Risk of extrahepatic cancer in a nationwide cohort of hepatitis C virus infected persons treated with direct-acting antivirals

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Summary

Background and aims: Direct-acting antivirals (DAAs) against HCV have an immune modulatory effect, this could possibly lead to a decreased tumour control. We, therefore, aimed to assess the risk of extrahepatic cancer (EHC) during and the first years after DAA treatment.

Methods and Results: This is a nationwide cohort study with prospectively collected data for 19,685 persons with HCV, 4013 DAA treated, 3071 interferon (IFN) treated and 12,601 untreated, from 2008 to 2016. Follow-up time was maximum 3 years. The risk for EHC was compared between the groups using Cox regression analyses, with adjustment for age and Charlson Comorbidity Index (CCI). The HCV-infected groups were also compared with matched cohorts without HCV from the general population. In total 341 EHCs were identified, 84, 43 and 214 EHC in the DAA, IFN and untreated group respectively. The EHC risk in DAA treated compared with IFN treated was doubled, but when adjusted for age and CCI the HR was 1.07 (95% CI 0.74-1.56). Compared with the general population, the HR of EHC for the DAA group was 1.45 (CI 1.13-1.86), with the difference remaining statistically significant after adjusting for CCI.

Conclusion: We found no increased risk for EHC associated with DAA therapy after adjustment for age and CCI. An increased risk of EHC in DAA treated compared with the general population was though seen, and attention should be paid to this association in the ageing population with a history of HCV infection.
1 | INTRODUCTION

Chronic HCV infection is a global health problem with an estimated 71 million people infected. After 20 years with chronic HCV infection, 20%-25% develop liver cirrhosis, with an increased risk of HCC, liver failure and liver-related death. Chronic HCV infection is also associated with an increased risk of some extrahepatic cancers (EHC) such as non-Hodgkin lymphoma and intrahepatic cholangiocarcinoma.

The treatment of chronic HCV infection was revolutionised in 2014 with the introduction of direct-acting antivirals (DAAAs) with cure rates of ≥95% and mild or no side effects. Previous interferon (IFN)-based therapies had low cure rates and serious side effects, and were not suitable for patients with conditions such as hepatic decompensation or some psychiatric disorders. With new treatment options, the World Health Organization (WHO) has set up a goal to eliminate hepatitis C by 2030 and many countries have scaled up DAA treatment, with approximately 5 million treated worldwide by the end of 2017.

Within a couple of years after the introduction of DAAAs, there were surprising reports of both occurrence and recurrence of HCC in patients recently treated with DAAAs. A substantial proportion of the detected HCCs had aggressive patterns and only palliative treatment could be offered. In contrast, other studies have shown long-term clinical benefits of DAA treatment, including decreased risk for HCC, lower risk of decompensation and a reduction in liver-related mortality. As a result, there have been different opinions about DAA treatment in relation to the risk for HCC and it has been proposed that further studies of safety are required.

The immunological changes caused by DAAs and a possibly decreased tumour control have raised a question about the risk for EHC in relation to DAA treatment. Other publications have described an unexpectedly high occurrence or recurrence of EHC in small cohorts of DAA-treated patients. Large studies of EHC risk in relation to DAA treatment per se are still missing. In Sweden, the personal identification number (PIN) of all residents and the national registers with high coverage and prospectively collected data present a unique opportunity to study this.

The aim of this nationwide population-based register study was to study the risk of EHC during and shortly after DAA treatment by comparing the risks for EHC in DAA treated with IFN-treated and -untreated HCV-infected patients. Additionally, we compared these groups with matched controls without HCV from the general population.

2 | METHODS

2.1 | Study population and data sources

All Swedish residents have a personal identification number (PIN) which is used by all authorities, health care and in national registers, enabling identification of individuals and linkage of registers for research.

Hepatitis C is a notifiable disease since 1990 in Sweden, with mandatory reporting (with PIN) of new cases (positive HCV-antibody, HCV RNA) from both the clinician and the laboratory to the register for Surveillance of Communicable Diseases at the Public Health Agency of Sweden. Hepatitis B virus (HBV) infection is a notifiable disease since 1967. A nationwide dataset totaling 64 149 records with all HCV notifications from 1990 to 2015, including information on date of notification (assumed to be the date of HCV diagnosis) and route of transmission, was extracted from the Public Health Agency, as described in previous studies. This dataset was sent to Statistics Sweden (SCB), where duplicates and notifications with incomplete PIN were excluded (n = 5174) and information on date of death, emigration/immigration and country of origin were extracted (Figure 1).

Individuals who did not live permanently in Sweden at the time of HCV-notification (n = 1935), and individuals co-infected with HBV-infection (n = 4038) were excluded. The remaining HCV-cohort comprised 53 002 individuals. The reported routes of transmission were unknown/missing in 50%, injection drug use in 40%, transfusion of blood/blood products before 1992 in 5%. From 1992 injection drug use is the assumed route of transmission in the majority of new infections.

An individually matched comparison-cohort without HCV diagnosis from the general population was obtained from SCB. For each HCV-infected person, 10 individuals, alive on the date of notification and matched for sex, birth year and county of residence, were collected. All comparators with inconsistent data, no observation-time, past/present EHC, liver cancer, or liver transplantation at inclusion, or who had died prior to or at the same day as inclusion date were excluded.

2.2 | Linkage with national registers

The data from the HCV-infected cohort and the matched comparison-cohort were linked to national registers with prospectively collected data held at the National Board of Health and Welfare; the Prescription Register (PrR), the Patient Register (PR), the Cancer Register (CR) and the Cause of Death Register (DR). The PrR contains data on all prescriptions dispensed from Swedish pharmacies since July 2005, including data on age, sex, dispensed item, amount, dosage, prescription and dispensing dates, with a coverage of more than 99%. Information on prescriptions of pegylated IFN (alfa-2a and alfa-2b), and 2nd or 3rd wave DAAs (sofosbuvir, simeprevir, daclatasvir, omibitasvir, paritaprevir, ritonavir, dasabuvir, ledipasvir, elbasvir, grazoprevir, velpatasvir, glecaprevir, pibrentasvir and voxilaprevir) were extracted to form the treatment groups for the study.

Individuals treated with 1st wave DAAs (telaprevir or boceprevir, n = 697) were excluded since these drugs were used in combination with IFN. The PR, containing diagnoses from all inpatient care and outpatient specialist care (classified according to International Classification of Diseases [ICD]), added information on comorbidity,
date of liver transplantation, other liver complications and cancer diagnoses. All hospital discharge diagnoses are registered in the PR with a coverage of >99% and high validity for most diagnoses, generally exceeding 85%.33

The primary outcome of the study was incident cases of EHC recorded after inclusion. Recurrence of cancer was not examined, since such data are lacking/insufficient in the registers. From the CR, all incident cancers and date of diagnosis were identified (using the seventh edition of ICD [ICD-7] to include both older cancer diagnoses and newer, converted into ICD-7). Only cancers recorded as malignant were included. The completeness of the CR is estimated to be high, with approximately 96% of cancers reported.34

For sensitivity analyses, EHC diagnoses from the PR and DR were identified (ICD-10) and added when missing in the CR. The accuracy of the PR and DR is lower than the accuracy of the CR (with high proportion of histological diagnoses), therefore EHC diagnoses from the PR were included only when registered at least twice.34,35

2.3 Treatment groups

The three therapy groups, DAA-treated, IFN-treated and the untreated group were identified among eligible subjects in the total HCV-cohort (Flow chart, Figure 1). Each individual could only be included in one group. Everyone with a liver cancer, EHC, or liver transplantation before the start of follow-up was excluded.

The DAA-treated group consisted of patients treated with IFN-free regimes with 2nd/3rd wave DAAs. Those who had been treated
with IFN less than 90 days before, together with, or after 2nd/3rd wave DAAs were excluded (n = 45). The IFN group consisted of patients treated with IFN who did not receive DAAs during follow-up. The untreated group had not received IFN (from July 2005) nor DAA, but had visited specialist outpatient care for a chronic HCV-diagnosis.

2.4 | Start and end of the study

Inclusion in the IFN- and untreated group started in January 2008 and in the DAA group in January 2014 when the first 2nd/3rd wave DAA was launched in Sweden. Individuals who had died or had no patient visits after 2007 could not be included (n = 27 109). Inclusion and start of follow-up in the DAA and IFN group was at the first date the drug was dispensed, which was considered equal to the start of treatment. Individuals in the untreated group were included at the date of the first outpatient visit with chronic hepatitis C diagnosis (ICD10: B18.2) after January 1, 2008. Data from the PrR were available from July 2005, but to avoid including patients that had been treated and cured in 2004-2005, with follow-up visits in 2006-2007, the inclusion started in 2008. This was also to set time periods as close as possible for the treatment groups. The last date for inclusion was December 31, 2016, to allow at least 1 year of follow-up.

The maximum follow-up time was limited to 3 years, to make the groups more comparable and limit the eventual time-dependent trend of the risk. The retrieved register data contained data on EHC until December 31, 2017. Observation time ended at outcome (date of EHC diagnosis), at censoring due to emigration, death, liver cancer, liver transplantation or start of DAA treatment after last date of EHC diagnosis), at censoring due to emigration, death, liver cancer, liver transplantation or start of DAA treatment after last date of inclusion (December 31, 2016), whichever occurred first, otherwise at maximum 3 years after inclusion or December 31, 2017.

2.5 | Charlson Comorbidity Index

Charlson Comorbidity Index (CCI) is a method of categorising comorbidity based on ICD-codes in outpatient and inpatient discharge diagnoses, with each comorbidity category having an associated weight, based on the adjusted risk of mortality or resource use. The sum of all weights results in a single comorbidity score for the patient.36 Data for calculating CCI for HCV-infected individuals and comparators were retrieved from the PR. The CCI-diagnoses were included from 5 years before start of the study, until end of follow-up. Malignancies were excluded from the CCI-diagnoses in this study.

2.6 | Ethics

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden. The linkage of the register-files was handled by the Public Health Agency, Statistics Sweden and the National Board of Health and Welfare. Only anonymised data were used for analyses.

2.7 | Statistical analysis

Information about cancers was retrieved from the CR, and patients were described by sex and therapy group in absolute numbers and per 1000 person years (PY). For cancers in breast and female/male genital organs, total PY were calculated for only women or men.

Cox proportional hazards models were used to compare the risk, expressed as HR with 95% CI, for incident EHC (identified from CR) between the three treatment groups. The proportional hazards assumption was examined for each covariate by plotting the time-varying coefficients and by performing a correlation test between the weighted Schoenfeld residual and failure time. The risks were calculated for men and women combined and stratified by sex. To adjust for the difference in age in the treatment groups, age was used as the underlying time-scale, and CCI to adjust for comorbidity. Furthermore, Cox regression analyses, using internal stratification for matching characteristics, were used to assess the relative risks of EHC in the three treatment groups compared with their matched general population.

Sensitivity analyses by adding cancers from the PR and DR (when missing in the CR) and with shorter follow-up times, to study HRs after 1 and 2 years of follow-up, were conducted.

Kaplan-Meier curves were used to estimate EHC free follow-up time for the different treatment groups, also stratified for different age groups (age [at start of follow-up] 35-49, 50-64 and ≥65 years), and for the matched general population, and log-rank tests to compare EHC-free follow-up time between the groups.

The data management and statistical analyses were carried out using the statistical software SAS® (Version 9.4, SAS Institute Inc). A two-sided P < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of study population

In total, 19 685 individuals with HCV mono-infection were included in the study population, with 4013 in the DAA group, 3071 in the IFN group and 12 601 in the untreated group.

Characteristics of the study population are shown in Table 1. The mean age at inclusion was highest in the DAA group with 54.2 (SD 11.1) years, whereas it was 44.2 (SD 12.1) and 43.6 (SD 14.2) years for the IFN-treated and untreated groups, respectively. High CCI score ≥3 was present in 14.5% of the DAA treated, whereas 5.1% and 7.9% in the IFN treated and untreated respectively. Censoring due to liver cancer occurred in 2.4% of DAA treated, 1.0% of IFN treated and 1.4% of untreated. The total years of follow-up was 50 345 person-years, with 8032 for DAA treated, 8956 IFN treated and 33 357 for untreated. The median observation time was 2.0 PY
in the DAA group, 3.0 PY in the IFN group and 3.0 PY in the untreated group; that is shortest in the DAA group, since the earliest inclusion in this group was January 2014.

The comparison groups without HCV diagnosis from the general population consisted of 35 650, 28 905 and 117 900 individuals matched to the DAA group, IFN group and untreated group respectively.

### 3.2 | Occurrence of extrahepatic cancer

Among 19 685 HCV-infected persons, there were 341 with malignant EHC (230 men, 111 women) reported to the CR during follow-up, with 84, 43 and 214, in the DAA, IFN and untreated groups respectively. The median time between study inclusion and cancer diagnosis was 1.1, 1.6 and 1.3 (range 0-3 for all groups) PY for DAA, IFN and untreated groups, respectively. The type and number of EHC diagnoses from the CR (in absolute numbers and per 1000 PY) by treatment group are presented in Table 2. The most common cancer types were cancer of the digestive tract, male genital organs and respiratory organs/chest. Cancer diagnoses from the CR, PR and DR are shown in Table S1. There were too few cases of each cancer type to perform reliable risk calculations for separate types of cancer.

### 3.3 | Comparison of risk in DAA treated vs other treatment groups

The results of the Cox regression analyses by treatment group, for the whole group and by sex, are presented in Table 3. The unadjusted results showed higher HRs in the DAA group with a statistically significant higher risk, but when adjusting for age, or age and CCI, there was no statistically significant difference in EHC risk between the three

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**TABLE 1** Patient characteristics, extrahepatic cancers and censoring reasons by study group

| Therapy group | Total cohort | DAA     | IFN     | Untreated |
|---------------|-------------|---------|---------|-----------|
| Total number, n | 19 685     | 4013    | 3071    | 12 601    |
| Sex, n (%)     |            |         |         |           |
| Women          | 7157 (36.4)| 1419 (35.4)| 1242 (40.4)| 4496 (35.7)|
| Men            | 12 528 (63.6)| 2594 (64.6)| 1829 (59.6)| 8105 (64.3)|
| Age at inclusion, mean (SD), y  | 45.8 (14.0) | 54.2 (11.1) | 44.2 (12.1) | 43.6 (14.2) |
| Observation time, median (range) person-y | 3 (0-3) | 2.0 (0-3) | 3.0 (0-3) | 3.0 (0-3) |
| CCI (0, 1, 2, 3+) |          |         |         |           |
| 0              | 14 051 (71.4%) | 2300 (57.3%) | 2443 (79.6%) | 9308 (73.9%) |
| 1              | 2937 (14.9%) | 842 (21.0%) | 372 (12.1%) | 1723 (13.7%) |
| 2              | 958 (4.9%) | 288 (7.2%) | 98 (3.2%) | 572 (4.5%) |
| 3+             | 1739 (8.8%) | 583 (14.5%) | 158 (5.1%) | 998 (7.9%) |
| Time to EHC diagnosisb, median (range) y | 1.2 (0-3) | 1.1 (0-3) | 1.6 (0-3) | 1.3 (0-3) |
| No. of EHC during follow-upb, n (%) | 341 (1.7) | 84 (2.1) | 43 (1.4) | 214 (1.7) |
| Persons censored by reason, n (%) |          |         |         |           |
| All            | 1929 (9.8) | 228 (5.7) | 130 (4.2) | 1571 (12.5) |
| Death          | 1069 (5.4) | 115 (2.9) | 66 (2.2) | 888 (7.1) |
| Liver cancer   | 299 (1.5) | 95 (2.4) | 32 (1.0) | 172 (1.4) |
| Liver transplantation | 31 (1.6) | 18 (0.5) | 4 (0.1) | 9 (0.1) |
| Migration      | 155 (0.8) | 0 (0.0) | 26 (0.9) | 129 (1.0) |
| Start of DAA treatmentc | 375 (1.9) | - | 2 (0.1) | 373 (3.0) |

Abbreviations: CCI, Charlson Comorbidity Index; DAA, direct-acting antiviral; EHC, extrahepatic cancer; IFN, interferon; y, years.

*bIncluding only individuals with a cancer event during follow-up.

*cCancer diagnoses retrieved from the cancer register. Unadjusted for age and co-morbidity (CCI).

Start of DAA treatment after the last date of inclusion on December 31, 2016.
treatment groups. The age and CCI-adjusted HR for DAA treated vs IFN treated was 1.07 (95% CI 0.74-1.56, \( P = 0.72 \)). The corresponding HR for DAA treated vs untreated was 0.83 (95% CI 0.64-1.07, \( P = 0.15 \)).

Sensitivity analyses using cancer diagnoses from the CR, PR and DR (adding 59 EHCs that were missing in CR) and analyses with shorter observation times did not notably change the results (Tables S2 and S3).

### TABLE 2
Type and number of cancer diagnoses extracted from the Cancer Register

| Type of cancer                                                                 | Number of cancers | Number of cancers per 1000 person-years |
|--------------------------------------------------------------------------------|-------------------|----------------------------------------|
|                                                                                | DAA | Interferon | Untreated | Total | DAA | Interferon | Untreated |
| Blood and lymphatic                                                           | 7   | 3          | 30        | 40    | 0.87 | 0.33       | 0.90      |
| Breast                                                                         | 5   | 5          | 21        | 31    | 1.74 | 1.37       | 1.71      |
| Digestive tract                                                               | 17  | 9          | 50        | 76    | 2.12 | 1.00       | 1.50      |
| Female genital organs                                                         | 4   | 2          | 10        | 16    | 1.39 | 0.55       | 0.82      |
| Lip, mouth, pharynx                                                           | 1   | 2          | 7         | 10    | 0.12 | 0.22       | 0.21      |
| Male genital organs                                                           | 18  | 9          | 25        | 52    | 3.49 | 1.70       | 1.18      |
| Malignant neoplasms of eye, brain and other parts of CNS                      | 5   | 2          | 5         | 12    | 0.62 | 0.22       | 0.15      |
| Malignant neoplasms of mesothelial and soft tissue                           | 1   | 1          |           | 1     | 0.62 | 0.11       | 0.18      |
| Malignant neoplasms of thyroid and other endocrine glands                     | 5   | 6          | 11        | 18    | 0.75 | 0.33       | 0.27      |
| Melanoma and other malignant tumours of the skin                              | 6   | 3          | 9         | 18    | 0.75 | 0.33       | 0.27      |
| Respiratory organs, chest                                                     | 10  | 4          | 28        | 42    | 1.24 | 0.45       | 0.84      |
| Urinary organs                                                                | 4   | 1          | 8         | 13    | 0.50 | 0.11       | 0.24      |
| Other cancers                                                                 | 2   | 2          | 15        | 19    | 0.25 | 0.22       | 0.45      |
| **In total**                                                                  | 84  | 43         | 214       | 341   |

### TABLE 3
Cox regression models showing HR for extrahepatic cancer in patients treated with direct-acting antivirals (DAA), interferon (IFN) or untreated

| Variables | Unadjusted | Adjusted for age\(^a\) | Adjusted for age and CCI\(^a\) |
|-----------|------------|-------------------------|-------------------------------|
|           | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value |
| DAA vs IFN\(^b\) |             |          |            |          |            |          |
| All       | 2.15 (1.49-3.12) | <0.001 | 1.10 (0.75-1.60) | 0.63    | 1.07 (0.74-1.56) | 0.72    |
| Men       | 2.09 (1.33-3.27) | 0.002  | 1.01 (0.64-1.59) | 0.96    | 0.99 (0.63-1.55) | 0.98    |
| Women     | 2.25 (1.16-4.36) | 0.02   | 1.27 (0.65-2.49) | 0.48    | 1.23 (0.63-2.40) | 0.55    |
| Untreated vs IFN\(^b\) |             |          |            |          |            |          |
| All       | 1.32 (0.95-1.84) | 0.09    | 1.31 (0.94-1.83) | 0.11    | 1.30 (0.93-1.81) | 0.12    |
| Men       | 1.23 (0.82-1.83) | 0.31    | 1.19 (0.80-1.78) | 0.39    | 1.18 (0.79-1.77) | 0.42    |
| Women     | 1.53 (0.87-2.72) | 0.14    | 1.58 (0.89-2.82) | 0.12    | 1.55 (0.87-2.76) | 0.14    |
| DAA vs untreated\(^c\) |             |          |            |          |            |          |
| All       | 1.63 (1.26-2.10) | <0.001  | 0.84 (0.64-1.08) | 0.17    | 0.83 (0.64-1.07) | 0.15    |
| Men       | 1.70 (1.25-2.31) | <0.001  | 0.85 (0.62-1.16) | 0.3     | 0.84 (0.62-1.15) | 0.28    |
| Women     | 1.47 (0.93-2.33) | 0.1     | 0.81 (0.5-1.29)  | 0.37    | 0.79 (0.5-1.27)  | 0.33    |

Abbreviation: CCI, Charlson comorbidity index.

\(^a\)Age is used as the underlying time scale.

\(^b\)IFN used as reference.

\(^c\)Untreated used as reference.
Kaplan-Meier curves of EHC free survival time for the three treatment groups and the DAA-matched controls, divided in three age groups, are demonstrated in Figure S1A-C. Log-rank tests showed statistically significant risk difference for the DAA group vs untreated group and for the untreated vs IFN group in the age-group 50-64 (P = 0.003 and <0.001 respectively), but not in the other age groups.

3.4 | Comparison of risk in the DAA- or IFN-treated groups with matched comparators from the general population

Cox regression analyses comparing the risk of EHC in DAA and IFN groups with their 10 times larger matched comparison cohort showed a statistically significantly increased age and CCI-adjusted HR for DAA-treated men (HR 1.50; 95% CI 1.10-2.05; P = 0.01), but not for women (HR 1.12; 95% CI 0.70-1.78; P = 0.65) (Table 4). For IFN-treated men the HR is elevated, however, not statistically significant; HR 1.44 (95% CI 0.94-2.21; P = 0.09).

When stratified for the age-groups (not sex stratified), the cumulative risk for EHC after 3 years was 3.3% in the DAA group and 2.2% in the matched general population (log-rank P = 0.001) (Figure 2A–D).

When stratified for the age-groups (not sex stratified), the cumulative risk for EHC after 3 years was 3.3% in the DAA group and 2.2% in the matched general population (log-rank P = 0.001) (figure not shown). Log-rank tests in the sex-stratified estimations showed a statistically significantly shorter EHC free survival time for men in both the DAA and IFN group compared to the matched comparison groups (P = 0.01 and P = 0.032, respectively), but not for women (P = 0.33/P = 0.68) (Figure 2A–D).

When stratified for the age-groups (not sex stratified), the cumulative risks for EHC in the age-group 50-64 years were 1.0%, 1.8% and 3.3% for the DAA group, and 0.6%, 1.6% and 2.2% for DAA-matched controls, at 1, 2 and 3 years follow-up respectively (DAA vs DAA-matched controls at 3 years follow-up: P ≤ 0.001) (Figure S1A-C). The corresponding figures for those aged ≥65 years were 2.3%, 5.5% and 7.5% for DAA group and 1.9%, 3.4% and 5.9% for DAA-matched controls, at 1, 2 and 3 years follow-up respectively (P = 0.065 at 3 years follow-up).

**TABLE 4** Cox regression models showing HR for extrahepatic cancer in hepatitis C patients treated with direct-acting antivirals (DAA) or interferon (IFN), compared to matched control groups from the general population

| Variables          | Adjusted for age<sup>a</sup> | Adjusted for age and CCI<sup>b</sup> |
|--------------------|------------------------------|-------------------------------------|
|                    | HR (95% CI)                  | P-value                             | HR (95% CI)                  | P-value                             |
| DAA vs controls<sup>b</sup> |                              |                                     |                              |                                     |
| All                | 1.45 (1.13-1.86)             | 0.004                               | 1.36 (1.05-1.76)             | 0.02                                |
| Men                | 1.58 (0.17-2.14)             | 0.003                               | 1.50 (1.10-2.05)             | 0.01                                |
| Women              | 1.22 (0.78-1.90)             | 0.40                                | 1.12 (0.70-1.78)             | 0.65                                |
| IFN vs controls<sup>b</sup> |                              |                                     |                              |                                     |
| All                | 1.23 (0.87-1.74)             | 0.23                                | 1.19 (0.84-1.69)             | 0.32                                |
| Men                | 1.50 (0.98-2.29)             | 0.06                                | 1.44 (0.94-2.21)             | 0.09                                |
| Women              | 0.90 (0.50-1.63)             | 0.73                                | 0.88 (0.48-1.59)             | 0.67                                |

Abbreviations: CCI, Charlson comorbidity index; DAA, direct-acting antivirals; IFN, interferon.

<sup>a</sup>Age is used as the underlying time scale.

<sup>b</sup>Controls were used as a reference. The controls were matched for age, sex and residence county, and further adjusted for age.

4 | DISCUSSION

This nationwide study of 19,685 individuals with hepatitis C, including 4013 DAA treated, demonstrates a doubled risk for EHC in DAA treated compared with IFN-treated patients during the first years after treatment initiation. After adjustment for age and co-morbidity there was no statistically significant difference in risk between the treatment groups. This indicates that there is no association between DAA treatment and the short-term risk for EHC. However, there was an increased risk of EHC among the DAA treated compared with the matched general population without HCV diagnosis, which highlights the need for further studies of cancer risk in the ageing population with a history of HCV infection.

Our findings are in contrast with a recently published large population-based study of patients with liver disease by Kim D et al, using US Census and the National Center for Health Statistics from 2007-2017, where a decrease in liver-related mortality was seen after the introduction of DAAs, nevertheless the EHC mortality increased in the DAA era. Data on HCV treatment were though missing, making it difficult to assess the real association between DAAs and the risk for EHC. Such assessment was possible in our nationwide study with data of HCV treatment on an individual level; however, we did not find any association between DAAs and the risk for EHC, after adjusting for age and CCI. Recently, Wang et al published a register-based study investigating the impact of HCV treatment on the risk of extrahepatic cancer among HCV-infected patients in the United States and found an 11% decreased incidence when comparing patients treated with any kind of HCV therapy with untreated patients. The association was not observed with only DAA treatment due to the short follow-up time for this group (12.7 months). Treatment response was not known in this study. Another study showed that IFN-induced SVR was significantly associated with a reduction of the risk of lymphoma, multiple myeloma, MGUS and haematological malignancies combined, but these associations were not observed with DAA-induced SVR during a mean follow-up of...
Neither IFN-α-induced SVR nor DAA-induced SVR was associated with the risk of colon cancer or prostate cancer. A strong association between viral eradication and a better outcome of HCV-positive B-cell non-Hodgkin lymphoma has also been shown in a meta-analysis, but due to lack of data, separate analyses on DAA-treated patients were not possible.

The three treatment groups in our study differed in several aspects. The DAA group had a 10-year higher mean age, higher CCI and more liver cancers than the other groups. This could be explained both by the ageing of the HCV population, being older with a longer duration of HCV infection in the DAA era, and the restrictions with the availability of DAAs only for patients with advanced fibrosis until the end of 2017. The untreated group was a heterogenic group including individuals who had not yet received treatment, possible reasons include the presence of mild liver disease, ongoing injection drug use or psychiatric disorders. In our study, the mortality rate was higher in the untreated group than in the other groups, possibly caused by drug-related events such as overdoses or accidents. To adjust for the differences in the treatment groups, adjustments for age and comorbidity were applied.
Our study showed an about 1.5-fold higher risk of EHC for both DAA- and IFN-treated men compared with matched comparators without HCV diagnosis from the general population (statistically significant for DAA treated). This could possibly be related to a lifestyle with higher alcohol and tobacco consumption than the matched comparison cohort, such information was not available since these risk factors seldom are registered as ICD-codes in clinical praxis. Additionally, surveillance bias of the DAA- and IFN-treated groups compared with the uninfected comparison group could possibly contribute to this finding. No decline of the increased risk for EHC in DAA treated compared with the general population was seen after 3 years of follow-up, even though ≥95% of DAA treated in Sweden have been cured. This implies that viral eradication does not diminish the elevated risk of EHC during the first years after treatment. A French prospective study found that HCV cirrhosis was associated with a higher risk of EHC compared to the French general population, even after cure with IFN.

The reports about a possible increased risk of early recurrence and more aggressive HCCs after DAA treatment raised a concern about the safety of DAAs. Many studies of DAAs and HCCs have been conducted the last years, and most of them showed no elevated HCC-risk after DAA treatment, but a diminishing HCC-risk over time. Hepatitis C virus infects not only hepatocytes but also extrahepatic cells and could induce alterations of the immune system and local tissue microenvironment, with increased risk of various extrahepatic neoplasia. Immunological studies have shown that chronic HCV patients have an altered natural killer (NK) cell phenotype, which is exacerbated during IFN therapy, but normalised with DAA therapy. The fast suppression of the virus reduces the level of inflammatory cytokines in DAA treated and is associated with a suppression of NK cells, which may reduce immunosurveillance of precancerous clones. It has also been shown that treatment-induced HCV clearance decreases the strength of HCC-specific CD8 T-cell response in cirrhotic patients and may fail to recover after DAA therapy. Interferons have a well-known role in promoting antitumor responses and have been extensively used for the treatment of several cancers, and there are theories of a protective effect of IFN on HCC development that is not seen with DAA treatment.

Strengths in our study are the nationwide approach and the rather large sample size. Other advantages are that the data were prospectively recorded, and the high completeness of the Swedish HCV surveillance register with notifications from both the clinician and the diagnosing laboratory, as well as the other national registers in Sweden. The use of PINs provides a unique opportunity to conduct register studies in Sweden, since many different registers can be combined and provide extensive information on each individual included.

A possible limitation is the lack of data regarding cure after treatment. This is a concern for the IFN group with cure rates around 50%, varying by HCV genotype and stage of liver fibrosis, in contrast to ≥95% in the DAA group. Cure of HCV results in a lower incidence of HCC and liver-related mortality, and longer survival may result in more EHC in the long term due to ageing without liver-related complications. However, the cure rate may not be an important factor if cancer is suspected to arise from immune-modulation early after initiation of therapy, which has been the concern. Another limitation is the lack of information about IFN treatment prior to 2005, before the start of the PrR. The impact of this limitation is probably low, since inclusion in the study started in 2008 and all individuals with a malignancy prior to inclusion were excluded. Additionally, the lack of data on smoking and alcohol consumption is a limitation when studying the risk of EHC in HCV-infected individuals compared with the general population. Nevertheless, in this study, the main objective was to investigate if there was an increased risk of EHC associated with DAA treatment by comparing the HCV treatment groups, assuming similar alcohol and tobacco use. The comparison with the general population for both DAA and IFN treated was added to illustrate the risk from another point of view, but still with the focus on DAA treatment in relation to IFN treatment.

This study estimates the EHC risk early after DAA treatment with follow-up time limited to a maximum of 3 years. This time frame is though sufficient to study the early incidence of cancer, which is important since previous reports suggested an increased risk for HCC during or soon after treatment start. In our study, sensitivity analyses with follow-up limited to 1 and 2 years were performed, but no statistically significant difference was found at any time-point. However, studies with longer follow-up, to estimate the long-term risk, are needed when DAAs have been used for a longer time.

In this nationwide register study of people with HCV diagnosis, there was no statistically significant increased risk for EHC after DAA treatment when adjusted for age and comorbidity. This suggests that DAA treatment is safe in respect to EHC early after treatment, but confirmatory studies and studies with longer follow-up time are needed to fully explore this issue. The study indicated an increased risk of EHC in people with an HCV diagnosis compared with members of the general population without HCV. This association remained after cure from HCV and further studies of the ageing population with a history of HCV infection would be of interest.

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CONFLICT OF INTEREST

A-S. D. has served as a speaker and/or consultant for AbbVie, BMS, Gilead and MSD. SA has served as a speaker and/or consultant for AbbVie, BMS, Gilead, Janssen, MSD/Merck, Medivir, Roche and Tillotts Pharma and has received research grants from AbbVie and Gilead. CL has served as a speaker for MSD. SM and DB reports no conflicts of interest.
AUTHORS’ CONTRIBUTIONS
A-SD, SA and CL contributed to the concept of the study. All authors contributed to the design of the study and interpretation of data. A-SD, SA and CL made the data collection. DB performed the statistical analyses. The manuscript was drafted by CL, and critically revised and approved by all authors.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/ygh2.456.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES
1. World Health Organization. Global Hepatitis Report, 2017. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/ Accessed May 5, 2018.
2. Westbrook RH, Dusheiko G. Natural history of hepatitis C. J Hepatol. 2014;61:558-568.
3. De Sanjose S, Benavente Y, Vajdic CM, et al. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clin Gastroenterol Hepatol. 2008;6:451-458.
4. Huang J, Magnusson M, Torner A, Ye W, Duberg AS. Risk of pancreatic cancer among individuals with hepatitis C or hepatitis B virus infection: a nationwide study in Sweden. Br J Cancer. 2013;109:2917-2923.
5. Pozzato G, Mazzaro C, Dal Maso L, et al. Hepatitis C virus and non-Hodgkin lymphoma: meta-analysis of epidemiology data and therapy options. World J Hepatol. 2016;8:107-116.
6. Mahale P, Torres HA, Kramer JR, et al. Hepatitis C virus infection and the risk of cancer among elderly US adults: a registry-based case-control study. Cancer. 2017;123:1202-1211.
7. Pol S, Vallet-Pichard A, Hermine O. Extrahepatic cancers and chronic HCV infection. Nat Rev Gastroenterol Hepatol. 2018;15:283-290.
8. Fiorino S, Bacchi-Reggiani L, de Blase D, et al. Possible association between hepatitis C virus and malignancies different from hepatocellular carcinoma: a systematic review. World J Gastroenterol. 2015;21:12896-12953.
9. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. J Hepatol. 2017;66:153-194.
10. Hoofnagle JH, Seeff LB. Peginterferon and ribavirin for chronic hepatitis C. N Engl J Med. 2006;355:2444-2451.
11. World Health Organisation. Global Health Sector Strategy on Viral Hepatitis 2016–2021. Towards Ending Viral Hepatitis. https://apps.who.int/iris/bitstream/handle/10665/246177/WHO%E2%80%90HV%E2%80%902016.06%E2%80%90eng.pdf?sequence=11 Accessed April 1, 2021.
12. World Health Organisation. Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c Accessed April 1, 2021.
13. Reig M, Maríño Z, Perelló C, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol. 2016;65:719-726.
14. Conti F, Buonfiglioli F, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016;65:727-733.
15. ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts). Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. J Hepatol. 2016;65:734-740.
16. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology. 2017;153:996-1005.e1.
17. Waziry R, Hajarizadeh B, Grebely J, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, and meta-regression. J Hepatol. 2017;67:1204-1212.
18. Carrat F, Fontaine H, Dorival C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet. 2019;393:1453-1464.
19. Trotter JF. Pro: direct-acting antivirals are associated with occurrence and recurrence of hepatocellular carcinoma. Liver Transpl. 2017;23:1593-1595.
20. Mehta N, Yao FY. Con: treating hepatitis C virus With direct-acting antivirals: fear not the perceived threat of hepatocellular carcinoma. Liver Transpl. 2017;23:1596-1600.
21. Gagliò PJ. Extrahepatic and intrahepatic malignancies in patients with HCV who achieve an SVR with directly acting antiviral agents: should we be concerned that DAA therapy contributed to this phenomenon? J Clin Gastroenterol. 2017;51:657-658.
22. Kim D, Adejumo AC, Yoo ER, et al. Trends in mortality from extrahepatic complications in patients with chronic liver disease, from 2007 through 2017. Gastroenterology. 2019;157:1055-1066.e11.
23. Lin RJ, Moskovits T, Diefenbach CS, Hymes KB. Development of highly aggressive mantle cell lymphoma after sofosbuvir treatment of hepatitis C. Blood Cancer J. 2016;6:e402.
24. Khoury J, Nassar G, Kramsky R, Saadi T. Extrahepatic malignancies after treatment with direct antiviral agents for chronic HCV infection. J Gastrointest Cancer. 2019;51:584-590.
25. Public Health Agency of Sweden. Hepatitis C statistics. https://www.scb.se/en/ Accessed March 1, 2019.
26. Duregh S, Nordström M, Törner A, et al. Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. Hepatology. 2005;41:652-659.
27. Simon TG, Duregh AS, Aleman S, et al. Lipophilic statins and risk for hepatocellular carcinoma and death in patients with chronic viral hepatitis: results from a nationwide Swedish population. Ann Intern Med. 2019;171:318-327.
28. Simon TG, Duregh AS, Aleman S, Chung RT, Chan AT, Ludvigsson JF. Association of aspirin with hepatocellular carcinoma and liver-related mortality. N Engl J Med. 2020;382:1018-1028.
29. Statistics Sweden. Homepage. https://www.scb.se/en/ Accessed March 1, 2019.
30. Batyrbekova N, Aleman S, Lybeck C, Montgomery S, Duregh AS. Hepatitis C virus infection and the temporal trends in the risk of liver cancer: a national register-based cohort study in Sweden. Cancer Epidemiol Biomarkers Prev. 2020;29:63-70.
31. Socialstyrelsen. The National Board of Health and Welfare; homepage Sweden. http://www.Socialstyrelsen.se/english Accessed March 1, 2019.
32. Sveriges Kommuner och Landsting och Socialstyrelsen. Kvalitet i sjukvårdsdata, indikatorbeskrivningar och vårdkonsumtion Sweden2008.
33. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.

34. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48:27-33.

35. The National Board of Health and Welfare H. The Swedish Cancer Register. https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/swedish-cancer-register/ Accessed December 3, 2019.

36. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali WA. New ICD-10 version of the Charlson comorbidity index predicted in-hospital mortality. J Clin Epidemiol. 2004;57:1288-1294.

37. Wang W, Lo Re V 3rd, Guo Y, Xiao H, Brown J, Park H. Impact of hepatitis C virus treatment on the risk of non-hepatic cancers among hepatitis C virus-infected patients in the US. Aliment Pharmacol Ther. 2020;52:1592-1602.

38. Ioannou GN, Green PK, Berry K, Graf SA. Eradication of hepatitis C virus is associated with reduction in hematologic malignancies: major differences between interferon and direct-acting antivirals. Hepatol Commun. 2019;3:1124-1136.

39. Masarone M, Persico M. Hepatitis C virus infection and non-hepatocellular malignancies in the DAA era: a systematic review and meta-analysis. Liver Int. 2019;39:1292-1306.

40. Frisk P, Agefors K, Cars T, et al. Introduction of the second-generation direct-acting antivirals (DAAs) in chronic hepatitis C: a register-based study in Sweden. Eur J Clin Pharmacol. 2018;74:971-978.

41. Allaire M, Nahon P, Layese R, et al. Extrahepatic cancers are the leading cause of death in patients achieving hepatitis B virus control or hepatitis C virus eradication. Hepatology. 2018;68:1245-1259.

42. Spaan M, van Oord G, Kreeft K, et al. Immunological analysis during interferon-free therapy for chronic hepatitis C virus infection reveals modulation of the natural killer cell compartment. J Infect Dis. 2016;213:216-223.

43. Serti E, Chepa-Lotre X, Kim YJ, et al. Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function. Gastroenterology. 2015;149:190-200.e2.

44. Owusu Sekyere S, Schlevogt B, Mettke F, et al. HCC immune surveillance and antiviral therapy of hepatitis C virus infection. Liver Cancer. 2019;8:41-65.

45. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol. 2006;6:836-848.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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