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Progression of the FIB-4 index among patients with chronic HCV infection and early liver disease

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ABSTRACT

Background and aims Historical paired liver biopsy studies are likely to underestimate current progression of disease in patients with chronic hepatitis C virus (HCV) infection. We aimed to assess liver disease progression according to the non-invasive Fibrosis-4 (FIB-4) index in patients with chronic HCV and early disease.

Methods and results Patients diagnosed with chronic HCV and FIB-4 <3.25 from four international liver clinics were included in a retrospective cohort study. Follow-up ended at start of antiviral therapy resulting in sustained virological response, at time of liver transplantation or death. Primary outcome of advanced liver disease was defined as FIB-4 >3.25 during follow-up. Survival analyses were used to assess time to FIB-4 >3.25.

In total, 4286 patients were followed for a median of 5.0 (IQR 1.7–9.4) years, during which 41 071 FIB-4 measurements were collected. At baseline, median age was 47 (IQR 39–55) years, 2529 (59.0%) were male, and 2787 (65.0%) patients had a FIB-4 <1.45. Advanced liver disease developed in 821 patients. Overall, 10-year cumulative incidence of advanced disease was 32.1% (95% CI 29.9% to 34.3%). Patients who developed advanced disease showed an exponential FIB-4 increase. Among patients with a presumed date of HCV infection, cumulative incidence of advanced disease increased 7.7-fold from 20 to 40 years as opposed to the first 20 years after HCV infection.

Conclusions The rate of advanced liver disease is high among chronic HCV-infected patients with early disease at time of diagnosis, among whom liver disease progression accelerated over time. These results emphasise the need to overcome any limitations with respect to diagnosing and treating all patients with chronic HCV across the globe.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The WHO has set the goal to eliminate viral hepatitis as a public health threat by 2030, but recent estimates indicate a global treatment uptake of only ~50%.

⇒ Historical liver biopsy studies indicated that ~16% of patients develop cirrhosis within 20 years of infection with hepatitis C virus (HCV).

⇒ Updated details on the course of HCV-related liver disease progression are important for policymakers to allocate resources for the implementation of the required HCV screening programmes.

WHAT THIS STUDY ADDS

⇒ Among chronic HCV-infected patients with a Fibrosis-4 (FIB-4) index <3.25 at diagnosis, the 10-year incidence of advanced disease is approximately 30% in case of ongoing HCV infection.

⇒ Among patients who developed advanced liver disease during follow-up, an accelerated increase of the FIB-4 index over time was observed, which suggests a non-linear pattern of disease progression.

⇒ Based on the presumed date of infection, the cumulative proportion of patients with advanced liver disease increased 7.7-fold from 20 years (3.8%) to 40 years (29.5%) after HCV infection.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results emphasise the need to rapidly diagnose and treat all patients with chronic HCV to maximise the benefit and cost-efficacy of antiviral therapy.

⇒ Limitations regarding the access to direct-acting antiviral should be overcome across the globe.

INTRODUCTION

Fortunately, almost all patients with chronic hepatitis C virus (HCV) infection would be able to attain sustained virological response (SVR) with direct-acting antivirals (DAAs), which has been extensively related to an improved clinical outcome.[1 2] There remain, however, important barriers to treatment. A recent meta-analysis indicated a global DAA treatment rate of only 53.6%, with non-adherence to clinical follow-up and lack of disease awareness as contributing factors.[3] In addition, there is underdiagnosis of HCV infection. Without successful treatment, however, patients may progress to cirrhosis with the risk of liver failure, hepatocellular

carcinoma (HCC) and death. In addition, in an advanced stage of disease, SVR reduces but not eradicates the risk of these complications.[4] Continued long-term and costly biannual surveillance then remains recommended.[5] Nevertheless, strategies to enable early diagnosis and treatment have not been widely introduced or adopted.

While the clinical benefits of SVR have been well recognised, physicians and policymakers may need a better understanding of the course of HCV-related liver disease to allocate time and resources to improve HCV screening and treatment. A meta-analysis of older liver biopsy studies indicated that 16% of patients develop cirrhosis within 20 years of infection.[6] However, this estimate likely underestimates the average disease progression among patients with early-stage HCV infection today. These patients have already been infected for longer duration and were subject to changing epidemiological characteristics.[7–9]

Non-invasive validated alternatives to stage liver disease have been developed and offer the opportunity of repeated measurement analyses.[10] The Fibrosis-4 (FIB-4) index is easily calculated based on readily available clinical parameters and represents one of the best validated non-invasive markers of disease stage.[11] Importantly, the FIB-4 index has a well-documented association with clinical outcome and is recommended as a tool to determine the need for HCC surveillance post-SVR.[12–15] Therefore, the aim of this international multicentre study was to assess the progression of the FIB-4 index during chronic HCV infection among patients with early-stage liver disease at diagnosis.

METHODS

Study population

In this retrospective international cohort study, all identifiable patients with chronic HCV infection (HCV RNA positive for at least 6 months) and a FIB-4 index <3.25 at presentation from 1990 to 2017 in four large hepatology clinics were eligible for inclusion. Patients were excluded in case of no FIB-4 measurements during follow-up, less than 6 months of follow-up, age <18 years, active coinfection with the hepatitis B virus (HBV) or HIV, other relevant liver disease(s) (including Wilson disease, hereditary haemochromatosis, primary biliary cholangitis, primary sclerosing cholangitis, or autoimmune hepatitis), or histological proof of advanced hepatic fibrosis prior to baseline (METAVIR F3–F4). Electronic medical records were reviewed between 2018 and 2021 to obtain clinical baseline and outcome data (online supplemental methods).

Patient and public involvement

Patients were not involved in the design or conduct of the study.

Observation period

The index date (time=0) was defined as the date of the first available FIB-4 measurement that complied with the inclusion criteria. Baseline characteristics were collected at the time of the index date, or within 12 months before that time. The follow-up ended at the start of antiviral therapy which resulted in SVR, as this has a profound beneficial impact on further liver-related endpoints, or at the time of liver transplantation (LT) or death.[16] Patients were considered to have attained SVR in case of absence of circulating HCV RNA 12–24 weeks after cessation of antiviral therapy. In case antiviral therapy was not successful, patients remained in follow-up.

FIB-4 index

For this study, all available alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and platelet counts were collected during the observation period, so the FIB-4 index could be calculated on multiple time points: FIB-4=[age (in years)×AST (IU/L)]/[platelet count ($\times 10^9$/L)×(ALT$^{0.5}$ (in IU/L)]. Because the FIB-4 has not been validated in the setting of severe hepatic inflammation, the FIB-4 index was not calculated for ALT levels >207 IU/L and/or AST levels >139 IU/L.[11] In a large cohort of chronic HCV-infected patients, the FIB-4 index had accurate discriminative ability for advanced hepatic fibrosis (METAVIR F3–F4) with an area under the receiver operating characteristic curve of 0.85 (95% CI 0.82 to 0.89). A FIB-4 index >3.25 had a specificity of 98.2% and a sensitivity of 37.6% for the presence of advanced hepatic fibrosis.[13] For the current study, patients were considered to have advanced liver disease in case of a FIB-4 index >3.25 during follow-up, which was the primary endpoint of the current study.

During follow-up, patients with chronic HCV infection may have received one or multiple regimens of antiviral therapy (interferon-based therapy or DAAs, with or without ribavirin). As these treatments may impact the laboratory values, all laboratory results during or within 12 weeks of antiviral therapy which did not result in SVR were excluded (follow-up was censored at start of treatment resulting in SVR).

AST to Platelet Ratio Index

In addition to the FIB-4 index, the AST to Platelet Ratio Index (APRI) was calculated at all available FIB-4 time points.[17] Combining APRI ([AST (IU/L)/AST upper limit of normal (IU/L)]/platelet count ($\times 10^9$/L)) with FIB-4 may improve the reliability of non-invasive assessment of liver disease severity and is a strategy which is more frequently adopted in HCV cohort studies.[18 19] As a sensitivity analysis, a stricter definition of advanced liver disease was assessed (the combination of a FIB-4 >3.25 and an APRI ≥1.5).

Clinical endpoints

Clinical disease progression was the combined endpoint of liver failure, HCC, LT and death, whichever came first. The definition of liver failure included an episode of either ascites confirmed by ultrasonography, bleeding varices, jaundice or overt hepatic encephalopathy. The

definition of HCC was in concordance with international clinical practice guidelines.[20]

Statistical analyses

Data are presented as median and IQR or proportions. Baseline characteristics were compared between patients with a FIB-4 index <1.45 and patients with an intermediate FIB-4 index between 1.45 and 3.25 using the Mann-Whitney U test, Student's t-test, or χ^2 test, as appropriate.

The cumulative incidence of a FIB-4 index >3.25 was estimated using the Kaplan-Meier method and compared between subgroups using the log-rank test. Bayesian proportional hazards models were used to assess the association between the development of a FIB-4 >3.25 during follow-up (binary, time-dependent) and clinical disease progression. Two models were fitted to this end, one only including the time-varying FIB-4 score, and one that additionally included baseline variables. The Bayesian framework allowed us to simultaneously estimate model parameters and impute missing covariate values.[21] Where the baseline values of albumin, total bilirubin and creatinine were missing, their value was imputed based on all available measurements ±2 years before/after the index date. Patients were censored when they started treatment leading to SVR, or at the time of the last available FIB-4 measurement. To assess the association between baseline characteristics and time to a FIB-4 index >3.25, another Bayesian multivariable proportional hazards model was fitted. We performed several sensitivity analyses for this model, applying more stringent criteria for inclusion and event definition. Details can be found in the online supplemental materials.

To investigate the pattern of liver disease development, we fitted a Bayesian linear mixed model to the natural logarithm-transformed repeated measurements of FIB-4 for those patients who, at some point, had FIB-4 >3.25. Time was modelled using a natural cubic spline with 4 df to allow for a flexible non-linear shape of the subject-specific trajectories. Due to the lack of a common baseline time, we used a reverse time approach with the last FIB-4 measurement as time=0. For visualisation, we plot the expected FIB-4 over time with corresponding 95% credible intervals (CIs). Additionally, the Kaplan-Meier method was used to estimate the cumulative incidence of a FIB-4 >3.25 from HCV infection onwards for the subset of patients for which an estimated year of infection was available.

P values below 0.05 were considered statistically significant. Results from Bayesian analyses are presented as the posterior mean and 95% CI. SPSS V.25 and R V.4.0.3 (R Core Team (2020) and the R package JointAI (V.1.0.2)) were used for the analyses.[21]

RESULTS

Patient and cohort characteristics

After application of the exclusion criteria, the total study population included 4286 patients (figure 1). The median year of inclusion was 2006 (IQR 2002–2010). Baseline characteristics are shown in table 1, overall and stratified by a FIB-4 index <1.45 (n=2787; 65.0%) or FIB-4 1.45–3.25 (n=1499; 35.0%). Median age was 47.0 years (IQR 38.9–54.7), 2529 (59.0%) patients were male, and the majority were infected with HCV genotype 1 (n=1879; 43.8%). The median FIB-4 index at baseline was 1.2 (IQR 0.8–1.7). Patients with a FIB-4 index of 1.45–3.25 were older and more frequently diagnosed with diabetes mellitus (DM).

Accounting for censoring at the time of SVR or LT, 41 071 FIB-4 measurements were registered during a median follow-up of 5.0 years (IQR 1.7–9.4). A total of 2747 (64.1%) patients received antiviral treatment during follow-up: 1778 (41.5%) received one treatment regimen, 661 (15.4%) received two treatment regimens, and 308 (7.2%) received three or more treatments. In total, 2191 (79.8%) patients attained SVR. The median year that successful antiviral therapy was started was 2015 (IQR 2010–2016). In total, 113 patients were diagnosed with HCC, 91 patients developed liver failure, 17 patients underwent LT, and 125 patients died. Clinical disease progression, as a combined endpoint, was experienced by 209 patients within the study period, of which 12 patients reached this endpoint because of death as the first registered event. Of these 12 deaths, 2 were considered liver related, 6 were considered non-liver related (2 because of cancer, 2 because of cardiovascular disease, and 2 for other reasons), and for 4 patients, the cause of death was unknown.

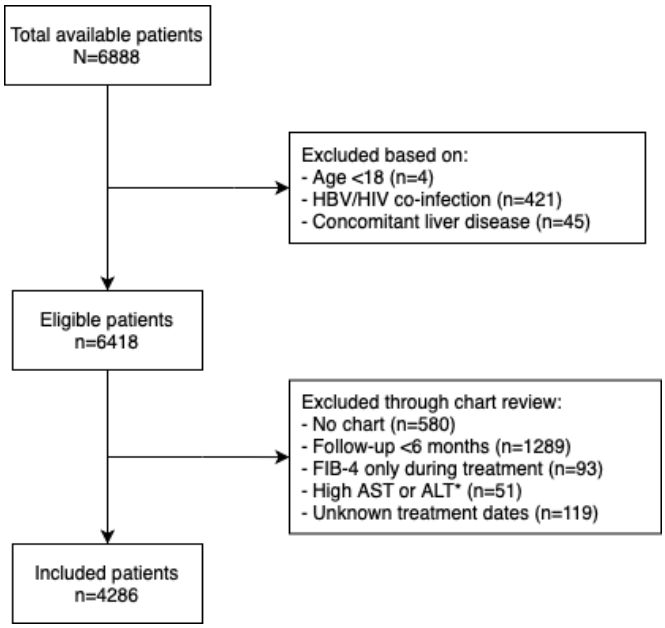

Figure 1 Flow chart with included and excluded patients. *Exclusion of patients who only had FIB-4 measurements with ALT levels >207 IU/L and/or AST levels >139 IU/L. ALT, alanine aminotransferase; AST, aspartate aminotransferase; FIB-4, Fibrosis-4; HBV, hepatitis B virus.

Table 1 Patient characteristics at the start of follow-up

	Overall (n=4286)	FIB-4 <1.45 (n=2787)	FIB-4 1.45–3.25 (n=1499)	P value
Age, median (IQR), years	47.0 (39–55)	43.1 (35–50)	53.7 (48–60)	<0.001
Male	2529 (59%)	1628 (59%)	901 (60%)	0.30
BMI, median (IQR)	25.5 (23–29)	25.3 (22–29)	25.9 (23–29)	0.001
Diabetes mellitus	359 (10%)	197 (9%)	162 (13%)	<0.001
Positive anti-HBc	853 (20%)	508 (18%)	345 (23%)	0.001
History of alcohol abuse	616 (14%)	395 (14%)	221 (15%)	0.15
Genotype HCV				0.03
1	1879 (44%)	1237 (44%)	642 (43%)	
2	213 (5%)	125 (5%)	88 (6%)	
3	434 (10%)	307 (11%)	127 (9%)	
4	140 (3%)	97 (4%)	43 (3%)	
5	8 (0.2%)	5 (0.2%)	3 (0.2%)	
6	19 (0.4%)	12 (0.4%)	7 (0.5%)	
Mixed	5 (0.1%)	2 (0.1%)	3 (0.2%)	
Unknown	1587 (37%)	1001 (36%)	586 (39%)	
Laboratory results, median (IQR)				
Platelets, $\times 10^9$/L	225 (187–272)	249 (214–295)	185 (158–215)	<0.001
ALT, IU/L	55 (35–90)	48 (31–77)	71 (43–113)	<0.001
AST, IU/L	41 (30–60)	35 (27–48)	58 (42–80)	<0.001
Albumin, g/L	43 (41–45)	43 (41–45)	42 (40–45)	<0.001
Total bilirubin, µmol/L	8 (5–12)	8 (5–11)	9 (6–13)	<0.001
INR	1.00 (0.96–1.06)	1.00 (0.95–1.04)	1.01 (0.97–1.09)	<0.001
Creatinine, µmol/L	67 (54–79)	66 (53–79)	68 (57–80)	0.002
FIB-4 index, median (IQR)	1.18 (0.79–1.69)	0.91 (0.66–1.16)	1.95 (1.66–2.41)	<0.001
APRI, median (IQR)	0.54 (0.34–0.84)	0.41 (0.29–0.59)	0.91 (0.64–1.26)	<0.001

Data are presented as no (%) unless otherwise noted. Data were available for 100% of the patients, except for the following characteristics in which it was available for: BMI in 2859 (66.7%) patients, genotype in 2699 (63.0%) patients, HBV anti-core positive 3893 (90.8%), alcohol abuse 4034 (94.1%), albumin in 3778 (88.1%), total bilirubin in 3960 (92.4%) patients, INR in 3471 (81.0%) patients and creatinine in 3708 (86.5%) patients.
ALT, alanine aminotransferase; anti-HBc, anti-hepatitis B core antigen; APRI, AST to Platelet Ratio Index; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, Fibrosis-4; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalised ratio.

Development of advanced liver disease

Overall, 821 (19.2%) patients developed a FIB-4 index >3.25 (advanced liver disease) during follow-up: 259 (9.3%) patients with a baseline FIB-4 index <1.45 and 562 (37.5%) patients with a baseline FIB-4 index of 1.45–3.25. The overall cumulative 10-year incidence of advanced liver disease was 32.1% (95% CI 29.9% to 34.3%) (figure 2A): 15.3% (95% CI 13.0% to 17.4%) among patients with a FIB-4 index <1.45 at baseline vs 61.4% (95% CI 57.3% to 65.0%) among those with a baseline FIB-4 index of 1.45–3.25 (p<0.001, figure 2B). In the subgroup of 1539 patients who remained untreated during follow-up, the cumulative 10-year incidence of advanced liver disease was 35.1% (95% CI 31.4% to 38.8%).

In the 719 (87.6%) patients with a FIB-4 >3.25 during follow and a subsequent FIB-4 measurement, the median following FIB-4 index was 3.51 (IQR 2.66–4.92). Online supplemental figure 1 shows the increase in FIB-4 following the first measurement >3.25. Adjusted for age, albumin, alcohol abuse, antibodies to hepatitis B core antigen, total bilirubin, body mass index, creatinine, DM, genotype 3, male sex and year of FIB-4 measurement, a FIB-4 index >3.25 during follow-up was associated with an independent increased risk of cirrhosis-related complications or mortality (adjusted HR (aHR) 5.3, 95% CI 3.9 to 7.3).

In a sensitivity analysis among 4054 patients with a baseline FIB-4 index <3.25 and an APRI <1.5, a total of 543 (13.4%) patients fulfilled the stricter definition for advanced liver disease during follow-up (FIB-4 >3.25 and APRI >1.5): 201 (7.2%) patients with a baseline FIB-4 index <1.45 and 342 (26.7%) patients with a baseline FIB-4 index of 1.45–3.25. The overall cumulative 10-year incidence was 21.9% (95%

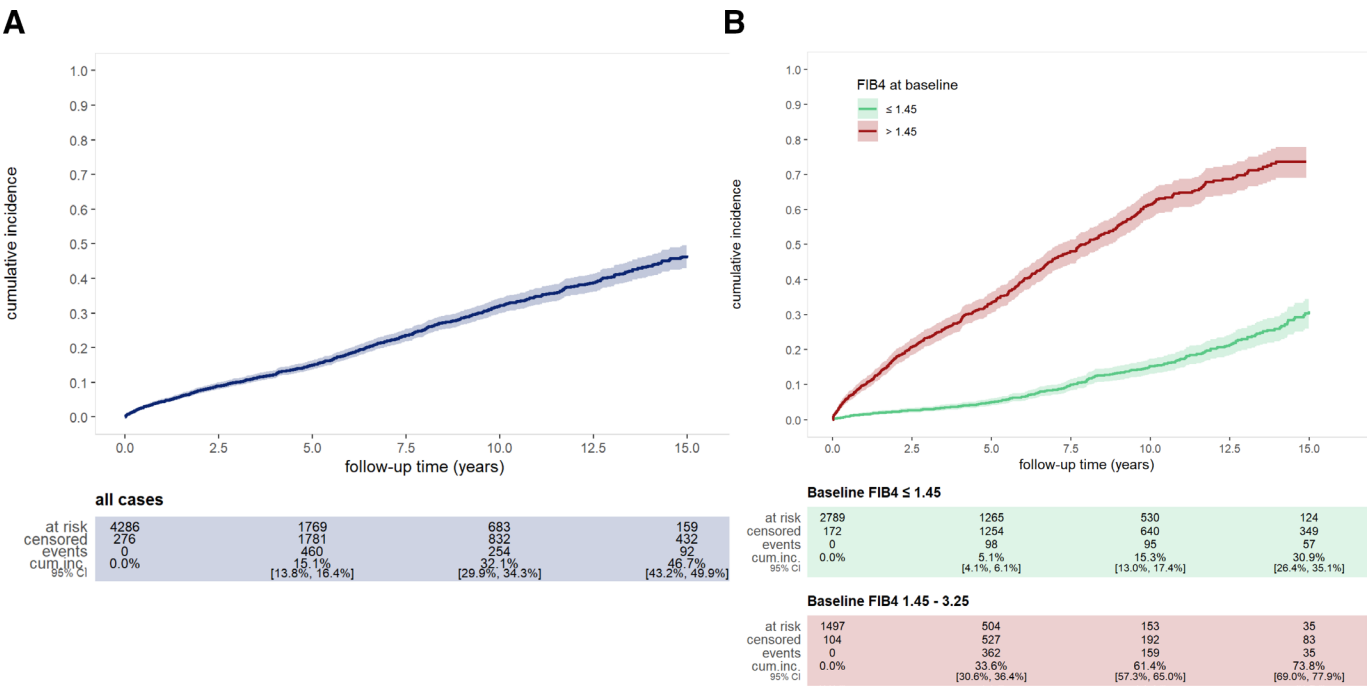

Figure 2 Time to development of advanced liver disease. (A) Overall and (B) stratified for patients with an index FIB-4 <1.45 or an index FIB-4 1.45–3.25. FIB-4, Fibrosis-4.

CI 19.9% to 23.9%), which differed by FIB-4 category (11.3%, 95% CI 9.3% to 13.3%, for a FIB-4 index <1.45 vs 44.3%, 95% CI 40.0% to 48.6%, for a FIB-4 index of 1.45–3.25 at baseline, p<0.001).

If the FIB-4 index during follow-up was calculated with baseline age as a constant, allowing only the laboratory parameters to change over time, 709 of the 821 patients remained with advanced liver disease during follow-up. In this analysis, the overall 10-year incidence of advanced liver disease was 28.0% (95% CI 25.9% to 30.1%): 12.6% (95% CI 10.6% to 14.5%) for those with a baseline FIB-4 index <1.45 vs 55.1% (95% CI 51.0% to 58.9%) for those with a FIB-4 index of 1.45–3.25 (p<0.001).

Pattern of development of advanced liver disease

Among the 821 patients who developed a FIB-4 index >3.25 during follow-up, the linear mixed model for repeatedly measured FIB-4 showed an exponential increase of the FIB-4 index over a 10-year time period (figure 3).

The (presumed) year of HCV infection could be derived for 1730 patients (40.4%). In this group, the median year of infection was 1982 (IQR 1975–1990). Online supplemental table 1 shows the baseline characteristics of the patients with a presumed date of HCV infection and those for whom the date of infection could not be estimated. The median age at the estimated time of HCV infection was 22.5 years (IQR 17.3–29.9). Figure 4 shows the cumulative incidence of FIB-4 >3.25 when the presumed date of infection was set as time 0. Twenty years following HCV infection, the cumulative incidence of advanced liver disease was 3.8% (95% CI 2.8% to 4.7%), which increased 7.7-fold to 29.5% (95% CI 26.1% to 32.8%) at 40 years.

Factors associated with the development of advanced liver disease

Bayesian multivariable proportional hazard analysis indicated that a history of alcohol abuse (aHR 1.36, 95% CI 1.13 to 1.65), baseline FIB-4 (aHR 3.9, 95% CI 3.6 to 4.3), albumin (aHR 0.93, 95% CI 0.92 to 0.95) and the year of inclusion (aHR 1.03, 95% CI 1.01 to 1.05) were independently associated with the time to advanced liver disease (table 2A). The estimated HRs were comparable in the sensitivity analyses in which

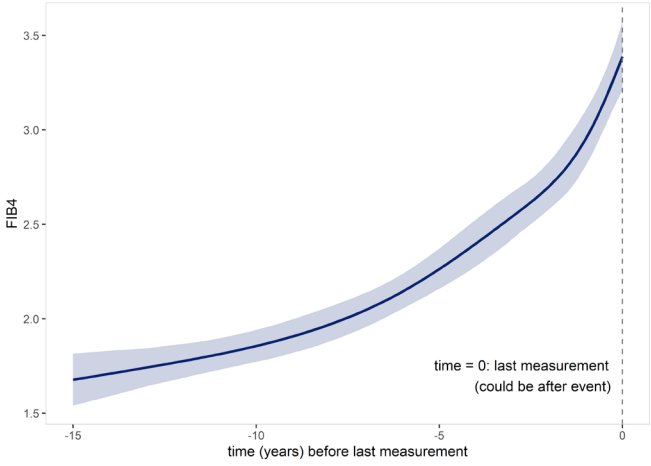

Figure 3 Reverse time analysis regarding the FIB-4 measurements. Time zero was set as the first FIB-4 >3.25 during follow-up over time among patients who developed advanced liver disease during follow-up. FIB-4, Fibrosis-4.

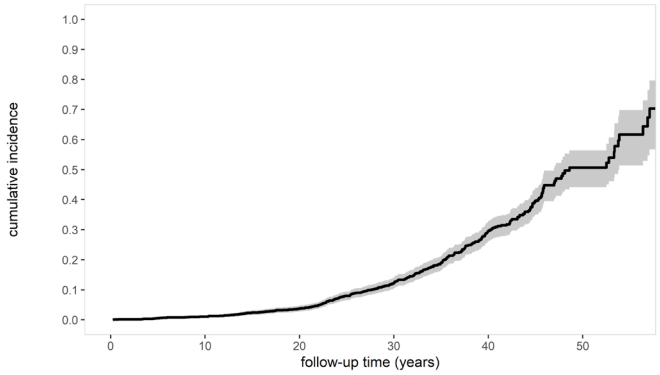

Figure 4 Time to development of advanced liver disease from the estimated year of HCV infection. Kaplan-Meier survival graph for the development of a FIB-4 >3.25 during follow-up among patients for whom an estimated year of infection could be derived from their medical chart. FIB-4, Fibrosis-4; HCV, hepatitis C virus.

FIB-4 and APRI were combined as criteria for inclusion and achievement of the study endpoint, and in the sensitivity analysis in which baseline age was held constant for the calculation of the FIB-4 index during follow-up (online supplemental tables 2 and 3). In the subgroup of patients with a FIB-4 index <1.45 at baseline, FIB-4 (aHR 8.17, 95% CI 5.30 to 12.72), albumin (aHR 0.91, 95% CI 0.88 to 0.94) and alcohol abuse (aHR 1.78, 95% CI 1.27 to 2.47) were associated with the time to advanced disease (table 2B). The overall 10-year cumulative incidence of advanced liver disease was 29.5% (95% CI 27.2% to 31.9%) among patients without a history of alcohol abuse.

DISCUSSION

In this large international multicentre cohort study, including over 4000 chronic HCV-infected patients with a FIB-4 index <3.25 at diagnosis, advanced liver disease developed in almost one-third of the patients within 10 years. The 10-year incidence of advanced disease remained as high as 15% in the subgroup of patients with the lowest FIB-4 levels (<1.45) at first presentation. An accelerated increase of the FIB-4 index was observed among patients who developed advanced liver disease during follow-up, which suggests a non-linear pattern of disease progression within individual patients. This was in line with exponential increase in the cumulative proportion of patients with advanced liver disease (7.7-fold increase from 20 to 40 years after HCV infection). These results are especially worrying as the global uptake of DAAs was recently estimated to be only ~50%.[22] With so many patients currently left at risk of accelerated disease progression, this study urges us to implement strategies to pick up the pace in the elimination of HCV as a public health threat.

Our estimated progression to advanced liver disease is higher than that in the meta-analysis of earlier liver biopsy studies (16% in 20 years).[6] However, the invasive nature of liver biopsy can introduce selection bias and there is a 20% risk that cirrhosis is incorrectly rejected due to sampling error.[10 23] In addition, histopathological assessment of fibrosis is scored categorically, depends on the individual pathologist, and limits repeated measurements. Objective non-invasive markers of hepatic fibrosis may overcome several of these limitations.[10] With a similar FIB-4 approach as presented here, a recent cohort study among American veterans indicated that as many as

Table 2 Cox proportional hazards regression for the development of advanced liver disease

	A. Overall	B. Among patients with an index FIB-4 <1.45
	HR (95% CI)	HR (95% CI)
Female sex	1.03 (0.89 to 1.19)	0.89 (0.67 to 1.18)
BMI, kg/m^2	1.00 (0.98 to 1.02)	0.99 (0.96 to 1.02)
HCV genotype 3 (vs other HCV genotypes)	0.92 (0.67 to 1.24)	1.25 (0.71 to 2.10)
History of alcohol abuse*	1.36 (1.13 to 1.65)	1.78 (1.27 to 2.47)
DM*	0.99 (0.79 to 1.23)	1.47 (0.99 to 2.16)
Year of inclusion*	1.03 (1.01 to 1.05)	0.99 (0.96 to 1.03)
Positive anti-HBc	0.94 (0.79 to 1.11)	0.96 (0.69 to 1.33)
FIB-4*	3.93 (3.58 to 4.32)	8.17 (5.30 to 12.72)
Total bilirubin, µmol/L	1.00 (0.99 to 1.01)	0.99 (0.96 to 1.01)
Albumin, g/L*	0.93 (0.92 to 0.95)	0.91 (0.88 to 0.94)
Creatinine, µmol/L	1.00 (1.00 to 1.00)	1.00 (1.00 to 1.00)

Missing values in covariates were imputed during the estimation procedure.
*Statistically significant in one or both analyses.
anti-HBc, anti-hepatitis B core antigen; BMI, body mass index (included as continuous covariate); DM, diabetes mellitus; FIB-4, Fibrosis-4; HCV, hepatitis C virus.

18.4% of the 1840 patients had developed cirrhosis within 10 years of HCV infection.[24] In contrast, advanced liver disease had developed in only 4% of patients at 20 years after the presumed date of infection in our cohort. The fact that the Veterans Affairs cohort consisted predominantly of males with high rates of excessive alcohol (53%) and drug use (58%) may explain this difference, as our study indeed confirmed the strong association between alcohol misuse and liver disease. Still, also in patients without a history of alcohol abuse, the rate of advanced liver disease was high. Our results are in line with the 20-year cirrhosis rate of 2–3% in female cohorts with iatrogenic HCV infection.[25] Looking forward from the time of an early HCV diagnosis rather than the time of infection, which was the main objective of this study as it should have the clearest implications for physicians and policymakers, up to one-third of patients developed advanced liver disease within 10 years. In addition, this estimate appeared to increase as HCV was diagnosed more recently. Our results thus emphasise that implementation of HCV screening and treatment programmes to enable early antiviral therapy is urgently needed.

The exponential pattern in the cumulative incidence of advanced liver disease has been described by prior natural history studies as well.[6 25 26] In line with our estimates, the recent update of the Irish cohort of patients who were administered HCV-contaminated anti-D immunoglobulin between 1977 and 1979 indicated that the percentage with cirrhosis increased 8.5-fold from 17 years (2%) to 36 years (19%) after HCV infection.[26] Due to the repeated measurement analysis, we had the opportunity to further elaborate on the pattern of disease progression within individual patients. This showed an accelerated increase of the FIB-4 index over a period of approximately 10 years prior to advanced liver disease, which may be explained by the association between hepatic fibrogenesis and angiogenesis. The altered blood flow through vascularised fibrotic septa results in further hypoxia, oxidative stress and associated inflammation, which may drive the histopathological abnormalities within the liver into a vicious circle.[27] This exponential increase in liver disease severity is also likely to explain the reason why patients included at a later calendar time had a statistically significantly short time to advanced liver disease. Due to the strong reduction of HCV transmission in the early 90s, the population with HCV is ageing. More recently included patients are thus, on average, further along in the natural history of their chronic HCV infection and closer to the point of exponential FIB-4 increase.

The strengths of our study include the large international cohort with many (>40 000) repeated FIB-4 assessments over a long follow-up period, with the possibility of various sensitivity analyses. There are also limitations to consider. First, although the FIB-4 index was repeatedly shown to be a reliable non-invasive surrogate of hepatic fibrosis, misclassification remains a possibility.[10 13 28] For this reason, we performed a sensitivity analysis in which the combination of a FIB-4 >3.25 and an APRI >1.5 was considered to be the definition of advanced liver disease. The overall cumulative incidence of advanced liver disease was lower in this sensitivity analysis (22% vs 32%), which is in part due to the exclusion of patients with a FIB-4 <3.25 but an APRI >1.5 at baseline, but would not change our interpretation of the results.[18 19 21] In addition, the relevance of our primary endpoint was confirmed by the strong association between a FIB-4 >3.25 during follow-up, as a time-dependent covariate, and liver failure, HCC, LT or death (aHR 5.3). Second, there were missing data at baseline, but this could be handled by imputation due to the substantial cohort size. Third, a bias may have been introduced by successful antiviral therapy during follow-up, as there is an overlap in patient characteristics associated with interferon-induced SVR and long-term outcome. However, in the interferon era, patients with early disease were not always treated because of the side-effects and limited virological efficacy.[29] While this might explain the low treatment rate in our study, a recent global meta-analysis indicated a poor DAA treatment rate of just above 50% as well.[3] Importantly, a sensitivity analysis among untreated patients showed similar estimates of disease progression. Last, it is relevant to consider that our study was performed in a cohort of patients who were primarily diagnosed through regular clinical care pathways, outside of the context of screening. Less progressive fibrosis development has been suggested among patients who were identified through screening, although the exponential pattern of disease progression is likely to remain.[6]

In conclusion, we report a high incidence of advanced liver disease following a diagnosis of early-stage chronic HCV infection, with an exponential progression of liver disease from the perspective of the individual patient as well as the population. Considering the limited global uptake of DAAs and the persistent need of HCC surveillance in case of advanced disease, these results indicate that HCV screening programmes are urgently needed to enable virological cure in an early stage of disease.

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SUPPLEMENTARY MATERIALS

Supplementary methods

Data collection

Electronic medical records were reviewed between 2018 and 2020 to acquire details on demographics, virologic and clinical data. Data were obtained on patient characteristics (sex, age, ethnicity, body mass index [BMI]), comorbidities (diabetes mellitus [DM] and a history of alcohol abuse as determined by the treating physician), HCV genotype and liver-related laboratory parameters. When possible, the presumed year in which the patient was infected with HCV was recorded based on the presence of well-known risk factors during their medical history, such as the transfusion of blood products pre-1992, injection drug use (IDU), tattoos, use of needles during medical procedures and having an HCV-positive partner. For patients in which a specific risk factor was present during a time period of more than 1 year, the middle year was considered as the presumed year of infection.

Sensitivity Analyses for development of FIB-4 > 3.25

We performed several sensitivity analyses for the multivariable proportional hazards model for a FIB-4 >3.25. First, the model was re-fitted using only patients with a FIB-4 index <1.45 at baseline in order to restrict the estimates to a group of patients with the highest probability of early liver disease, as a FIB-4 <1.45 had a negative predictive value of 94.7% with respect to advanced hepatic fibrosis and a sensitivity of 74.3%. Second, the development of advanced liver disease, based on the stricter definition of FIB-4 and APRI was investigated among patients with a FIB-4 <3.25 as well as a APRI <1.5 at the index date. Third, we excluded patients with a history of alcohol abuse and re-fitted the Bayesian multivariable model, and estimated the cumulative incidence stratified by the index FIB-4 (<1.45 vs 1.45-3.25) using the log-rank test to test for significance. Finally, the model was performed while baseline age was kept stable over the duration of follow-up, allowing only the changes in the laboratory parameters to influence the FIB-4 index.

Reversed-time model for the development of advanced liver disease

Since the observation period of patients started at various points during the course of their chronic HCV infection, a common baseline time is lacking and it is not clear how to align the trajectories of different patients with respect to the time scale. We opted for using the time of the last available FIB4 measurement (i.e., the time the FIB4 reached the threshold of 3.25, the last available FIB4 that did not meet one of the exclusion criteria given in the methods section of the manuscript, or the end of follow-up) as ”baseline” (i.e., time = 0) and to count time backwards from this point. Thus, a FIB-4 measurement 5 years before the last measurement has time=-5.

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Supplementary table 1. Baseline characteristics in patients with and without an estimated year of HCV infection.

	Estimated year of infection (n=1730)	No estimated year of infection (n=2556)
Age, median (IQR), years	47.5 (40-54)	46.6 (38-55)
Male	1077 (62%)	1452 (57%)
BMI, median (IQR)	25.7 (23-29)	25.4 (23-28)
Diabetes Mellitus	359 (10%)	197 (9%)
History of alcohol abuse	616 (14%)	395 (14%)
Genotype HCV 1	875 (51%)	1004 (39%)
2	86 (5%)	127 (5%)
3	154 (9%)	280 (11%)
4	27 (2%)	113 (4%)
5	2 (0.1%)	6 (0.2%)
6	4 (0.2%)	15 (0.6%)
Mixed	3 (0.2%)	2 (0.1%)
Unknown	579 (34%)	1008 (40%)
Laboratory markers, median (IQR) Platelets, x10^9/L	225 (186-270)	226 (188-274)
Albumin, g/L (n=3778)	43 (41-45)	43 (41-45)
Total bilirubin, µmol/L (n=3960)	9 (7-13)	8 (1-11)
FIB-4 index	1.21 (0.83-1.71)	1.16 (0.76-1.17)

IQR, interquartile range; BMI, body mass index; HCV, hepatitis C virus; FIB-4, Fibrosis-4.

Supplementary table 2. Cox proportional hazards regression analyses for the development of advanced liver disease

Including only patients with a baseline APRI <1.5 and advanced disease progression is defined by both FIB-4 >3.25 and APRI>1.5.

	HR (95%CI)
Female sex	0.94 (0.78-1.13)
BMI	1.01 (0.99-1.03)
Genotype 3	1.37 (0.97-1.92)
History of alcohol abuse	1.46 (1.16-1.83)
DM	1.02 (0.78-1.33)
Year of inclusion	1.02 (1.00-1.05)
Positive Anti-HBc	0.93 (0.75-1.15)
FIB-4	3.61 (3.21-4.06)
Total Bilirubin, µmol/L	1.00 (0.99-1.01)
Albumin, g/L	0.94 (0.92-0.96)
Creatinine, µmol/L	1.00 (1.00-1.00)

HR, hazard ratio; CI, credible interval; BMI, body mass index; DM, diabetes mellitus; Anti-HBc, anti-hepatitis B core antigen; FIB-4, Fibrosis-4; APRI, AST to platelet ratio index.

Supplementary table 3. Cox proportional hazards regression for the development of advanced liver disease in patients with age set at baseline

	HR (95%CI)
Female sex	0.99 (0.84-1.16)
BMI	1.00 (0.98-1.02)
HCV Genotype 3 (vs. other HCV genotypes)	0.94 (0.68-1.30)
History of alcohol abuse	1.33 (1.08-1.64)
DM	0.97 (0.78-1.22)
Year of inclusion	1.03 (1.01-1.05)
Positive Anti-HBc	0.90 (0.74-1.09)
FIB-4	4.04 (3.65-4.45)
Total Bilirubin, µmol/L	1.00 (0.99-1.01)
Albumin, g/L	0.93 (0.91-0.94)
Creatinine, µmol/L	1.00 (1.00-1.00)

HR, hazard ratio; CI, credible interval; BMI, body mass index; HCV, hepatitis C virus; DM, diabetes mellitus; Anti-HBc, anti-hepatitis B core antigen; FIB-4, Fibrosis-4. Missing values in covariates were imputed during the estimation procedure.

Supplementary figure 1. FIB-4 progression among patients reaching a FIB-4 >3.25 during follow-up, with the time of the first FIB-4>3.25 set as time zero