Research Article

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Factors associated with spontaneous clearance of chronic hepatitis C virus infection

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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 cases as individuals who spontaneously resolved CHC, and controls as individuals who 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 $\geqslant$2 sequential samples positive for HCV RNA $\geqslant$6 months apart followed by $\geqslant$1 negative test, and those with $\geqslant$2 positive samples $\geqslant$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. 2,518 untreated, chronically infected controls were identified, contributing 13,766 person-years follow-up, from whom 200 controls were randomly selected. The incidence rate of spontaneous clearance was 0.36/100 person-years follow-up, occurring after a median 50 months' infection. Spontaneous clearance was positively associated with female gender, younger age at infection, lower HCV RNA load and co-infection with hepatitis B virus. It was negatively associated with current intravenous drug use.

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

Lay summary: Clearance of hepatitis C virus infection without treatment occurs rarely once chronic infection has been established. We interrogated a large Scottish patient cohort and found that it was more common in females, patients infected at a younger age or with lower levels of HCV in the blood, and patients co-infected with hepatitis B virus. Patients who injected drugs were less likely to spontaneously clear chronic infection.

Keywords: HCV; Spontaneous clearance; Gender; HBV/HCV co-infection.
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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 and 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.

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 185 million individuals worldwide and 37,000 persons in Scotland [3]. Chronicity 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 20–40% of people who acquire HCV infection [2,5]. Although predictors of clearance remain poorly elucidated, 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 relevant. Host genetics are also important, 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]. Case reports have described clearance in the context of liver transplantation or surgery [12,13], following the development of hepatocellular carcinoma [14] or the withdrawal of immunosuppressive medication [15], and during pregnancy/-parturition [16,17]. It has been reported in the literature following superinfection with hepatitis B virus (HBV) [18,19] or

following hepatitis delta virus (HDV) superinfection of human immunodeficiency virus (HIV)-HBV co-infected subjects [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 35,474 cases of HCV antibody positivity diagnosed in Scotland as of December 2013, 14,076 (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 selected from the comparison group using random 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 was used as a proxy for duration of infection and 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 *U* test. *p* values are 2-sided and values of <0.05 were considered significant. IBM SPSS Statistics 22 software was used for data analysis and missing variables were handled by listwise deletion.

Results

Derivation of final sample (Fig. 1)

A total of 25,113 samples were positive for HCV RNA, relating to 10,318 patients. Of these, 1,430 patients had 2 sequential positive results followed by a negative result. Following linkage to the Scottish Hepatitis C Clinical Database 1,314 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 injecting drug use. 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, 3,329 patients with 2 positive HCV RNA samples at least 6 months apart with no subsequent negative samples were identified of whom 955 were treatment expe-