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DOI:
10.1016/j.jhep.2016.04.030

Publication date:
2016

Document Version
Peer reviewed version

FACTORS ASSOCIATED WITH SPONTANEOUS CLEARANCE OF CHRONIC HEPATITIS C VIRUS INFECTION: A RETROSPECTIVE CASE CONTROL STUDY

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Electronic word count: 5,966 words

Figures: 3

Tables: 2 (2 supplementary tables)

Abbreviations: HCV, hepatitis C virus; CHC, chronic hepatitis C virus infection; IL28B, interleukin-28B; Gt1, HCV genotype 1; HBV, hepatitis B virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; WoSSVC, West of Scotland Specialist Virus Centre; NHSGGC, NHS Greater Glasgow & Clyde; DBS, dried blood spot; HPS, Health Protection Scotland; BMI, body mass index; Gt3, HCV genotype 3; HBsAg, hepatitis B surface antigen; IFN, interferon; LPS, lipopolysaccharide; PWID, people who inject drugs

Keywords: HCV; spontaneous clearance; gender; HBV/HCV coinfection

Conflict of interest: The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support: No specific funding source for this project.

Authors contributions: STB and NB concepted and designed the study. PS and NB were responsible for the data acquisition. NB analysed the data and prepared the paper. All authors provided critical revisions and approved the final manuscript.

Abstract

Background & Aims: Spontaneous clearance of chronic hepatitis C virus (HCV) infection (CHC) is rare. We conducted a retrospective case control study to identify rates and factors associated with spontaneous clearance of CHC.

Methods: We defined a case as an individual who spontaneously resolved CHC, and a control as an individual that remained chronically infected. We used data obtained on HCV testing between 1994 and 2013 in the West of Scotland to infer case/control status. Specifically, untreated patients with ≥ 2 sequential samples positive for HCV RNA ≥ 6 months apart followed by ≥ 1 negative test, and those with ≥ 2 positive samples ≥ 6 months apart with no subsequent negative samples were identified. Control patients were randomly selected from the second group (4/patient of interest). Case notes were reviewed and patient characteristics obtained.

Results: 25,113 samples were positive for HCV RNA, relating to 10,318 patients. 50 cases of late spontaneous clearance were identified, contributing 241 person-years follow-up. 2518 untreated, chronically infected controls were identified, contributing 13,766 person-years follow-up, from whom 200 controls were randomly selected. Spontaneous clearance was positively associated with female gender, hepatitis B co-infection, younger age at infection and lower HCV RNA load. Spontaneous clearance was negatively associated with current intravenous drug use. The incidence rate of spontaneous clearance was 0.36/100 person-years follow-up, occurring after a median 50 months diagnosis.

Conclusions: Spontaneous clearance of CHC occurs infrequently but is associated with identifiable host and viral factors. More frequent RNA monitoring may be appropriate in selected patients.

Introduction

Hepatitis C virus (HCV) is an enveloped, positive sense, single stranded RNA virus which causes both acute and chronic hepatitis [1, 2]. Chronic HCV infection (CHC) is a global public health problem, estimated to affect approximately 185 million individuals worldwide and 37,000 persons in Scotland [3]. Chronic hepatitis C develops in around 75% of people who acquire HCV infection, and it is defined as viral persistence beyond six months post exposure [3, 4].

Spontaneous clearance of HCV in the acute phase (<6 months) occurs in around 20-40% of people who acquire HCV infection [2, 5]. Predictors of clearance remain poorly elucidated, however host factors, including gender [2, 6-8] and immune response [9], and viral factors, such as HCV genotype and quasispecies diversity [2], appear to be important. Host genetics are relevant, and the strongest host factor associated with clearance is a favourable interleukin-28B (IL28B) gene polymorphism [2, 8, 10].

Spontaneous clearance of HCV in the chronic phase is less well understood [11]. It has been reported in the literature following superinfection with hepatitis B virus (HBV) [12, 13] or following hepatitis delta virus (HDV) superinfection of human immunodeficiency virus (HIV)-HBV co-infected subjects [14]. Case reports have also described clearance following the withdrawal of immunosuppressive medication [15], in the context of liver transplantation or surgery [16, 17], following the development of hepatocellular carcinoma [18] and during pregnancy/parturition [19, 20]. Additionally, spontaneous HCV RNA negativity has been described in HIV-HCV co-infected patients, including those with hepatic decompensation, following initiation or optimisation of antiretroviral therapy [21-23].

Host factors may be important predictors of clearance in the chronic phase as well as the acute phase; Raghuraman et al reported a case of HCV clearance at 65 weeks post infection

which was associated with reversal of T cell exhaustion and the appearance of neutralising antibodies [24] and two recent studies looking at HIV-HCV co-infected patients found that late clearance was associated with a favourable IL28B-CC genotype [5, 23]. However, interpretation of these studies is limited by the small number of cases.

We sought to establish the incidence and factors associated with spontaneous clearance of CHC amongst a large Scottish cohort.

Patients and methods

Study design and population:

The West of Scotland Specialist Virus Centre (WoSSVC) is part of the NHS Greater Glasgow & Clyde Health Board (NHSGGC) which serves a population of > 1 million. Of the 35474 cases of HCV antibody positivity diagnosed in Scotland as of December 2013, 14076 (40%) reside within NHSGGC [25]. The WoSSVC provides the majority of the diagnostic virology service for the West of Scotland and is the sole provider of HCV RNA testing. Data were obtained from the WoSSVC on HCV testing over a 20 year period between 1994 and 2013. The study followed a retrospective case-control design; cases were individuals who spontaneously resolved CHC, and controls were individuals who did not.

Identifying cases and controls:

All patients must have been tested on either serum or dried blood spot (DBS) for HCV RNA as part of their clinical care. Patients with a minimum of 2 sequential samples positive for HCV RNA at least 6 months apart, followed by at least one negative test for HCV RNA, were identified. These patients were linked with national treatment data obtained from the Scottish Hepatitis C Clinical Database. This database is held by Health Protection Scotland (HPS) and contains clinical and treatment data for HCV infected patients attending outpatient specialist clinics across Scotland [26]. Patients with a history of HCV treatment were then excluded to create a cohort of individuals with potential spontaneous clearance of chronic HCV. Clinical records of potential spontaneous clearers were reviewed to confirm the clinical scenario. Individuals in the spontaneous clearance group with > 1 negative HCV RNA sample were subcategorised as ‘confirmed’ clearers.

Patients with 2 positive HCV RNA samples at least 6 months apart with no subsequent negative samples were identified as our comparison group. To create a control group of

chronically infected patients, individuals were randomly selected from the comparison group using number generation with a frequency of 4 controls per patient of interest.

Clinical, demographic and exposure data on cases and controls:

Demographic patient data (age at infection, sex, ethnicity, alcohol intake, body mass index (BMI), source of infection), HCV markers (liver enzymes, HCV genotype, IL28B genotype, HCV RNA and history of cirrhosis), HIV, HBV and HDV serostatus and IL28B genotype were obtained from the Scottish Hepatitis C Clinical Database, augmented by case note review. Where available, biochemical and haematologic variables were recorded at the time of the last positive HCV RNA test for all patients, and concurrently with the first negative HCV RNA test for spontaneous clearers. The date of HCV clearance was estimated using the midpoint between the time at which the last positive HCV RNA and the first negative HCV RNA samples were collected. Duration of diagnosis (which serves as a proxy of duration of infection) was calculated as the interval between the first positive HCV RNA and the time of HCV clearance for spontaneous clearers; for the control group this was defined as the interval between the first positive and the last positive HCV RNA results. Follow up was censored at the last positive HCV RNA test for the control group. Clinical records for case patients were reviewed and data were collected on hospitalisations or acute events in the 12 months prior to clearance.

Incidence of spontaneous resolution of CHC:

The incidence density rate of spontaneous clearance of CHC amongst untreated individuals was calculated as the number of cases of spontaneous clearance over the total number of person years follow up.

Laboratory testing:

All patients had been tested for HCV RNA as part of their clinical care. Viral load samples logged as 'positive' or 'detectable' were recorded as the upper limit of sensitivity for the given assay. Patients underwent HCV genotyping as part of their routine clinical care.

Statistical analysis:

Continuous variables are expressed as medians and interquartile ranges, and categorical variables are recorded as number and percentages. Categorical variables were compared using chi-square testing and continuous variables were analysed using the exact Wilcoxon Mann-Whitney test. P values are 2-sided and values of <0.05 were considered significant. The IBM SPSS Statistics 22 software was used for data analysis and missing variables were handled by listwise deletion.

Results

Derivation of final sample (Figure 1):

A total of 25,113 samples were positive for HCV RNA, relating to 10,318 patients. Of these, 1430 patients had 2 sequential positive results followed by a negative result. Following linkage to the Scottish Hepatitis C Clinical Database 1314 patients were identified as treatment experienced and were thus excluded, leaving 116 patients of interest. Ten patients were excluded following case note review as examination of full laboratory data showed that the HCV RNA positive samples were not sequential, suggesting 2 or more episodes of spontaneous clearance during acute infection rather than spontaneous clearance of CHC. A further 48 patients had exposure to HCV treatment that had not yet been recorded on the national database. For 7 patients, patient identifiers held in the database did not link with a clinical record. One patient had been incorrectly coded as negative, but on review of the laboratory data was found to have quantifiable HCV RNA. After these exclusions, 50 case patients remained and were included in downstream analysis, contributing 241 person-years follow up. Two patients were classified as spontaneous clearers solely on the basis of DBS testing, 1 of whom went on to have a positive serum HCV RNA test in the absence of ongoing risk exposure. A further 2 patients who were classified as spontaneous clearers on the basis of serum HCV RNA testing developed HCV RNA positivity > 1 year post probable clearance; 1 patient admitted to ongoing IDU. Twenty-seven patients went on to have at least 1 further negative HCV RNA test (26 serum samples and 1 DBS) and were subcategorised as 'confirmed' clearers.

For the comparison group, 3329 patients with 2 positive HCV-RNA samples at least 6 months apart with no subsequent negative samples were identified of whom 955 were treatment experienced. The remaining 2374 were untreated, contributing 13766 person-years

follow up. Our control population comprised 200 randomly selected patients from this untreated cohort.

Incidence of spontaneous clearance of CHC:

The overall incidence density rate of spontaneous clearance of CHC amongst the untreated patient population was 0.36 per 100-person-years follow up. When restricting the analysis to patients with 'confirmed' clearance, the incidence rate was 0.19 per 100-person-years follow up.

Characteristics of cases and controls:

Table 1 summarises the main demographic and clinical characteristics of the study populations. The majority of patients were white, with a history of IDU as the risk factor for acquisition of HCV. There was a similar incidence of Gt1 and genotype 3 (Gt3) infections. There were no significant differences in HCV genotype, ethnicity or risk group between the two populations. Ongoing IDU was positively associated with chronicity of infection (p=0.034).

Patients who spontaneously cleared CHC were more likely to be female (p = 0.001) and to have been diagnosed at a younger age (28.5 years vs. 33 years; p = 0.022). Median age at diagnosis in females was not significantly different between the two groups (27 years vs. 31.5 years; p=0.144). The age at which males and females were diagnosed in each group was similar (cases, p=0.200; controls, p=0.108).

There was no difference in the distribution of duration of diagnosis between groups (median duration 50 months v 50 months; p= 0.854) (Figure 2). The minimum duration of diagnosis in the spontaneous clearance group was 9 months and the maximum duration was 182 months, compared with 7 months and 195 months in the comparator group. As spontaneous clearance may be more likely in early infection, a subgroup analysis was performed for case patients

(n=41) and control patients (n=144) with at least 24 months confirmed viraemia and showed identical findings (Supplementary data: Table 1).

Median ALT levels were similar between cases and controls at the time of the last positive HCV RNA test (47.5 IU/L v 42.5 IU/L, p=0.560). There was a significant decrease in the ALT level between the last positive and the first negative HCV RNA test for case patients, providing further evidence of spontaneous clearance (47.5 IU/L v 20 IU/L, p<0.001).

Of those subjects who had been tested, quantitative HCV RNA levels were significantly lower amongst cases versus controls (p<0.001) however spontaneous clearance in the context of high-level viraemia (>10000 IU/mL) was observed in 7 patients (Figure 3). IL28B genotyping was performed on 1 case patient; this patient was found to carry the IL28B-CC allele.

27 of the cases had repeated negative RNA testing. Demographic and virologic characteristics of these are compared with controls in Table 2. On analysis of this more strictly defined cohort of spontaneous clearers, only female gender (p=0.006) and a lower median HCV viral load (p=0.001) remained significantly associated with clearance of CHC.

Co-infection with HIV and hepatotropic viruses:

Amongst those tested, patients who spontaneously cleared CHC were significantly more likely to be hepatitis B surface antigen (HBsAg) positive (5/48 (10.4%) vs 0/99 (0%); p<0.001). Eight case patients and 28 patients in the control group were positive for hepatitis B core antibody and negative for HBsAg indicating past infection. One HBsAg+ patient was co-infected with hepatitis delta virus. Rates of HIV IgG positivity were similar between the two groups (p=0.518).

Acute events:

In 5 patients, 4 of whom had documentation of ongoing alcohol abuse, spontaneous clearance of CHC followed admission to hospital with decompensated liver disease. In 2 of these cases there was intercurrent sepsis and in 1 case the patient was admitted twice; once as a result of a staggered paracetamol overdose and several months later due to alcoholic hepatitis with queried spontaneous bacterial peritonitis. The abstinent patient decompensated due to gram negative bacteraemia. Of the decompensated patients, two had significant ALT rises (>5 times the upper limit of normal).

Discussion

This is the largest cohort of patients with evidence of spontaneous clearance of chronic HCV infection studied to date. We have demonstrated that spontaneous clearance of CHC is rare, with an incidence rate of 0.19 – 0.36 per 100-person-years follow up. We found that spontaneous clearance of CHC was associated with female gender, HBsAg positivity, younger age at diagnosis and lower HCV RNA titres. It was negatively associated with current IDU. We observed that a proportion of cases occurred in the context of significant intercurrent illness and hepatic decompensation.

The incidence rate of spontaneous HCV clearance in our cohort is similar to that described in a previous Japanese study which demonstrated an annualized incidence rate of spontaneous CHC clearance of 0.5%/year/person and found that clearance was associated with milder liver disease [11]. In contrast, a recent study by Scott et al., [27] found that a significant percentage of Alaska Natives with CHC experienced HCV RNA negativity, corresponding to a rate of 1.15 per 100 persons per year. This variation in rates of spontaneous clearance may reflect the different genetic background of the study populations together with different incidences of factors associated with clearance of CHC. In addition, repeat HCV RNA testing in patients with established CHC in whom treatment is not immediately anticipated is performed rarely in our clinical practice, in accordance with international guidelines [4]. Infrequent repeat testing of HCV RNA may have led to an underestimate of the true incidence of spontaneous clearance in our cohort.

Concurrent with our study, Scott et al., [27] found that spontaneous HCV clearance was associated with a lower HCV viral load and a trend towards younger age at infection. Older age at acquisition is independently associated with a faster progression to fibrosis, even when controlling for duration of infection [28], and children who are vertically infected appear to

have a very slow progression to cirrhosis [29]. The presence of significant fibrosis is associated with a poorer response to HCV therapy [4] and may be negatively associated with spontaneous clearance of CHC [11]. The reasons for the importance of age as a predictor of progressive fibrosis are undetermined, but may relate to changes in immune function and reduced hepatic blood flow [30].

Female sex was significantly associated with spontaneous clearance of CHC in our study. This result remained significant when restricting the analysis to 'confirmed' clearers. These results mirror findings in the acute setting [2, 6-8], and are supported by data from Scott et al., [27] who found that all patients in their cohort with late sustained HCV RNA negativity were female. It has been postulated that gender-based differences in immunity may underlie the association between female sex and acute spontaneous clearance [2], and the same may hold true for clearance in the chronic setting. Additionally, male sex is associated with a faster progression to cirrhosis, even after controlling for age, duration of infection, alcohol intake and metabolic factors [31, 32]. It is possible that male gender was associated with increased disease severity in our study, and therefore a lower rate of clearance. Furthermore, Grebely et al., [2] demonstrated that the effect of IL28B and HCV genotype on clearance in the acute phase was greater among females than males. IL28B-CC genotype has also been associated with spontaneous clearance of HCV in HCV-HIV co-infected patients (5, 24), a finding we are unable to confirm due to infrequent testing amongst our cases.

Gt1 and Gt3 were equally distributed in our cohort, reflecting the distribution in Scotland [25]. We did not find an increased likelihood of spontaneous clearance associated with Gt1 infection, as has previously been described in both the acute and the chronic setting [2, 27]. However, as only a third of patients in our cohort had viral genotyping performed it is possible that this null result reflects limited statistical power.

Hepatitis B surface antigen positivity was significantly associated with spontaneous clearance of CHC ($p = 0.001$). HCV clearance in the context of HBV superinfection has been described in several case reports [12, 13] and may occur as a bystander effect of antiviral cytokine release [13]. It has been suggested that release of type 1 interferons (IFN) from the liver during acute infection may contribute to clearance [33] and that HBV may monopolise the synthetic machinery of the hepatocyte, thus interrupting the HCV replication cycle [33].

Despite the negative association previously described between fibrosis and spontaneous clearance of CHC [11], one third of our case patients had a diagnosis of cirrhosis. Additionally, we identified a unique cohort of patients who cleared HCV following decompensation of their cirrhosis, most commonly in the context of alcohol excess and bacterial infection. The mechanisms underlying spontaneous clearance in this setting are unclear. Cirrhosis is associated with a reduction in the number of functional hepatocytes, potentially limiting viral replication and whilst HCV RNA quantification was performed too infrequently in our study to explore this hypothesis, patients with cirrhosis have been found to have lower HCV viral loads than non-cirrhotic subjects in a large Scottish mixed infection database (unpublished data [34]). Furthermore, cirrhosis is associated with immune dysregulation and predisposition to bacterial infection [35]. Bacterial translocation occurs as a result of intestinal bacterial overgrowth and increased intestinal permeability, and results in endotoxaemia [35, 36]. Bacterial lipopolysaccharide (LPS) triggers production of inflammatory cytokines, including IFN-γ from hepatic lymphocytes, resulting in acute hepatic injury. Chronic alcohol ingestion enhances immune cell sensitivity to LPS resulting in increased production of inflammatory cytokines [37]. We hypothesise that HCV RNA clearance may occur in this setting as a result of non-specific stimulation of the immune system on a background of lower baseline viral load [27]. In support of this, two of the

decompensated patients in our study had significant hepatitis flares preceding clearance suggesting the development of a vigorous Th1 cytopathic immune response.

Finally, we present the tentative finding that ongoing IDU is negatively associated with spontaneous clearance of CHC. People who inject drugs (PWIDs) are at risk of superinfection with distinct HCV strains which may negatively impact the likelihood of spontaneous clearance [38, 39]. We also accept the possibility that a high HCV re-infection rate post clearance amongst PWIDs may be masking the incidence of spontaneous clearance in our cohort [6, 40]. However, one study based in NHSGGC reported a trend towards a lower incidence of re-infection post spontaneous clearance [41] as described in previous studies [42].

There are a number of limitations to our study as a consequence of its observational and retrospective design. Our study is strengthened by the inclusion of patients presenting and followed up over two decades. Inherent in this however, is considerable variation in the utilisation of different laboratory tests over time, reflecting changing advice from clinical guidelines [43, 44] and the development and introduction of new technologies. As a consequence of the missing data, multivariate analysis was not deemed appropriate and statistical inferences must be interpreted with caution.

We accepted HCV RNA testing on both serum and DBS in our study design to increase our study population. DBS testing may increase the uptake of screening in PWIDs, in whom venepuncture is often difficult and who may be less likely to attend clinic [45, 46]. However, HCV RNA testing on DBS has reduced sensitivity compared to the serum assay; one patient in our cohort who was classified as a spontaneous clearer on the basis of DBS HCV RNA testing was found to have detectable HCV RNA on a subsequent serum sample. Additionally, the sensitivity of the serum quantitative HCV RNA assays varied over the course of follow

up (supplementary data) and earlier samples may have been more likely to be falsely negative. Additionally, fluctuating and low level viraemia is common in the early stages of infection. As we relied on only one negative HCV RNA for the definition of spontaneous clearance, it is possible that we misclassified these patients as spontaneous clearers. However, restricting the analysis to patients with at least 24 months confirmed infection did not change our findings and the normalisation of liver biochemistry provides further support for clearance.

Follow up of patients with presumed late spontaneous clearance was poor; only 60% of spontaneous clearers had follow up HCV-RNA testing performed at any time point to confirm clearance. To address this limitation we performed an additional analysis of patients with persistent HCV RNA negativity over time and found that only female gender and low HCV viral load remained significant.

We used age at diagnosis as a surrogate marker for age at infection. Many patients self-identified as having been at risk of exposure to HCV many years before they were first tested and therefore it is likely that we overestimated the true age at infection. Also, for many case patients there was a considerable duration between the last positive and the first negative HCV PCR, making it difficult to ascertain the true date of HCV clearance.

Finally, HCV RNA testing rates may be subject to bias. Repeat HCV RNA testing in CHC is only recommended in patients for whom treatment is anticipated [4]. Although we allowed testing on DBS to increase our study population, certain patient groups may have been less likely to have been tested, including patients with chaotic lifestyles who are not engaged in care, or patients with contraindications to therapy. However, despite the methodological drawbacks inherent in a retrospective study, the biological plausibility of our results and concordance with the precedent in the literature lead us to believe that our results are sound.

We conclude that spontaneous clearance of CHC is more common in females and patients with a low HCV viral load, and that previously described factors including superinfection with HBV and younger age at infection may play a role. We report novel findings of a negative association with ongoing IDU, and describe a cohort of spontaneous clearance in the context of decompensated liver disease. Further work is required to identify the mechanisms underlying spontaneous clearance of chronic infection. Given that such clearance may occur after a prolonged duration of chronic infection, more regular serum HCV-RNA monitoring may be warranted, particularly in females, HBV co infection, patients with low level viraemia and those with decompensated liver disease.

Acknowledgements

The authors would like to extend their thanks to Dr David Young at the University of Strathclyde for his support with statistical analysis. We are grateful to the Hepatitis C Clinical Database Monitoring Committee for provision of the data used in this analysis.

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Author names in bold designate shared co-first authorship

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548 Tables

549 **Table 1: Univariate association between case-control status and demographic/clinical**
550 **factors**

	Late spontaneous clearance (n=50)	Chronically infected (n=200)	P value
Male sex [n (%)]	19 (38)	129 (65)	**0.001**
Median age at diagnosis [years (IQR)]	29 (25-36)	33 (28-38)	**0.022**
Ethnic group [n (%)]			0.719
White	48 (96)	194 (97)	
Asian	2 (4)	6 (3)	
Risk group [n (%*)]			0.789
Intravenous drug use	41 (89)	161 (90)	
Other	5 (11)	17 (10)	
Unknown	4	22	
HCV genotype [n (%*)]			0.713
1	7 (41)	61 (52)	
2	1 (6)	5 (4)	
3	9 (53)	52 (44)	
Unknown	33	82	
Serum HIV IgG [n(%*)]			0.518
Positive	2 (5)	3 (3)	
Negative	36 (95)	98 (97)	
Not tested	12	99	
Serum HBsAg [n (%*)]			**0.001**
Positive	5 (10)	0 (0)	
Negative	43 (90)	99 (100)	
Not tested	2	101	
Current IDU [n (%*)]			**0.034**
Yes	15 (38)	97 (56)	
No	25 (62)	76 (44)	
Unknown	10	27	
History of alcohol excess/ALD [n (%*)]			0.236
Yes	21 (47)	64 (36)	
No	24 (53)	109 (64)	
Unknown	5	27	
Cirrhosis [n (%*)]			0.238
Yes	13 (34)	34 (25)	
No	25 (66)	104 (75)	
Unknown	12	62	
Median duration of diagnosis [months (IQR)]	50 (31-81)	50 (19-103)	0.854
HCV VL (IU/ml)			**<0.001**
Median	1000†	341142†	
Interquartile range	1000 - 83293	59496 - 1517864	

551 *Percentage related to the actually recorded data; missing data handled by listwise deletion

552 †Data on HCV VL only available for 19 patients and 138 patients respectively

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554

555 **Table 2: As per Table 1, but where cases are confined to "confirmed clearers"**

	'Confirmed' clearance (n=27)	Chronically infected (n=200)	P value
Male sex [n (%)]	10 (37)	129 (65)	**0.006**
Median age at diagnosis [years (IQR)]	29 (25-37)	33 (28-38)	0.142
Ethnic group [n (%)]			0.362
White	27 (100)	194 (97)	
Asian	0 (0)	6 (3)	
Risk group [n (%*)]			0.803
Intravenous drug use	23 (92)	161 (90)	
Other	2 (8)	17 (10)	
Unknown	2	22	
HCV genotype [n (%*)]			0.784
1	6 (55)	61 (52)	
2	0 (0)	5 (4)	
3	5 (45)	52 (44)	
Unknown	16	82	
Serum HIV IgG [n (%*)]			0.765
Positive	1 (4)	3 (3)	
Negative	23 (96)	98 (97)	
Not tested	3	99	
Serum HBsAg [n (%*)]			0.055
Positive	1 (4)	0 (0)	
Negative	26 (96)	99 (100)	
Not tested	0	101	
Current IDU [n (%*)]			0.126
Yes	9 (39)	97 (56)	
No	14 (61)	76 (44)	
Unknown	4	27	
History of alcohol excess/ALD [n (%*)]			0.500
Yes	11 (44)	64 (36)	
No	14 (56)	109 (64)	
Unknown	2	27	
Cirrhosis [n (%*)]			0.638
Yes	7 (29)	34 (25)	
No	17 (71)	104 (75)	
Unknown	3	62	
Median duration of diagnosis [months (IQR)]	46 (29-76)	50 (19-103)	0.593
HCV VL (IU/ml)			**0.001**
Median	1000†	341142†	
Interquartile range	763 - 131242	59496 - 1517864	

556 *Percentage related to the actually recorded data; missing data handled by listwise deletion

557 †Data on HCV VL available for 10 patients and 138 patients respectively

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559

Figure legends

Figure 1. Derivation of case and control patient cohorts

Figure 2. Box-whisker plot of duration of diagnosis by group

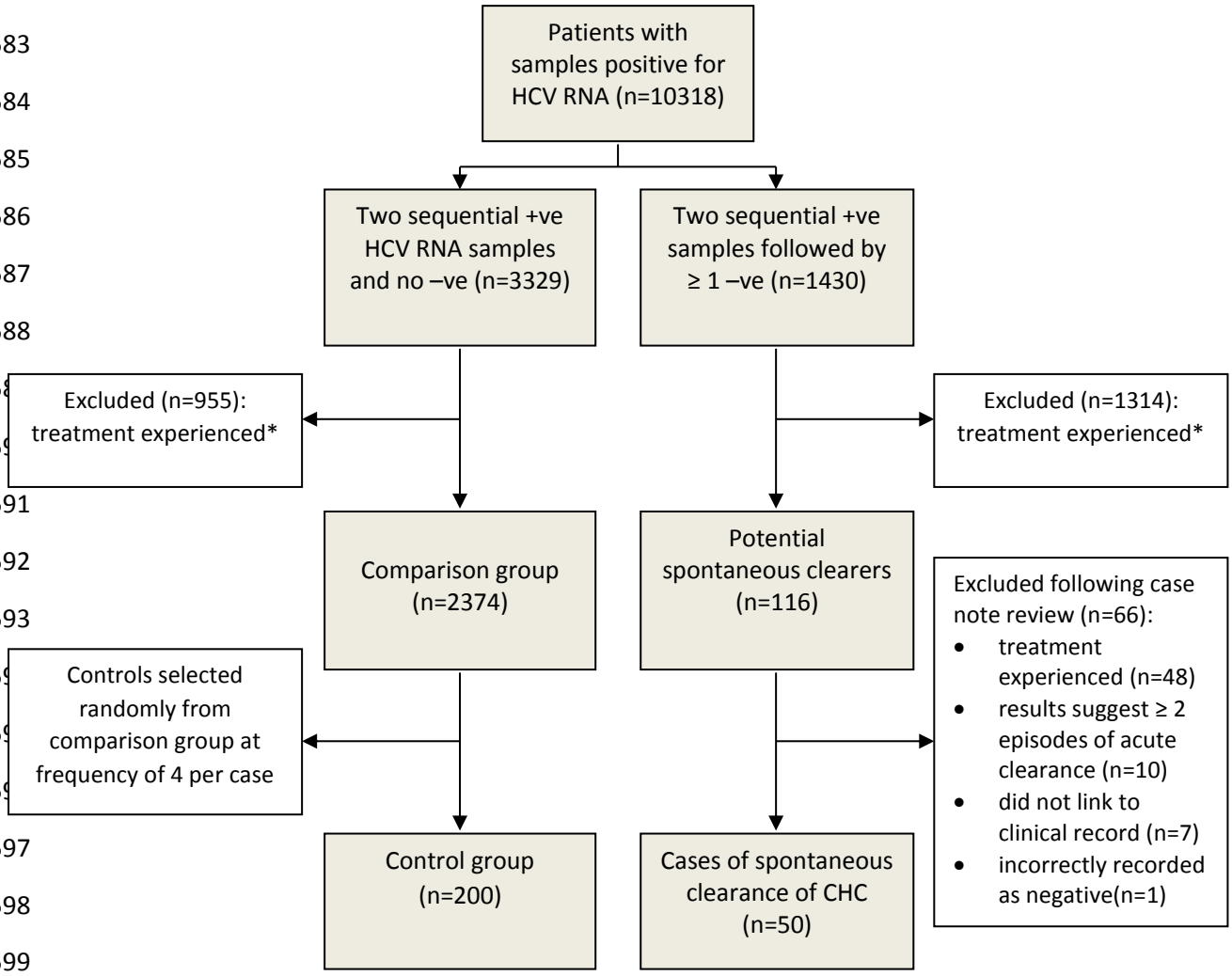
Box whisker plots of duration of diagnosis in months by group. Boxes represent 25th and 75th percentile, whiskers range and horizontal lines represent the median. Outliers are shown as circles.

Figure 2. Changes in HCV RNA levels against time since diagnosis for individuals showing spontaneous clearance of HCV RNA

Panel A: Changes in HCV RNA against time since diagnosis for all individuals with PCR results available (n=19). Point 0 represents the date of diagnosis.

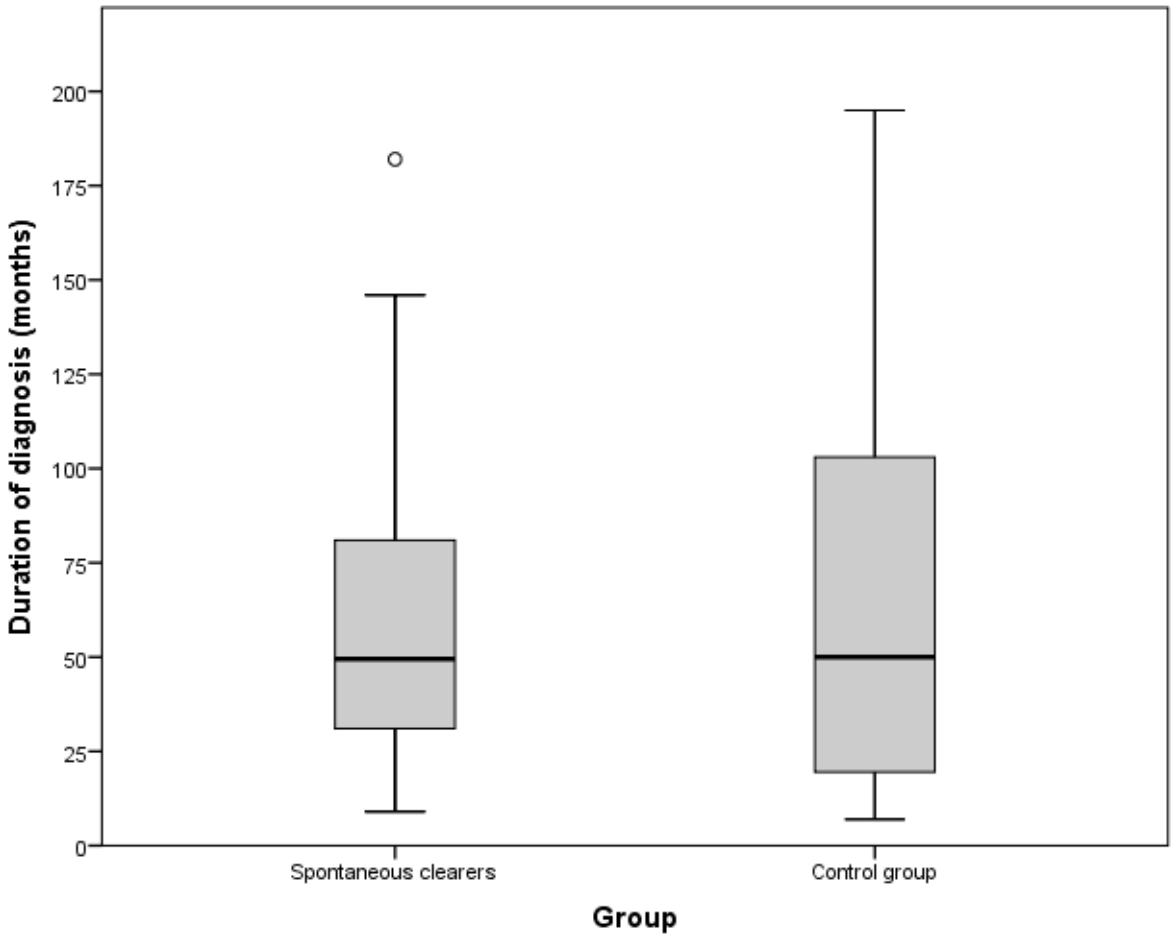
Panel B (insert) shows the same data, excluding patients with peak viraemia > 60,000 IU/mL (n=2).

Figure 1



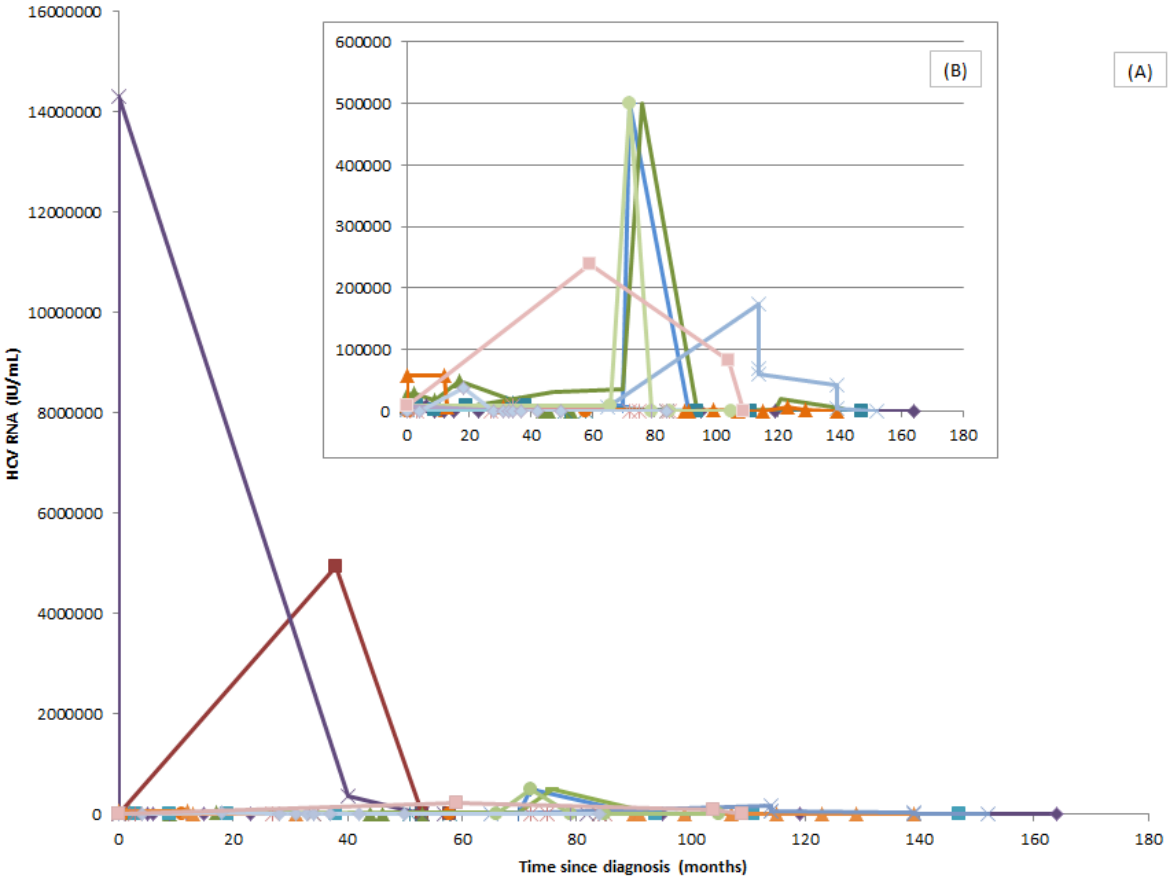
*Patients excluded following linkage to treatment data held in the Scottish Hepatitis C Clinical Database

Figure 2


Duration of diagnosis (months)
200
175
150
125
100
75
50
25
0
Spontaneous clearers
Control group
Group

619 **Figure 3**

620



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