No Impact of Hepatitis C Virus Infection on Mortality Among Drug Users During the First Decade After Seroconversion

BART GRADY,[*,‡] CHARLOTTE VAN DEN BERG,[*,‡,§] JANNIE VAN DER HELM,[*,‡] JANKE SCHINKEL,[‡,‖] ROEL COUTINHO,[*,‡,¶] ANNEKE KROL,[*] and MARIA PRINS[*,‡]

[*]Department of Research, Cluster of Infectious Diseases, Public Health Service, Amsterdam; [‡]Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Amsterdam; [§]Department of Internal Medicine, Academic Medical Center, Amsterdam; [‖]Department of Medical Microbiology, Academic Medical Center, Amsterdam; and [¶]Center for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

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BACKGROUND & AIMS: Most studies of progression of chronic hepatitis C virus (cHCV) infection were conducted in hospital settings and were therefore biased for patients with severe disease. We evaluated the long-term outcomes of hepatitis C virus (HCV) infection among injecting drug users, recruited from outside the hospital setting, and examined the effect of cHCV on mortality after seroconversion. **METHODS:** We studied data from 106 seroconverters with a documented or estimated date of HCV seroconversion. Cox proportional hazards analysis was used to determine the effect of HCV persistence, compared with HCV clearance, on survival after HCV seroconversion. The median follow-up time was 14.8 years (interquartile range, 7.8–19.6). **RESULTS:** cHCV infection developed in 71 of the subjects (67%; 95% confidence interval [CI], 57%–76%); 33 subjects died. One HCV-related death was observed 23 years after HCV seroconversion. Most causes of death were non-natural (n = 12) or acquired immune deficiency syndrome–related (n = 8). The effect of cHCV on mortality was nonproportional over time. When survival time was analyzed separately for 0–5 years, >5—10 years, and >10 years after HCV seroconversion, the age-adjusted hazard ratios for cHCV were 0.59 (95% CI, 0.16–2.20), 1.76 (95% CI, 0.36–8.53), and 8.28 (95% CI, 1.10–64.55), respectively, compared with resolved HCV infection. **CONCLUSIONS: cHCV infection does not affect overall mortality in the first decade after seroconversion, compared with individuals who resolve HCV infection; however, during the second decade after infection, individuals with cHCV have an increased risk for all-cause mortality. Mortality from liver-related causes was low but might have been masked by competing mortality.**

Keywords: Cause-Specific Mortality; Community-Acquired; Liver Disease; Substance Abuse.

Twenty years after the discovery of the hepatitis C virus (HCV), the progression rate to chronic liver disease and mortality remains uncertain. Although it has been shown that after primary HCV infection, persistent viremia will develop in 60%–85% of patients, putting them at risk for progressive liver disease,[1,2] data on the long-term outcomes are difficult to interpret. First, the severe sequelae of chronic HCV (cHCV) have been studied chiefly in hospital settings, and thus the results might have been biased because of the unknown duration of cHCV infection and event-biased referrals.[3–5] In these studies, estimates of the percentage of patients who developed cirrhosis range from 4%–22% after 20 years of cHCV infection.[6] In contrast, observational cohort studies of women in Ireland and Germany who became infected with HCV via administration of HCV-contaminated anti-D immunoglobulins showed cirrhosis rates of 0%–0.5% after a minimum of 20 years of HCV infection.[7–9] Second, death from liver-related causes attributable to cHCV is poorly defined, and the analyses of outcomes vary by study design.[10–13] Third, more than two-thirds of HCV infections in high-income countries are associated with the use of injected drugs, and injecting drug users (IDUs) are at increased risk of premature mortality compared with other HCV-infected groups because of their lifestyle.[14] Consequently, studies on the impact of cHCV on mortality rates in IDUs are complicated by competing causes of mortality. Fourth, to determine the impact of cHCV on mortality in community-acquired HCV infection, the impact on mortality in this group should be compared with that in HCV-resolvers, because these 2 groups most likely share important characteristics, unlike a population that is HCV-negative.[15,16] Recently, longitudinal community-based studies showed an impact on all-cause mortality in patients with cHCV, as compared with HCV resolvers.[13,17,18] However, referral bias and bias by the unknown duration of cHCV infection might have been present.

Worldwide, the prevalence of HCV in IDU ranges from 44% to more than 95%.[19,20] It is crucial, therefore, to provide accurate estimates of the future burden of HCV-related disease in IDUs. The Amsterdam Cohort Studies (ACS) on human immunodeficiency virus (HIV) is an ongoing (since 1985) prospective cohort study among drug users, which has retrospectively identified incident HCV and hepatitis B virus (HBV) infections. Because the ACS has the advantage of extensive follow-up of individuals with a well-defined HCV seroconversion date, we had the unique opportunity to document the long-term outcome of HCV on survival in IDUs with acute HCV, in particular to determine the effect of

Abbreviations used in this paper: ACS, Amsterdam Cohort Studies; AIDS, acquired immune deficiency syndrome; ALFO, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cART, combination antiretroviral therapy; cHCV, chronic hepatitis C virus; CI, confidence interval; COD, cause(s) of death; GGT, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, hazard ratio; IDU, injecting drug user; IQR, interquartile ratio.

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cHCV on mortality compared with resolved HCV. In addition, we examined the progression of liver disease during the first decades of infection by measuring fibrosis markers in the cohort of IDUs.

Methods

The ACS is an open and ongoing prospective cohort study among drug users that was initiated in 1985.[21] Participation is voluntary, and informed consent is obtained at intake for every participant. ACS participants visit the Public Health Service of Amsterdam every 4–6 months. Standardized questionnaires about their health, risk behavior, and sociodemographic situation are completed at each visit. Questions about current behavior refer to the period between the present and the preceding ACS visit. Questions at baseline refer to the period since 1980, since the start of regular use of hard drugs, or the last 6 months. At intake, blood is drawn to test for HIV antibodies by enzyme-linked immunosorbent assays and for storage. In 2006 and 2007, all ACS participants who had had at least 2 visits between December 1985 and November 2005 (n = 1276) were retrospectively tested for HCV and HBV by using the first sample in each IDU.[22] Third-generation tests were used to detect HCV antibodies (AxSym HCV version 3.0; Abbott, Wiesbaden, Germany). Patients who were anti-HCV negative at ACS entry were tested for anti-HCV at their most recent ACS visit. HCV seroconversion was defined as the presence of anti-HCV in a previously seronegative individual. On finding HCV seroconversion, we tested samples taken between these 2 visits to determine the moment of seroconversion.

The population in the present study comprises all IDUs who seroconverted for HCV during follow-up (n = 59) and IDUs who were anti-HCV positive at ACS entry (n = 58) but who had started using injected drugs within 2 years before study entry. We defined the probable date of HCV seroconversion as the midpoint between the last seronegative visit and first seropositive visit. For those who entered the cohort anti-HCV positive within 2 years after they started injecting drugs, we estimated the date of infection as the midpoint between the date of starting injecting and the study entry date. HCV viral clearance was defined as 2 consecutive HCV RNA-negative visits within 2 years after HCV seroconversion.

To determine HBV status, stored serum samples were retrospectively tested for anti-hepatitis B core by the same algorithm as for HCV (axSym Core; Abbott and Hepanostika; Organon Technika, Arnhem, Netherlands). To identify individuals with active HBV infection, the presence of HBV surface antigen (HBsAg) was determined (AxSym HBsAg; Abbott).

Reverse-Transcription Polymerase Chain Reaction Methods

We measured HCV RNA at a minimum of 4 time points around acute infection: the last visit before HCV seroconversion (the last anti-HCV–negative visit), 2 visits shortly after the estimated date of HCV seroconversion, and a visit approximately 2 years after HCV seroconversion. In those individuals who were anti-HCV positive at entry, HCV RNA was measured at study entry and at 1 visit at about 2 years after study entry. HCV RNA was also measured at their last visit or second-to-last visit before death. In addition, as reported in a previous study, a subset of HCV seroconverters from the ACS, mainly HCV resolvers, had additional serial serum samples measured for HCV RNA.[23] All serum samples were tested for the presence of HCV RNA by using an in-house quantitative real-time polymerase chain reaction based on the conserved 5′-untranslated region, and genotyping was performed as described in detail by van de Laar et al.[23]

Causes of Death

Every year, those lost to follow-up are matched against the local and national registries to obtain information about their vital status. Cause(s) of death (COD) were actively and systematically obtained, if available, from hospitals, general practitioners, the national HIV monitoring foundation,[24] or coroners. Data were collected on the primary COD, contributing COD, and underlying COD. For the present study, COD were grouped into 5 categories on the basis of the primary COD: liver-related, AIDS/HIV-related, natural COD, non-natural COD (overdose, accident, and suicide), and unknown COD.

Biological Markers of Fibrosis

To determine progression of liver disease, we analyzed fibrosis markers at 2 time points, namely within 2 years after HCV seroconversion (T = 1) and the second-to-last sample before death or a serum sample obtained at the last visit before December 2005 (T = 2). By using stored sera, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and albumin were determined by spectrophotometry on a P800 unit of a modular analytics serum work area from Roche Diagnostics (Basel, Switzerland). Haptoglobin was measured by turbidimetry on a P800 unit of a modular analytics serum work area from Roche Diagnostics. Alpha-fetoprotein (ALFO) was measured by electro-chemiluminescence on an E170 unit of a modular analytics serum work area from Roche Diagnostics. Alpha$_2$-macroglobulin was measured in serum samples by nephelometry on BN ProSpec (Siemens, Munich, Germany). Apolipoprotein A1 was measured by immunoturbidimetry on Architect (Abbott Laboratories, Abbott Park, IL).

Because single markers are not a satisfactory tool for assessing the amount of liver damage, we used Fibrotest, an algorithm that combines the effects of multiple biomarkers to assess for liver damage.[25] Fibrotest has been validated in several hepatitis C cohorts, although an independent study could not confirm this.[26] Because we did not have serum levels of bilirubin, we used a fixed level of 12 μmol/L for the algorithm. The formula used is available on the USPTO Web site (http://www.uspto.gov; patent no. 6,631,330).

Statistical Analysis

By using the Kaplan–Meier method, we estimated mortality from any cause. Cumulative incidence curves were estimated for the 5 categories of COD within a competing-risks framework. We determined the effect of cHCV versus HCV clearance on survival by using Cox proportional hazards analysis. Follow-up time was calculated from the estimated HCV seroconversion date until death, HCV treatment start, loss to follow-up, or date of analysis (September 1, 2009), whichever occurred first. IDUs who started injecting within 2 years before inclusion into the ACS were included in the risk set on their enrollment date (ie, correction for left truncation was applied).

Including cHCV versus HCV clearance and age as fixed variables in the model, multivariate models were built by using backward-stepwise techniques. Likelihood ratio tests were used to derive *P* values. Variables with a univariate *P* value $<.20$ were considered as