%, CI 0% - 7%) among the HCV RNA negative subjects.

The proportion of advanced fibrosis (F3-F4) was 10/61 (16%, CI 7% - 26%) among patients with CHC, and 1/41 (2%, CI 0% - 7%) among HCV RNA negative subjects (p=0.026).

The proportion of advanced fibrosis increased according to the time since HCV exposure (Table 2). Among 26 subjects with CHC autopsied up to 15 years after HCV exposure, none had F3-F4. Among the individuals who died 15 - 25 years after exposure, 4/18 (22%, CI 3% - 41%) had F3-F4, and after more than 25 years since exposure 6/17 (35%, CI 13% - 58%) had F3-F4. On the other hand, 16/26 (62%, CI 43% - 80%) of subjects with CHC autopsied more than 20 years after exposure had no fibrosis (F0) or only portal fibrosis (F1) (Table 2).

Median time from HCV exposure to autopsy for subjects with F0-F2 was 14.8 years (interquartile range (IQR) 8.5 – 22.5) and for F3-F4 26.8 years (IQR 23.1 – 28.9).

Development of advanced fibrosis (F3-F4) is illustrated by the Kaplan-Meier plot in Fig. 2. End point in the figure is the combination of death and advanced fibrosis (F3-F4), while those with stage F0-F2 were censored. The first two cases of advanced fibrosis occurred 17 and 19 years after HCV exposure, respectively. The number of patients at risk 30 years after HCV exposure was too small for reliable results. Fig. 3 demonstrates the stage of fibrosis in

different age groups among the HCV RNA negative and positive individuals respectively, and implies that 6/16 (38%, CI 14% - 62%) of HCV RNA positive subjects autopsied at age >45 years had advanced fibrosis .

Among the 41 HCV RNA negative subjects, one male had developed cirrhosis 33 years after HCV-exposure. This individual was both anti-HBc positive and anti-HCV positive but with no sign of chronic viral hepatitis. He died from opioid intoxication at age 50. There was no information regarding liver disease or use of alcohol in his death certificate.

The median rate of fibrosis progression (METAVIR units/year) for HCV RNA positive patients was 0.068 (IQR 0.038 – 0.128, range 0.0 – 0.63), mean 0.097 (SD 0.094).

Five subjects were HBsAg positive, two of whom had HBV/HCV coinfection. One patient with HBV/HCV coinfection autopsied 29 years after HCV exposure had F2. The other four HBsAg positive individuals autopsied 6-30 years after HCV exposure had F0 or F1.

Only one subject had received antiviral HCV-treatment. She died 27 years after HCV exposure from opioid poisoning, a few months after treatment. Her fibrosis score was F1.

One subject with CHC had HIV as cause of death. At autopsy she had F1 14 years after the HCV exposure.

Inflammatory activity and steatosis

There were non-significant associations between HCV RNA and both inflammatory activity and steatosis (Table 3a). However, the associations between inflammatory activity and fibrosis and between steatosis and fibrosis were clearly significant (Table 3b).

Study group compared to group of dead IDUs without liver tissue available for analysis

(comparison group)

The study group of 102 subjects with available liver tissue was compared with the 118 who died in the same period without liver tissue available for assessment of fibrosis (Fig. 1).

The study group and the comparison group did not differ concerning gender (males 74.5% and 72.0% respectively), mean age at HCV exposure (20.9 years and 19.8 years) or chronic HCV infection (HCV RNA positive 59.8% and 61.9%). However, the groups differed significantly regarding causes of death, with drug related deaths (mostly opioid poisoning) dominant in the autopsied study group and other causes more frequent in the comparison group (Table 4).

Quality of liver specimens and the evaluation of fibrosis

All sections had at least some signs of autolysis (grade 1). Of the 102 liver tissue specimens suitable for the assessment of fibrosis, seven were unsuitable for the evaluation of inflammatory activity and eight of steatosis assessment due to autolysis.

The scoring of fibrosis differed by one stage among the two pathologists in 33 cases, of which 23 were HCV RNA positive. Most discrepancies were between F1 and F2, but in three cases there were discrepancies between F2 and F3. In cases that differed with one stage, the lower score was selected. In four cases the scoring differed by two stages. These cases were reassessed by each pathologist separately, resulting in four scores for each case. The most frequent score was chosen.

Discussion

In this study of autopsied IDUs, chronic HCV infection was clearly associated with the development of advanced fibrosis, as 16% of those who were HCV RNA positive had advanced fibrosis compared to 2% of those who were HCV RNA negative, after a mean time of 16 years since HCV exposure.

About 1/3 of the subjects with a chronic infection, observed for more than 25 years, had developed septal fibrosis or cirrhosis. Thus, among drug users in Norway older than 45 years, one in three with chronic HCV infection may be expected to have, or be in the process of developing, cirrhosis. On the other hand it is also noticeable that 62% of subjects with CHC autopsied more than 20 years after exposure had no or minimal fibrosis

The median fibrosis progression rate of 0.07 per year observed in this cohort was similar to or lower than reported in other studies of patients with chronic HCV-infection [16-20], but the progression rate was higher than in studies of young females who contracted an HCV-infection accidentally as a result of infected anti-D immunoglobulin in Ireland and Germany [5, 21]. In a meta-analysis of the stage-specific fibrosis progression rate in different populations of HCV-patients, the mean progression rate among the IDUs were: F0-F1 0.116, F1-F2 0.085, F2-F3 0.085 and F3-F4 0.130 [20]. This corresponds well to the overall mean progression rate of 0.097 in our study.

Cirrhosis was observed in 8% of this cohort of 102 autopsied IDUs. This is in line with an Australian autopsy study where 11% of anti-HCV positive autopsy cases of drug-related deaths had cirrhosis [22]. In a similar Danish autopsy study of fatal opioid overdose cases cirrhosis was observed among 19% (6/32) of anti-HCV positive subjects after more than 20 years of drug use [23], compared to 13% (6/46) in our study (Table 2).

Among anti-HCV positive/HCV RNA positive individuals, 11% had cirrhosis compared to 2% of the anti-HCV positive/HCV RNA negative ones. We are not aware of other autopsy studies that stratified the prevalence of cirrhosis according to HCV RNA status.

A study of HCV-related mortality, based on the same Norwegian population of IDUs as this study, demonstrated increased liver-related mortality 25-30 years after HCV transmission [14]. This is in accordance with the results in the present study.

The lack of significant associations between HCV RNA and steatosis/inflammatory activity in this study might be due to Type 2 errors, as these associations are well-documented in the literature [2, 24]. The clear associations between inflammatory activity and fibrosis and between steatosis and fibrosis also are in line with previous studies.

The important strengths of this study were the unbiased recruitment to the study, the relevant comparison with anti-HCV positive/HCV RNA negative subjects, long observation time, and secure identification through the Norwegian 11 digit personal code which ensures correct identity. Patients were included in this cohort when they entered treatment for drug abuse, typically five years after having started intravenous drug abuse and even a shorter time after being exposed to HCV. Thus, the sample represents the full spectrum of HCV related fibrosis progression without the bias in a more serious direction seen in cross-sectional studies which tends to include patients with signs of liver disease as raised alanine transaminase (ALT) activity. The HCV RNA negative patients were anti-HCV positive and had the same experience regarding drug abuse, were of the same age and were admitted for drug abuse treatment at the same clinic during the same period. This may compensate for the lack of knowledge about factors known to influence development of HCV-related fibrosis, such as use of alcohol, insulin resistance and body mass index [18].

Another strength was the fact that the histological evaluations were based on full sections of liver, not on needle biopsies like in most other studies. Thus sampling bias which could interfere with the evaluation was avoided [25-27].

This is, to the best of our knowledge, the first time such information from autopsies has been used in a longitudinal study of fibrosis in IDUs infected by HCV. The importance of this is underlined by a recent study based on digitally simulated biopsies from liver tissue which demonstrated great variability in the scoring of fibrosis stage in biopsies as compared to the real "gold standard" when the examination of liver tissue is performed on liver tissue obtained by resection or transplantation [28].

A limitation of the present study was the relatively small sample size. This inhibits definite conclusions concerning rates of fibrosis, especially after more than 30 years of follow up. In addition, no conclusions can be made with regard to the lack of influence from chronic HBV-infection on fibrosis progression in this study.

We did not have direct information on HIV except in the single case where HIV was noted as cause of death (Table 1). Among Scandinavian IDUs HIV has played a minor role compared with IDUs in other parts of the world including southern Europe [29], and it seems probable that the number of HIV positive subjects in the study group was small.

Another weakness was that the estimation of the fibrosis progression rate was based on only one histologic examination, with no biopsies having been taken between the time of infection and death. Our conclusions lean on the assumption that fibrosis rates are linear throughout the different stages, which is not confirmed [10, 30]. This approximation, however, is of value for comparison between this and other studies.

There is also a possibility that patients registered as HCV RNA negative could have been reinfected after the serum used for our analysis were drawn, and hence may have developed chronic HCV infection later. This could explain the development of fibrosis and - in one case - cirrhosis among patients registered as HCV RNA negative. Extensive use of alcohol is another possible explanation.

The study was based on PCR analysis of HCV RNA on sera stored at -20 ^{o}C. This temperature might not be sufficient for obtaining correct results, and clearance of HCV is somewhat higher than in most studies. Degradation of HCV RNA in some cases cannot be excluded. However, HCV clearance in this study is the same as in another Norwegian study based on storage at -70 ^{o}C [31], which might indicate that our HCV RNA results are acceptable.

Out of 220 deaths, 102 were autopsied and included in the study. They had significantly more drug related deaths (mainly opioid intoxication) than the 118 with no liver tissue available for examination, because death by intoxication is associated with a much higher rate of autopsies – mainly forensic - than death caused by disease. It might be hypothesized that liver disease could increase the risk of opioid intoxication, resulting in a bias towards patients with advanced liver fibrosis in the study group. However, the occurrence of liver disease as a cause of death was higher in the comparison group of 118 who were not part in the study (Table 4). Instead, this could indicate a higher occurrence of advanced fibrosis in that group compared to the study group, and consequently this study may underestimate the true risk of advanced fibrosis among IDUs chronically infected by HCV.

We may also have underestimated the risk of advanced fibrosis (F3-F4) by our selection of the lower score in cases where there was disagreement between the pathologists. Three of these were between F2 and F3.

Conclusions

About 1/3 of IDUs chronically infected by HCV progressed to advanced liver fibrosis within three decades after exposure. Without antiviral treatment, a high rate of liver related mortality must be expected in this group.

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Table 1 Cause or manner of death according to HCV RNA

	HCV RNA + (CHC)		HCV RNA –		Total	
	N	%	N	%	N	%
Drug related	46	75.4	33	80.5	79	77.4
Suicide	3	4.9	2	4.9	5	4.9
Homicide	2	3.3	0	0.0	2	2.0
Accident	3	4.9	2	4.9	5	4.9
Liver disease	3	4.9	0	0.0	3	2.9
HIV	1	1.6	0	0.0	1	1.0
Other disease	3	4.9	4	9.8	7	6.9
Total	61	100.0	41	100.0	102	100.0

Table 2 METAVIR fibrosis score according to HCV RNA and duration of HCV-infection

	Years from HCV exposure to autopsy	METAVIR fibrosis score					
		F0	F1	F2	F3	F4	Total
HCV RNA + (CHC)	0-10	0	14	0	0	0	14
	10-20	5	14	0	0	2	21
	20-30	2	13	1	2	4	22
	30-40	0	1	1	1	1	4
	Total	7	42	2	3	7	61
HCV RNA -	0-10	3	4	0	0	0	7
	10-20	1	14	0	0	0	15
	20-30	1	13	2	0	0	16
	30-40	0	1	1	0	1	3
	Total	5	32	3	0	1	41

Table 3a Fibrosis, inflammatory activity and steatosis according to HCV RNA

		HCVRNA+ (CHC)		HCVRNA-		
		N	%	N	%	
Fibrosis	F0-F2	51	83.6	40	97.6	p=0.026
	F3-F4	10	16.4	1	2.4	
	Total	61	100.0	41	100.0	
Inflammatory activity	A0-A1	55	94.8	39	100.0	p=0.149
	A2-A3	3	5.2	0	0.0	
	Total	56	100.0	39	100.0	
Steatosis	S0-S1	45	80.4	36	92.3	p=0.109
	S2-S3	11	19.6	2	7.7	
	Total	56	100.0	38	100.0	

Table 3b Fibrosis according to inflammatory activity and steatosis

		Inflammatory activity				
		A0-A1		A2-A3		
		N	%	N	%	
Fibrosis stage	F0-F2	85	90,4	1	33,3	P=0.02
	F3-F4	9	9,6	2	66,7	
	Total	94	100,0	3	100,0	

		Steatosis				
		S0-S1		S2-S3		
		N	%	N	%	
Fibrosis stage	F0-F2	77	95,1	8	57,1	P<0.001
	F3-F4	4	4,9	6	42,9	
	Total	81	100,0	14	100,0	

Table 4. Autopsied study group compared with comparison group without liver tissue available for analysis

		Study group (Liver available) N=102		Comparison group (Liver not available) N=118		
		n	%	n	%	p-value
	Males	76	74.5	85	72.0	0.679
	HCV RNA positive	61	59.8	73	61.9	0.755
	HBsAg positive	5	6.0	13	13.0	0.109
Cause or manner of death	Drug related	79	77.5	30	25,4	<0.001
	Suicide	5	4.9	15	12.7	0.044
	Accident and homicide	7	6.9	16	13.6	0.105
	Liver disease	3	2.9	9	7.6	0.127
	HIV	1	1.0	11	9.3	0.007
	Other disease	7	6.9	37	31.4	<0.001
		Years	SD	Years	SD	
Mean time from HCV exposure to death		18.20	8,47	15.84	9,84	
Mean age at death		38.01	8,52	36.80	10,16	

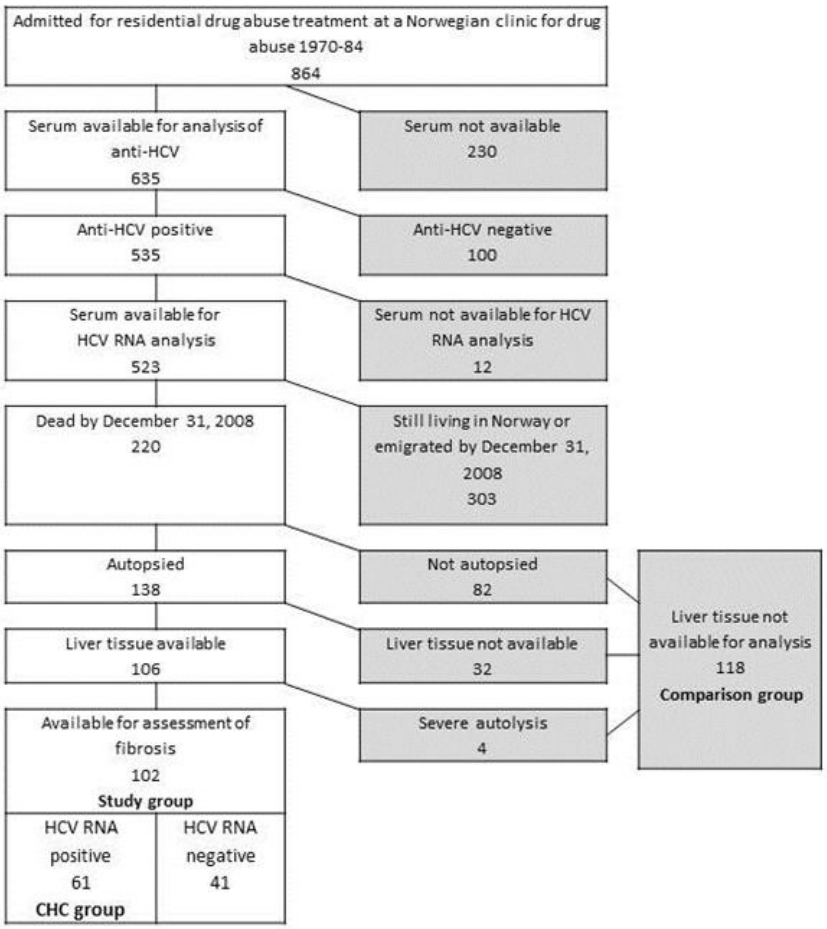

Figure 1 Selection of patients for fibrosis study based on liver tissue from autopsies

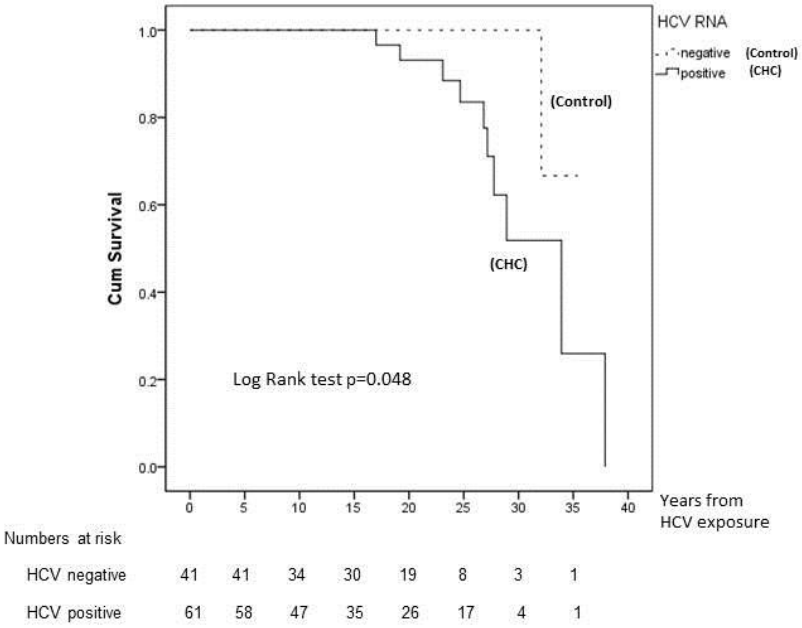

Figure 2 Kaplan-Meier plot on occurrence at autopsy of advanced fibrosis (METAVIR stages F3 or F4) according to HCV RNA. Subjects who at autopsy had METAVIR stages F0-F2 did not match the end point criteria and thus were censored

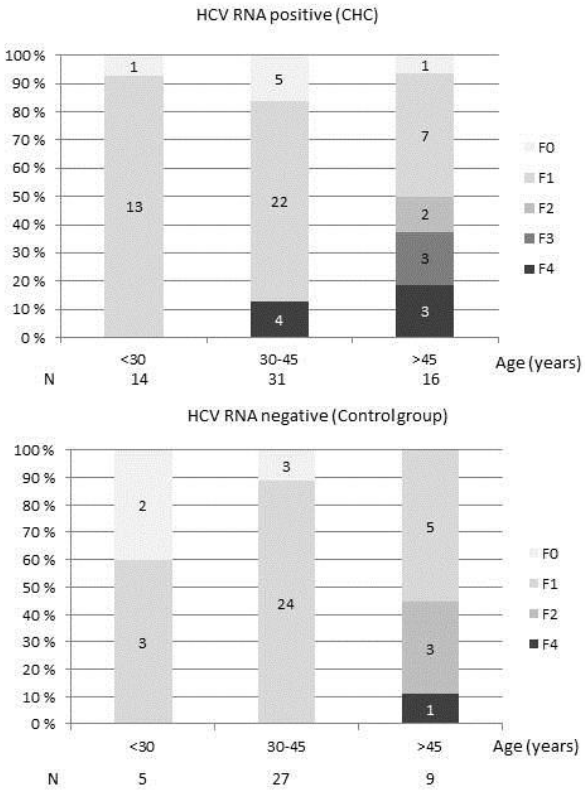

Figure 3 Stages of liver fibrosis at autopsy in different age groups according to HCV RNA