**Medicine**®

OPEN

# The elevated prevalence of risk factors for chronic liver disease among ageing people with hemophilia and implications for treatment

Christian Qvigstad, MD[a,b,*], Robert Campbell Tait, MBChB[c], Stephan Rauchensteiner, MD[d], Erik Berntorp, MD, PhD[e], Philippe de Moerloose, MD, PhD[f], Roger E. Schutgens, MD, PhD[g], Pål Andre Holme, MD, PhD[a,b], on behalf of the ADVANCE Working Group

## Abstract

Chronic liver disease (CLD) is frequently seen in the hemophilia population. The ADVANCE Working Group conducted a cross-sectional study in which people with hemophilia (PWH) aged ≥40 years were included. This study aimed to assess the associations between CLD and its risk factors using data from the H3 study, and to suggest implications for optimal care.

Data from 13 European countries were collected at a single time-point (2011–2013). Univariate and multivariate logistic regression (MLR) analyses were performed.

A total of 532 PWH were included with either hemophilia A (n = 467) or hemophilia B (n = 65). A total of 127 (24%) were diagnosed with CLD. Hepatitis C virus (HCV), human immunodeficiency virus (HIV), total cholesterol, and severe hemophilia were significant risk factors in univariate logistic regressions. In MLR, HCV Ab+/PCR+ (OR = 17.6, $P < .001$), diabetes (OR = 3.0, $P = .02$), and HIV (OR = 1.9, $P = .049$) were positively associated with CLD. Total cholesterol (OR = 0.6, $P = .002$) was negatively associated with CLD. We found no evidence of interaction effects among the explanatory variables. No significant associations with age and type of or severity of hemophilia were observed in MLR.

The main risk factors for CLD in this European cohort also apply to the general population, but the prevalence of HCV and HIV is considerably larger in this cohort. With new and improved treatment options, intensified eradication therapy for HCV seems justified to prevent CLD. Similarly, intensified monitoring and treatment of diabetes seem warranted.

**Abbreviations:** BMI = body mass index, CLD = chronic liver disease, CRF = case report form, DAA = direct-acting antiviral, ESLD = end-stage liver disease, HAART = highly active antiretroviral therapy, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HIV = human immunodeficiency virus, IR = insulin resistance, MLR = multivariate logistic regression, NRTI = nucleotide reverse transcriptase inhibitor, PWH = people with hemophilia, SVR = sustained virologic response, T2D = type 2 diabetes.

**Keywords:** chronic liver disease, CLD, diabetes, hemophilia, HCV, HIV, PWH

## 1. Introduction

Chronic liver disease (CLD) is recognized as a major cause of morbidity and mortality in people with hemophilia (PWH). Prior to the mid-1980s, factor concentrates contaminated with hepatitis C virus (HCV) were used for the treatment of PWH, resulting in mass infection and death of PWH worldwide, and many were coinfected with human immunodeficiency virus (HIV).[1,2] Except from viruses of contaminated transfusions, PWH are not known to have greater exposure to other risk factors for CLD than the general population.[3]

HCV infection is a major cause of cirrhosis, progression to end-stage liver disease (ESLD), hepatocellular carcinoma (HCC), and liver-related death in the Western world.[4] As a slowly progressive disease, cirrhosis develops in approximately 10% to 20% of patients over the course of 20 to 30 years. With cirrhosis established, the estimated annual risk of HCC is 1% to 5%, of hepatic decompensation 3% to 6%, and following an episode of decompensation, the risk of death in the following years is 15% to 20%. Studies of PWH have shown that HIV-HCV coinfection leads to accelerated HCV infection, and in 10% to 35% of the cases, ultimately ESLD.[5–10] Longer duration of HCV infection, alcohol abuse, HIV coinfection, older age at infection, and presence of HCV genotype 1 have been identified

as risk factors for rapid progression to ESLD.[8] The introduction of highly active antiretroviral therapy (HAART) in the mid-1990s has diminished the mortality due to HIV-AIDS (acquired immunodeficiency syndrome). For individuals with HCV, and in particular those with HIV-HCV coinfection, CLD is the leading cause of morbidity and mortality.[11–15]

The ADVANCE (age-related-developments-and-comorbidities-in-hemophilia) Working Group conducted an epidemiological study of hematuria and hypertension in hemophilia A and B (the H3 study), a multicenter, observational, noninterventional cross-sectional design.[16]

Using data from the H3 study, we have assessed the associations between CLD and clinically interesting risk factors and examined whether this European cohort differs from the general population and previous studies of PWH. The papers by Goedert et al and Posthouwer et al[7,8] are similar to ours but differ in some important respects. They have assessed the risk factors for developing ESLD using survival analysis, whereas we have studied risk factors for any type of CLD and disease progression using logistic regressions. Another difference is that they have considered only patients with HCV whereas our cohort included noninfected patients. Based on our findings and recent advances in treatment options, we have suggested implications for optimal care.

## 2. Methods

### 2.1. Data collection

The dataset, consisting of 532 patients aged 40 years and older, was collected between June 2011 and September 2013 from researchers in 16 participating centers in 13 European countries, where Germany had 3 centers and Italy had 2.[16] All data were gathered from consecutive PWH attending their routine clinical visit using a case report form (CRF) and from laboratory data collected no earlier than 1 year prior to the clinical visit. The CRF included items about patient characteristics, demographics, past and current treatment, and medical history including a lifetime history of comorbidities. Consenting patients with all severities of hemophilia A or B were included in the study and only patients below 40 years of age were excluded. In the CRF, the physician recorded the binary variable CLD as either present or absent. CLD, if present, was diagnosed as fibrosis or cirrhosis with ultrasound, transient elastography, biopsy, or as evidenced by portal hypertension/variceal disease. In addition, persisting (>6 months) abnormal/elevated liver transaminases qualified as chronic hepatitis. The presence or absence of HCV antibody and HCV RNA was used for the diagnosis of HCV infection and further categorized into 4 groups: HCV Ab positive PCR positive (Ab+/PCR+), HCV Ab negative PCR negative, patients with persistent response to antiretroviral drugs, and patients with natural clearance of HCV. Antibodies to HIV were determined by serological testing and reported in the CRF as existent or not. If applicable, HIV was reported together with the variable HAART. Diabetes was recorded as present if a patient had a known diagnosis or if currently on medication for diabetes. The study was approved by respective national ethical committees or the institutional review boards.

### 2.2. Statistical analysis

We have performed univariate and multivariate logistic regression (MLR) analyses to ascertain associations between CLD and its risk factors. Statistical analysis was performed with R version 3.5.0.[17] Throughout we have used a two-sided .05 significance level.

## 3. Results

### 3.1. Patient characteristics

A total of 98% of patients were Caucasian with median age 52 years (range 40–98 years). Each patient was characterized by type and severity of hemophilia: 467 (87.8%) hemophilia A, 65 (12.2%) hemophilia B, and 59% with severe, 11% with moderate, and 30% with mild hemophilia.

### 3.2. Descriptive results

There were 127 (24%) patients with CLD, 397 without, and 8 with missing CLD data. A total of 216 (42%) patients were HCV Ab positive PCR positive (Ab+/PCR+), 30% were HCV Ab negative PCR negative, 18% had persistent response to antiretroviral drugs, and 11% were reported with natural clearance of HCV. HIV infection was present in 106 (20%), 97% of whom were treated with HAART. HIV-HCV coinfection

---

**Table 1**

Descriptive statistics by country.

| Country | Patients | CLD | HCV | HIV | DIAB | CHOL |
|---|---|---|---|---|---|---|
| Austria | 20 | 0.00 | 0.75 | 0.20 | 0.05 | 4.72 |
| Germany | 149 | 0.20 | 0.33 | 0.28 | 0.08 | 4.90 |
| Great Britain | 46 | 0.35 | 0.43 | 0.13 | 0.13 | 4.48 |
| Greece | 5 | 0.40 | 0.40 | 0.40 | 0.20 | 4.74 |
| Israel | 22 | 0.05 | 0.55 | 0.27 | 0.18 | 4.59 |
| Italy | 78 | 0.32 | 0.42 | 0.19 | 0.13 | 4.91 |
| Netherlands | 45 | 0.36 | 0.36 | 0.11 | 0.09 | 4.65 |
| Norway | 42 | 0.00 | 0.33 | 0.05 | 0.15 | 4.79 |
| Poland | 20 | 0.35 | 0.68 | 0.00 | 0.10 | 3.87 |
| Slovenia | 19 | 0.16 | 0.42 | 0.05 | 0.05 | 4.44 |
| Spain | 31 | 0.48 | 0.48 | 0.42 | 0.13 | 4.26 |
| Sweden | 40 | 0.23 | 0.48 | 0.18 | 0.05 | NA |
| Switzerland | 15 | 0.33 | 0.40 | 0.27 | 0.07 | 4.79 |
| Total | 532 | 0.24 | 0.42 | 0.20 | 0.10 | 4.71 |

The patients column reports the number of patients by country. Columns CLD through DIAB report the fraction of patients with the condition, that is, having CLD, HCV equal to Ab+/PCR+, HIV positive, and having diabetes, respectively. The final column reports average total cholesterol (not recorded in Sweden). CHOL = total cholesterol, CLD = chronic liver disease, DIAB = diabetes, HCV = hepatitis C virus, HIV = human immunodeficiency virus.

### Table 2

**Descriptive statistics by age group.**

| Age group | Patients | CLD | HCV | HIV | DIAB | CHOL |
|---|---|---|---|---|---|---|
| 40–49 y | 222 | 0.24 | 0.43 | 0.32 | 0.04 | 4.79 |
| 50–59 y | 152 | 0.22 | 0.41 | 0.16 | 0.07 | 4.69 |
| 60–69 y | 89 | 0.30 | 0.48 | 0.10 | 0.15 | 4.66 |
| 70–79 y | 56 | 0.20 | 0.31 | 0.05 | 0.27 | 4.55 |
| 80–100 y | 13 | 0.31 | 0.46 | 0.00 | 0.46 | 4.44 |
| Total | 532 | 0.24 | 0.42 | 0.20 | 0.10 | 4.71 |

The patients column reports the number of patients by age group. Columns CLD through DIAB report the fraction of patients with the condition, that is, having CLD, HCV equal to Ab+/PCR+, HIV positive, and having diabetes, respectively. The final column reports average total cholesterol. CHOL = total cholesterol, CLD = chronic liver disease, DIAB = diabetes, HCV = hepatitis C virus, HIV = human immunodeficiency virus.

### Table 3

**Univariate logistic regression.**

| Variable | Level | Reference level | OR | 95% CI | P-value | n |
|---|---|---|---|---|---|---|
| HCV | Ab+/PCR+ | Not Ab+/PCR+ | 22.73 | (12.88, 43.03) | <.001 | 520 |
| HAART | Yes | No | 2.26 | (1.42, 3.58) | <.001 | 524 |
| HIV | Positive | Negative | 2.18 | (1.37, 3.44) | <.001 | 523 |
| Alcohol/week | 1–5 units | 0 units | 0.46 | (0.29, 0.71) | <.001 | 509 |
| Total cholesterol | | | 0.45 | (0.35, 0.57) | <.001 | 444 |
| Haemophilia severity | Severe | Mild | 2.05 | (1.28, 3.4) | .004 | 523 |
| Employment status | Early retirement | Full-time | 2.01 | (1.02, 3.87) | .04 | 519 |
| Diabetes | Yes | No | 1.60 | (0.84, 2.93) | .14 | 520 |

This table reports separate univariate logistic regressions with CLD as the response and the variables in the first column as covariates. The 2nd column lists the level which generated a significant coefficient for the categorical variables. The 3rd column lists the corresponding reference level. Each OR in the table is accompanied by a 95% confidence interval and the corresponding 2-sided P-value. The final column reports the number of observations used when estimating the model. Diabetes is included in the table for reference and comparison with the MLR, in which it became significant. CLD = chronic liver disease, CI = confidence interval, HAART = highly active antiretroviral therapy, HCV = hepatitis C virus, HIV = human immunodeficiency virus, OR = odds ratio.

was present in 89 patients (17%). A total of 52 (10%) of the patients were diagnosed with diabetes. The median value for total cholesterol was 4.63 mmol/L (range 2.15–8.32 mmol/L).

The highest prevalence of CLD was found in patients recruited from Spain (Table 1). There were no patients with CLD in either Austria or Norway. We attribute this to randomness, as there was no deliberate strategy in place for avoiding CLD patients. Diabetes ranged from 5% to 20% for the countries in this sample.

The number of recruited patients decreased by age group (Table 2), with most patients in the range 40 to 49 years. HIV was most prevalent in this age group (32%). In the age group 70 to 79 years, 31% had HCV, which was lower than the other groups that ranged between 41% and 48%. Prevalence of diabetes increased with age, from 4% in the 40 to 49 to 46% in the 80 to 100 years age group.

### 3.3. Univariate associations

In univariate logistic regressions, we found significant associations with $P < .01$ between CLD and severity of hemophilia, HIV, HAART, HCV, total cholesterol, and alcohol (Table 3). In unreported results, we found that the coefficient estimates for the HCV levels natural clearance and persistent response to antiviral drugs were statistically indistinguishable from the reference level, Ab negative/PCR negative. Hence, in Table 3 and the remainder, we have recoded HCV to a binary variable with HCV diagnosed as Ab+/PCR+ or not. One further significant association with $P < .05$ was found with employment status. No significant associations were found with age, diabetes, malignancy, time on current treatment regimen, body mass index (BMI), weight, NSAID use, hypertension, or smoking status.

### Table 4

**Prevalence of risk factors and CLD status.**

| CLD | HCV | HIV | DIAB | CHOL |
|---|---|---|---|---|
| A. Prevalence of risk factors by CLD status | | | | |
| No | 0.26 (103/393) | 0.17 (67/396) | 0.09 (35/394) | 0.26 (89/342) |
| Yes | 0.89 (113/127) | 0.31 (39/127) | 0.13 (17/126) | 0.52 (53/102) |
| All | 0.42 (216/520) | 0.20 (106/523) | 0.10 (52/520) | 0.32 (142/444) |
| B. CLD status by presence of risk factors | | | | |
| No | 0.05 (14/304) | 0.21 (88/417) | 0.23 (109/468) | 0.16 (49/302) |
| Yes | 0.52 (113/216) | 0.37 (39/106) | 0.33 (17/52) | 0.37 (53/142) |
| All | 0.24 (127/520) | 0.24 (127/523) | 0.24 (126/520) | 0.23 (102/444) |

Panel A reports the fraction of patients with the condition specified in the header grouped by CLD status. The numerator in the parentheses shows the number of patients with the condition for a particular CLD status. The denominator is the number of patients with valid observations for the corresponding CLD status. The number in the denominator is not constant across risk factors because of missing data. Panel B reports the fraction of patients without and with CLD conditional on the presence of the risk factor specified in the header. The numerator shows the number of patients with the specified CLD status and presence of the specified risk factor. The denominator is the number of patients with valid observations for the risk factor in question. The final row in each panel shows the results for all patients. The numbers in the final column correspond to the fraction of patients in the <33rd percentile. CHOL = total cholesterol, CLD = chronic liver disease, DIAB = diabetes, HCV = hepatitis C virus, HIV = human immunodeficiency virus.

**Table 5**

**Multivariate logistic regression.**

| Variable | Odds ratio | 95% CI | *P*-value |
|---|---|---|---|
| HCV Ab+/PCR+ | 17.57 | (9.15, 36.65) | <.001 |
| Total cholesterol | 0.62 | (0.46, 0.84) | .002 |
| HIV | 1.86 | (1, 3.44) | .049 |
| Diabetes | 2.96 | (1.19, 7.5) | .02 |

This table shows the results from a multivariate logistic regression with CLD as the response and the variables in the first column as covariates. Each odds ratio in the table is accompanied by a 95% confidence interval and the corresponding 2-sided *P*-value. The sample size is 437. The main reason for reduced sample size is due to some missing data for total cholesterol. CI = confidence interval, HCV = hepatitis C virus, HIV = human immunodeficiency virus.

**Table 7**

**Ordinal logistic regression with continuation ratio model.**

| Variable | Odds ratio | 95% CI | *P*-value |
|---|---|---|---|
| HCV Ab+/PCR+ | 15.75 | (7.53, 32.95) | <.001 |
| Total cholesterol | 0.58 | (0.43, 0.77) | <.001 |
| HIV | 1.97 | (1.17, 3.33) | .011 |
| Diabetes | 2.57 | (1.2, 5.49) | .015 |

This table shows the results from a continuation ratio logistic model. The model has been constrained to have a common vector of slope coefficients. Each odds ratio in the table is accompanied by a 95% confidence interval and the corresponding 2-sided *P*-value. The sample size is 418. The reason for reduced sample size compared to the multivariate logistic regression is that some patients have been diagnosed with an unspecified chronic liver disease. CI = confidence interval, HCV = hepatitis C virus, HIV = human immunodeficiency virus.

Panel A of Table 4 shows the prevalence of risk factors by CLD status. The percentages of patients with HCV Ab+/PCR+, diabetes, HIV, and lowest 3rd of total cholesterol were higher for patients with CLD than for those without. For example, when CLD is present 89% are HCV Ab+/PCR+ whereas only 26% are Ab+/PCR+ otherwise. Panel B shows that CLD increases with the presence of risk factors. In patients with HCV Ab+/PCR+, 52% are reported to have CLD and 5% not. The 33rd percentile, corresponding to 4.1 mmol/L, is a somewhat arbitrary cut-off value but serves to illustrate the strong association between low total cholesterol levels and CLD. In the logistic regressions, we have used the continuous variable total cholesterol as recorded in the dataset.

### 3.4. Multivariate associations

In MLR, we have assessed the associations between CLD and the covariates HCV, total cholesterol, HIV, and diabetes (Table 5). HCV Ab+/PCR+ was positively associated with CLD (OR = 17.57 [9.15, 36.65], *P* < .001). Diabetes was not significant in univariate logistic regression, but after controlling for HCV the coefficient estimate increased, revealing a positive association with CLD (OR = 2.96 [1.19, 7.5], *P* = .02). The interaction term for HCV and diabetes was insignificant implying that HCV was a confounder but not an effect modifier for diabetes. Total cholesterol was negatively associated with CLD with OR = 0.62 (0.46, 0.84), *P* = .002 for each 1.0 mmol/L increment. HIV was associated with an increased likelihood of CLD (OR = 1.86 [1, 3.44], *P* = .049). We found no evidence of interaction effects among the explanatory variables.

Several measures of goodness of fit for a logistic regression model exist. The widely recommended le Cessie–van Houwelingen–Copas–Hosmer unweighted sum of squares test[18] gave a *P*-value of .9, and hence we could not reject the null hypothesis that the model fitted. The Hosmer–Lemeshow test yielded the same conclusion.

**Table 6**

**Distribution of chronic liver disease type.**

| Liver status | Number of patients | Frequency |
|---|---|---|
| No CLD | 397 | 76 |
| Cirrhosis | 46 | 9 |
| Fibrosis | 41 | 8 |
| Not specified | 28 | 5 |
| Steatosis/hepatitis | 12 | 2 |
| Total | 524 | 100 |

This table shows the breakdown of patients by CLD type. The sum total of 524 is slightly less than the total number of patients due to some missing data points on CLD. CLD = chronic liver disease.

### 3.5. Risk factors across type of CLD

We have mapped the reported CLD type to 3 major categories: cirrhosis, fibrosis, and steatosis/hepatitis (Table 6). Patients with an unspecified CLD were disregarded. CLD can deteriorate according to no CLD ≺ steatosis/hepatitis ≺ fibrosis ≺ cirrhosis, where ≺ indicates that the condition on the right is more severe than that on the left side of the sign. Since a patient must pass through the previous stages of the disease in order to reach a certain level, we have used a continuation ratio ordinal model taking the rank ordering of the CLD type into account. This model assesses the probability of proceeding to the next stage of CLD, conditional on not having reached that stage yet, and the set of risk factors. We found all risk factors included in Table 5 statistically significant at the 5% level (Table 7). This model implies that at each stage of the disease, HCV, HIV, and diabetes increase the risk of the condition deteriorating. The results were consistent with and provided additional credence to the MLR in Table 5. The odds of proceeding from, for example, chronic hepatitis to fibrosis, or from fibrosis to cirrhosis are 15.75 times higher for a patient with HCV Ab+/PCR+. HIV and diabetes have odds ratios 1.97 and 2.57, respectively. When total cholesterol increases by 1 unit, the odds are reduced by a factor of 0.58. A likelihood ratio test showed that we could not reject that the model parameters were constant across CLD type.

## 4. Discussion

### 4.1. HCV

As expected, MLR demonstrated that HCV Ab+/PCR+ was positively associated with CLD. The majority (89%) with CLD in this cohort was HCV Ab+/PCR+, presumably due to the difficult management of HCV in PWH, as previous interferon-based treatments had limited efficacy and were poorly tolerated. However, since their introduction in 2011, treating HCV-infected with direct-acting antivirals (DAAs) offer high cure rates. Sustained virologic response (SVR) of more than 95% of the patients within 12 to 24 weeks of treatment has been reported in several papers, and similarly in patients with previous treatment failure, for coinfected patients, and also not limited by HCV genotype.[19,20] One of the main findings from the ongoing prospective Swiss HIV Cohort Study[21] was that treated patients with non-SVR compared to those with SVR had a higher risk of liver events, liver-related death, and diabetes. A significant decrease of liver-related disease and death, as well as a reduction of diabetes, was observed following viral eradication. In a recent study,[22] 120 patients with bleeding disorders (91% hemophilia) and HCV were treated with DAAs. SVR was achieved in 99% for the most common HCV genotypes after 12 weeks of treatment,

and the authors concluded that patients with bleeding disorders could receive successful treatment for HCV infection with DAA regimens without significant safety concerns.

As chronic HCV infection is a slowly progressive disease, and most PWH have had long-term exposure, early and intensified eradication therapy with DAAs seems justified to prevent CLD.

### 4.2. Diabetes

After controlling for HCV in MLR, we found diabetes positively associated with CLD. The mechanisms through which HCV induces type 2 diabetes (T2D) include direct viral effects, insulin resistance (IR), pro-inflammatory cytokines, and other immune-mediated processes.[23] Numerous studies indicate that HCV-associated IR/T2D leads to an increased risk of fibrosis progression,[24,25] and having T2D is associated with the progression of chronic HCV infection, decreased response to HCV treatment, and increased risk of HCC. A meta-analysis examining the relationship between HCV infection and risk of T2D[26] demonstrated a 2-fold significant increase in the risk of T2D with HCV infection, and a large population-based study[27] found that T2D and IR were independently associated with liver-related mortality in HCV-infected persons.

Diabetes is associated with increased risk of complications and mortality rates in patients with CLD. It enhances inflammation and fibrosis of the liver, and treatment has been difficult due to impaired hepatic function and drug toxicity. Now, new and safe treatment options exist with dipeptidyl peptidase-4 inhibitors that are only minimally metabolized in the liver and are excreted unchanged by the kidney.[28] Consequently, intensified monitoring and treatment of T2D seems warranted in PWH. It is particularly important to screen for non-alcoholic steatohepatitis in patients with T2D and additional risk factors such as dyslipidemia and obesity, especially in non-European populations where the prevalence is increasing significantly. Furthermore, curing HCV may result in a reduced incidence of T2D and HCV-infected diabetics may improve diabetes-related clinical outcomes if SVR is achieved.[29]

### 4.3. Cholesterol

Total cholesterol was negatively associated with CLD in both univariate and MLR. It is recognized that HCV infection results in lower cholesterol levels, and also that cholesterol levels in HCV Ab +/PCR+ subjects are significantly lower than in subjects who are HCV Ab+ but PCR negative.[30] There is a strong relation between lipid metabolism and HCV infection, with circulating virus being bound to lipoproteins in serum, and the HCV life cycle being dependent on lipids from entry to hepatocytes to viral replication.[31–33] In a study of more than 700 PWH,[34] total cholesterol levels were significantly lower in PWH than in the general population and mean total cholesterol levels were lower in patients with active chronic HCV infection than in patients without.

In our data, HCV is a categorical variable that gives no additional information about the severity of the infection. The mean mmol/L of total cholesterol decreases monotonically by severity of CLD, from no evidence of CLD (4.90), to steatosis/hepatitis (4.44), to fibrosis (4.10), and finally to cirrhosis (3.78). It is our conjecture that total cholesterol adds information about the degree of HCV infection, for example, an HCV Ab+/PCR+ patient with low total cholesterol is likely to have a serious infection. Therefore, total cholesterol in combination with HCV is an important risk factor that should be included in the model and

statistical tests confirmed that the model improved significantly by including total cholesterol. Nevertheless, we are aware that CLD may also yield lower cholesterol levels and we acknowledge that there may be instances of reverse causality as well. Omitting the 61 patients that used statins, only 13 of them HCV Ab+/PCR+, the significance of HIV is at the 10% level whereas the other risk factors, and especially total cholesterol, increase their significance. Our opinion is that intensified antiviral treatment seems needed for HCV-infected patients with low cholesterol.

### 4.4. HIV/HAART

HIV was significantly associated with an increased likelihood of CLD. This association has been recognized by other groups,[35,36] and also the potential of antiretroviral drugs to cause hepatotoxicity, as longer exposure to HAART has been associated with an increased mortality from liver-related disease. From the D:A:D study of HIV-positive and mainly HCV-negative people, the authors identified cumulative use of nucleotide reverse transcriptase inhibitors (NRTIs) to be independently associated with an increased rate of ESLD/HCC development. There was also limited evidence for reversibility of ESLD/HCC risk upon cessation of NRTIs.[37]

It has been debated whether HIV infection itself, the use of HAART, or immunodeficiency is the cause of CLD. The results in Table 5 are robust to replacing HIV with HAART, as all risk factors remain significant at the 5% level, and even with a slightly lower P-value for HAART than HIV. In either case, age-related effects of the liver in HIV-infected people, HCV-coinfection, and the toxicity of long-term HAART are of concern. Even though the use of NRTIs is reduced in modern HIV treatment, we might see more CLD due to NRTIs in the ageing HIV population.

### 4.5. Significant univariate associations not considered risk factors in this cohort

A considerable number of patients (39%) were abstinent from alcohol, and it seems reasonable to assume that several refrain from drinking because of CLD. Alcohol was therefore omitted as an explanatory variable in the MLR. Employment status reported as early retirement, approximately 82% in the age range 40 to 59 years, was significant in univariate logistic regression but became insignificant in the MLR. This could be due to early retirement being attributable to deteriorating health, captured by controlling for HCV, HIV, and diabetes. Severe hemophilia was also significant in univariate logistic regression, but insignificant after controlling for HCV in MLR. This is likely due to those with severe hemophilia receiving more transfusions, thus increasing the probability of a transfusion contaminated with HCV. The data confirm that HCV prevalence increases with severity of hemophilia. Beta blockers were significantly associated with increased likelihood of CLD, but they are often included in the treatment of patients already suffering from cirrhosis and consequently omitted from our model. Modification of diet in renal disease was statistically significant, but not included due to reverse causation as renal failure is known to be a common and severe complication of progressive cirrhosis.

### 4.6. Risk factors recognized in the literature but absent for this cohort

The set of risk factors we have identified for this European cohort is not exhaustive. Previous research has identified additional risk

factors, including, but not limited to excessive alcohol consumption, older age, and metabolic syndromes related to overweight and obesity, hypertension, hyperglycaemia, and dyslipidemia.

Posthouwer et al found that excessive alcohol consumption was a significant risk factor. Compared to abstinence, we found a significant risk-reducing effect from moderate alcohol consumption (1–5 units/week) and a risk-increasing but not significant adverse effect from heavy drinking (>20 units/week), a result we attribute to reverse causality. We found no evidence that age played a role in the development or progression of CLD, in contrast to Goedert et al and Posthouwer et al. Our cohort consisted of only adult PWH, whereas their cohorts included children as well as adults. Even though development of CLD is time dependent, this could suggest that there exists a threshold after which time since infection is no longer of significance as few, if any, were infected after the early 1990s. We did not find an association between CLD and BMI, although both mean and median BMI were >25 in this sample. A likely reason is that CLD was not from metabolic origin in this cohort but rather from HCV infection (89%). Apart from total cholesterol and T2D, further blood biochemical parameters and anthropometric measurements were not associated with CLD in this cohort of PWH.

### 4.7. Noninvasive assessment of liver fibrosis

Recent advances in pharmaceutical therapies have significantly improved life expectancy in PWH with HCV-related CLD, and non-invasive monitoring of disease progression has reduced the bleeding risk from biopsy. Although liver biopsy has been the gold standard for identifying and evaluating liver fibrosis, it is not required before starting antiviral treatment. Also, the need for factor concentrates and hospitalization due to the bleeding risk in PWH undergoing biopsy has been a significant disadvantage. However, transient elastography is a painless and quick procedure that can easily be performed in the outpatient clinic. As reviewed by Coppola et al,[38] several studies have recognized this noninvasive technique as a reliable alternative to liver biopsy. The need for biopsies in PWH has been greatly reduced as this method offers an accurate staging of fibrosis. In addition, good reproducibility and accessible repeated procedures have provided a risk-free method to monitor disease progression of fibrosis and cirrhosis, and it serves as both a diagnostic and prognostic tool.

### 4.8. Limitations

A cross-sectional study means that it is difficult to statistically differentiate between causes and consequences of CLD. However, theory and previous studies guide our hypotheses regarding causality. Another limitation was that most data were collected retrospectively. In our data, 21/46 (46%) of patients with cirrhosis were classified by Child–Pugh score (mainly class A), some patients were classified by MELD-score (Model for ESLD), and even more were classified by transient elastography for both fibrosis and cirrhosis. However, it is a limitation that some patients were only reported with either fibrosis or cirrhosis without any further information by what method they were classified. The specific field in the CRF for reporting on current CLD status and staging was text based, and only 1 patient was described as being evaluated for a liver transplant. A further limitation was the lack of data for hepatitis B virus (HBV). Even though HCV usually dominates and suppresses HBV replication,[39] we cannot dismiss an additional role of HBV in CLD.

Missing data caused the removal of some patients in the MLR. Despite these limitations, the H3 study[16] represents a comprehensive multicountry hemophilia sample which adds to the generalizability of the results.

## 5. Conclusion

We have confirmed previous research findings, both for PWH and the general population, that HCV and HIV coinfection/treatment are significant risk factors for CLD in this new dataset on PWH comprising several additional European countries. In addition, we found that diabetes and low levels of total cholesterol were further significant associated factors in multivariate analysis. In contrast to the general population, we usually know both status and time of HCV infection in PWH, and long before the development of CLD. The majority who contracted HCV in this cohort did so in the 1980s, and it may take 30 years to reach the final stages of CLD. Therefore, the prevalence and severity of CLD may not have reached its peak yet. Using an ordinal model, we have shown that the same set of risk factors is responsible for the deterioration of CLD, implying that treatment at any stage of the disease is paramount. With well-organized and swift implementation of treatment, the concept of microelimination is to completely eliminate HCV infection in targeted populations such as PWH. This is on-going in some countries, still all PWH certainly need to be allowed this therapy. Hence, intensified eradication therapy for HCV with DAAs seems justified to prevent the potentially fatal consequences of advanced CLD.

In addition, intensified monitoring and treatment of T2D appears warranted. This is particularly true in the new treatment era with dipeptidyl peptidase-4 inhibitors, considered safe to use in the treatment of diabetes in patients with CLD.

The ADVANCE group is currently conducting a prospective, longitudinal study that will offer the opportunity to study the causal relationships of CLD in PWH in more detail.

## Author contributions

CQ designed the research study, performed the research, analyzed the data and wrote the paper. RCT, SR, EB, PdM, RES, and PAH designed the research study and participated in writing the manuscript.

**Conceptualization:** Christian Qvigstad, Campbell Tait, Stephan Rauchensteiner, Erik Berntorp, Philippe de Moerloose, Roger E Schutgens, Pål Andre Holme.

**Data curation:** Christian Qvigstad, Campbell Tait, Stephan Rauchensteiner, Erik Berntorp, Philippe de Moerloose, Roger E Schutgens, Pål Andre Holme.

**Formal analysis:** Christian Qvigstad.

**Investigation:** Christian Qvigstad, Campbell Tait, Stephan Rauchensteiner, Erik Berntorp, Philippe de Moerloose, Roger E Schutgens, Pål Andre Holme.

**Methodology:** Christian Qvigstad, Pål Andre Holme.

**Project administration:** Campbell Tait, Stephan Rauchensteiner, Philippe de Moerloose, Pål Andre Holme.

**Software:** Christian Qvigstad.

**Validation:** Christian Qvigstad, Campbell Tait, Stephan Rauchensteiner, Erik Berntorp, Philippe de Moerloose, Roger E Schutgens, Pål Andre Holme.

**Visualization:** Christian Qvigstad.

**Writing – original draft:** Christian Qvigstad.

**Writing – review & editing:** Campbell Tait, Stephan Rauchensteiner, Erik Berntorp, Philippe de Moerloose, Roger E Schutgens, Pål Andre Holme.

Christian Qvigstad orcid: 0000-0003-1419-6465

## References

[1] Fletcher M, Trowell J, Craske J, et al. Non-a non-b hepatitis after transfusion of factor viii in infrequently treated patients. Br Med J (Clin Res Ed) 1983;287:1754–7.

[2] Schimpf K, Mannucci PM, Kreutz W, et al. Absence of hepatitis after treatment with a pasteurized factor viii concentrate in patients with hemophilia and no previous transfusions. N Engl J Med 1987;316: 918–22.

[3] Darby SC, Ewart DW, Giangrande PL, et al. Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. Lancet 1997;350: 1425–31.

[4] Westbrook RH, Dusheiko G. Natural history of hepatitis C. J Hepatol 2014;61:S58–68.

[5] Yee T, Griffioen A, Sabin C, et al. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. Gut 2000;47:845–51.

[6] Ragni MV, Belle SH. Impact of human immunodeficiency virus infection on progression to end-stage liver disease in individuals with hemophilia and hepatitis C virus infection. J Infect Dis 2001;183:1112–5.

[7] Goedert JJ, Eyster ME, Lederman MM, et al. End-stage liver disease in persons with hemophilia and transfusion-associated infections. Blood 2002;100:1584–9.

[8] Posthouwer D, Makris M, Yee TT, et al. Progression to end-stage liver disease in patients with inherited bleeding disorders and hepatitis C: an international, multicenter cohort study. Blood 2007;109:3667–71.

[9] van de Putte DE, Makris M, Fischer K, et al. Long-term follow-up of hepatitis C infection in a large cohort of patients with inherited bleeding disorders. J Hepatol 2014;60:39–45.

[10] Eyster ME, Kong L, Li M, et al. Long term survival in persons with hemophilia and chronic hepatitis C: 40 year outcomes of a large single center cohort. Am J Hematol 2016;91:

[11] Arnold DM, Julian JA, Walker IR, et al. Mortality rates and causes of death among all HIV-positive individuals with hemophilia in Canada over 21 years of follow-up. Blood 2006;108:460–4.

[12] van der Helm J, Geskus R, Sabin C, et al. Effect of HCV infection on cause-specific mortality after HIV seroconversion, before and after 1997. Gastroenterology 2013;144:751–60.

[13] Morlat P, Roussillon C, Henard S, et al. Causes of death among HIV-infected patients in France in 2010 (national survey): trends since 2000. AIDS 2014;28:1181–91.

[14] Mazepa MA, Monahan PE, Baker JR, et al. Men with severe hemophilia in the United States: birth cohort analysis of a large national database. Blood 2016;127:3073–81.

[15] Goehringer F, Bonnet F, Salmon D, et al. Causes of death in HIV-infected individuals with immunovirologic success in a national prospective survey. AIDS Res Hum Retroviruses 2017;33:187–93.

[16] Holme PA, Combescure C, Tait R, et al. Hypertension, haematuria and renal functioning in haemophilia – a cross-sectional study in Europe. Haemophilia 2016;22:248–55.

[17] R Core TeamR: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria:2018.

[18] Hosmer DW, Hosmer T, Le Cessie S, et al. A comparison of goodness-of-fit tests for the logistic regression model. Stat Med 1997;16:965–80.

[19] Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014;370:211–21.

[20] Naggie S, Cooper C, Saag M, et al. Ledipasvir and sofosbuvir for HCV in patients coinfected with HIV-1. N Engl J Med 2015;373:705–13.

[21] Kovari H, Rauch A, Kouyos R, et al. Hepatitis C infection and the risk of non-liver-related morbidity and mortality in HIV-positive persons in the Swiss HIV cohort study. Clin Infect Dis 2017;64:490–7.

[22] Walsh C, Workowski K, Terrault N, et al. Ledipasvir–sofosbuvir and sofosbuvir plus ribavirin in patients with chronic hepatitis C and bleeding disorders. Haemophilia 2017;23:198–206.

[23] Negro F. Facts and fictions of HCV and comorbidities: steatosis, diabetes mellitus, and cardiovascular diseases. J Hepatol 2014;61:S69–78.

[24] Hui JM, Sud A, Farrell GC, et al. Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis progression. Gastroenterology 2003;125:1695–704.

[25] Fartoux L, Poujol-Robert A, Guechot J, et al. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 2005;54:1003–8.

[26] White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. J Hepatol 2008;49:831–44.

[27] Stepanova M, Rafiq N, Younossi ZM. Components of metabolic syndrome are independent predictors of mortality in patients with chronic liver disease: a population-based study. Gut 2010;59:1410–5.

[28] García-Compeán D, González-González JA, Lavalle-González FJ, et al. Current concepts in diabetes mellitus and chronic liver disease: clinical outcomes, hepatitis C virus association, and therapy. Dig Dis Sci 2016;61:371–80.

[29] Vanni E, Bugianesi E, Saracco G. Treatment of type 2 diabetes mellitus by viral eradication in chronic hepatitis C: myth or reality? Dig Liver Dis 2016;48:105–11.

[30] Dai C-Y, Chuang W-L, Ho C-K, et al. Associations between hepatitis C viremia and low serum triglyceride and cholesterol levels: a community-based study. J Hepatol 2008;49:9–16.

[31] Popescu C-I, Dubuisson J. Role of lipid metabolism in hepatitis C virus assembly and entry. Biol Cell 2010;102:63–74.

[32] Negro F. Abnormalities of lipid metabolism in hepatitis C virus infection. Gut 2010;59:1279–87.

[33] Felmlee DJ, Hafirassou ML, Lefevre M, et al. Hepatitis C virus, cholesterol and lipoproteins – impact for the viral life cycle and pathogenesis of liver disease. Viruses 2013;5:1292–324.

[34] van de Putte DF, Fischer K, Makris M, et al. Unfavourable cardiovascular disease risk profiles in a cohort of dutch and british haemophilia patients. J Thromb Haemost 2013;109:16–23.

[35] Carr A, Cooper DA. Adverse effects of antiretroviral therapy. Lancet 2000;356:1423–30.

[36] Mocroft A, Soriano V, Rockstroh J, et al. Is there evidence for an increase in the death rate from liver-related disease in patients with HIV? AIDS 2005;19:2117–25.

[37] Ryom L, Lundgren JD, De Wit S, et al. Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons. AIDS 2016;30:1731–41.

[38] Coppola A, Di Capua M, Conca P, et al. Noninvasive assessment of liver fibrosis in patients with chronic hepatitis C (and congenital bleeding disorders): where do we stand? Semin Thromb Hemost 2013;39:803–15.

[39] Wiegand S, Jaroszewicz J, Potthoff A, et al. Dominance of hepatitis C virus (HCV) is associated with lower quantitative hepatitis B surface antigen and higher serum interferon-(-induced protein 10 levels in HBV/HCV-coinfected patients. Clin Microbiol Infect 2015;21:710.