Universitat Autònoma de Barcelona

Cancer incidence related to pharmacological treatment of hepatitis C virus infection

José Pedro Ríos Guillermo

Departament de Farmacologia, de Terapèutica i de Toxicologia

Universitat Autònoma de Barcelona

Programa de Doctorat en Farmacologia

1 Title Page

Cancer incidence related to pharmacological treatment of hepatitis C virus infection

Thesis to opt for the title of Doctor in Pharmacology of

José Pedro Ríos Guillermo

Directors: Dr Caridad Pontes García and Dr Ferran Torres Benítez

Barcelona, June 20th, 2022

2 Signature page:

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I

De Diógenes compré un día
la linterna a un mercader;
distan la suya y la mía
cuanto hay de ser a no ser.
Blanca la mía parece;
la suya parece negra;
la de él todo lo entristece;
la mía todo lo alegra.
Y es que en el mundo traidor
nada hay verdad ni mentira;
"todo es según el color
del cristal con que se mira".

II

– Con mi linterna – él decía-
no hallo un hombre entre los seres-.
¡Y yo que hallo con la mía
hombres hasta en las mujeres!
él llamó, siempre implacable,
fe y virtud teniendo en poco,
a Alejandro, un miserable,
y al gran Sócrates, un loco.
Y yo ¡crédulo! entretanto,
cuando mi linterna empleo,
miro aquí, y encuentro un santo,
miro allá, y un mártir veo.
¡Sí! mientras la multitud
sacrifica con paciencia
la dicha por la virtud
y por la fe la existencia,
para él virtud fue simpleza,
el más puro amor escoria,
vana ilusión la grandeza,
y una necedad la gloria.
¡Diógenes! Mientras tu celo
sólo encuentra sin fortuna,
en Esparta algún chicuelo
y hombres en parte ninguna,
yo te juro por mi nombre
que, con sufrir al nacer,
es un héroe cualquier hombre,
y un ángel toda mujer.

III

Como al revés contemplamos
yo y él las obras de Dios,
Diógenes o yo engañamos.
¿Cuál mentirá de los dos?
¿Quién es en pintar más fiel
las obras que Dios creó?
El cinismo dirá que él;
la virtud dirá que yo.

Y es que en el mundo traidor
nada hay verdad ni mentira:
"todo es según el color
del cristal con que se mira".

'Las dos linternas' (1846) - Ramón de Campoamor (1817-1901) –

Extraído de la Biblioteca Virtual 'Miguel de Cervantes'

3 Acknowledgements

A medida que pasan los años atesoramos experiencias. Experiencias que nos hacen más sabios pero que, por encima de todo, nos han permitido conocer a personas con trayectorias extraordinarias con las que he compartido momentos de extraordinaria riqueza y felicidad. Con ello, hacer un texto de agradecimientos, con los 50 años recién cumplidos se me hace una tarea difícil.

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A mis hijas, Jimena y Xilda. Pocas cosas existen que te cambien tanto la vida como tener descendencia, a partir de ese momento sabes que lo que hagas, en el futuro les afectará también a ellas. Ellas, que día a día, son motivo de alegría y ganas por continuar adelante.

A Gema, mi compañera de viaje en estos últimos años y que siempre ha estado a mi lado apoyándome en todo. Mi buena suerte permitió que me encontrara con ella.

En lo personal, en lo académico, en lo profesional… en ese conjunto de cosas que configuran un día a día, la mención es sin duda alguna a Ferran. Un buen día un profesor de la carrera, Miguel Martín, al que tanto debo también, me lo presentó. Ferran ha sido, es, y será, un referente para mi. Ha sido compañero, amigo, consejero y poco menos que un hermano. Con él he compartido experiencias de todo tipo a lo largo de casi tres décadas; he reído y llorado, me he emocionado y angustiado, me he esperanzado y me he desesperado, he estado de acuerdo y he discutido… pocas emociones creo que me faltan por compartir, pero muchas cosas aún por aprender y, este trabajo de Tesis forma parte de una de estas experiencias con él. Siempre ha tenido disponibilidad para ayudarme, aconsejarme y apoyarme, incluso en momentos en los que este tiempo que me ha destinado era muy valioso para él.

A la Universitat Autònoma de Barcelona, casa que desde estudiante nunca he dejado y que tantas cosas me ha dado que costaría enumerar. Pero sobretodo a las personas que a lo largo de los años he conocido en la Unitat de Bioestadística de la Facultat de Medicina y de las que de todas se podía aprender.

Mi crecimiento profesional, lo debo sin duda a muchas personas del Hospital Clínic, tantas que no sería capaz de nombrar sin olvidarme tantos nombres por escribir como nombres escritos. Como estadístico he intento aplicar conocimientos de mi área a la investigación clínica que he intentado plasmar en este trabajo, empatizando con el colectivo de facultativos y con los pacientes, el verdadero objetivo en que creo que debe centrarse la investigación. Desde que entré a formar parte en este concepto que se escucha de 'la familia Clínic' he intentado estar a la altura

de las expectativas que se esperan de alguien como yo. Tengo dudas de haberlo conseguido siempre, el reto me abrumó desde el primer día que entré, pero pocas dudas tengo de estar en uno de los mejores sitios para disfrutar de lo que hago. He intentado siempre embarcarme en todos los retos que podían estar a mi alcance: colaborar en proyectos con servicios poco representativos en investigación, una carrera de orientación, un concurso para incentivar la vacunación para la gripe estacional, colaborar con el CEIm… en el fondo el objetivo era intentar ser útil y aprender que todos sumamos y todos aportamos. En especial mi agradecimiento a toda la juventud que me ha permitido colaborar en sus proyectos y con los que he procurado que se lleven en la mochila algo de conocimiento de mi área. Que no la vean como una abstracción inabordable y que, al menos en la ilusión de sus proyectos, se sientan con ánimos y seguridad de explicar 'la parte de la estadística'. En el fondo, estoy convencido que he aprendido más de ellos y ellas de lo han sido conscientes.

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4 Abbreviations

95% CI: 95% confidence intervals

AECC: Spanish Association Against Cancer

AEEH: Spanish Association for the Study of the Liver

ALT: alanine transaminase

AST: aspartate aminotransferase

ATC: Anatomic Therapeutic Chemical Classification System

ATE: mean treatment effect

ATT: mean treatment effect for the treated

BCLC: Barcelona Clinic Liver Cancer

BMI: Body mass index (Kg/m2)

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration estimation for Glomerular Filtration Rate

DAA: direct-acting antivirals

DAP: Geographical code, , grouping healthcare units

eCAP: electronic health records in primary care of the ICS

ECDC: European Centre for Disease Prevention and Control (

EU: European Union

Events/100k*PY: new cancer diagnosis/100,000 patients-year of follow-up

GDPR: General Data Protection Regulation

GGT: gamma-glutamyl transferase

GPs: general practitioners

HBV: hepatitis B virus

HCC: Hepatocellular carcinoma

HCV: hepatitis C virus

HIV: human immunodeficiency virus

ICD: International Classification of Diseases

ICS: Catalan Institute of Healt

IDIAP: Institute for Primary Care Research

IFN: Interferon-based treatments

IgG-HCV: Inmunoglobuline G antiboby for hepatitis C virus

IPTW: inverse probability of treatment weighting

IQR: interquartile range

IVA: instrumental variable analysis

MBDR: Minimum Basic Data Registry

MEDEA: Mortalidad en áreas pequeñas españolas y desigualdades socioeconómicas y ambientarles Project

mRNA: messenger RNA

PADRIS: Public Data Analysis for Health Research and Innovation Programme

PCR: RNA Polymerase Chain Reaction

PS: Propensity Score

PSM: Propensity Score Matching

RPT: Patients and Treatments of CatSalut

RR: Rate ratio

RWE: Real world evidence

SEOM: Spanish Society of Medical Oncology

SIDIAP: System for the Development of Research in Primary Care

STD: standardized difference

SVR: sustained virologic response

WHO: World Health Organization

5 Summaries

5.1 Abstract (English)

Background & Aims: Chronic infection by hepatitis C virus (HCV) is a well-known cause of morbi-mortality, by causing liver cirrhosis and eventually hepatocellular carcinoma (HCC). First treatments aimed to eradicate HCV were interferon (IFN) based regimes, generally associated to ribavirin; these were poorly tolerated and thus were used only in very fit patients. Later, direct-acting antivirals (DAA) replaced IFN based regimes and provided a very high rate of HCV eradication with good tolerability, allowing a wide use in all types of patients. In routine care, after treatment patients are generally discharged and often lost to follow-up. Whether they may experience cancer later on is unknown, and some concerns on increased cancer risk after treatment despite virus eradication have been raised. An observational retrospective study was designed with the aim to compare the incidence of cancer between patients receiving antiviral treatment for HCV infection and matched controls.

Methods: We carried out a population-based study using real-world data sources of linked healthcare registries from the Catalan Health System (ICS), including patients treated for HCV infection between 2012 and 2016 with either interferon, usually combined with ribavirin, (IFN), IFN followed later on by DAA (IFN-DAA), or with DAA only (DAA), and their matched controls. Since treatments were not concurrent in time, but used at different times and in different types of patients, propensity score matching (PSM) of HCV patients with concurrent comparable controls was carried out for each group (IFN, IFN-DAA and DAA). Poisson regression models were used to determine the annual cancer incidence and the rate ratios (RR) between HCV-treated patients and controls. Hazard ratios (HR) from Cox proportional hazard models were estimated. To account for potential information and selection biases, a number of sensitivity and subgroup analyses were carried out.

Results: Estimated cancer incidences per 100,000 person-years (95% confidence intervals [CI]) were 596.1 (482.5-671.4) cases for IFN, 1255.3 (947.9-1662.2) cases for IFN-DAA, and 1552.0 (95% CI 1380.1-1745.3) for DAA. A modestly increased cancer risk as compared to matched controls was found for IFN- DAA (RR 1.77, 95% CI 1.27-2.46) and for DAA (RR 1.90, 95% CI 1.66-2.19), but not for IFN (RR 1.11, 95% CI 0.92-1.32). In DAA-treated patients, the cancer risk was increased mostly in the subgroup of patients with cirrhosis, and attributable to HCC.

Discussion: A slight increase in the incidence of cancer has been observed in patients treated for HCV infection shortly after completion of their treatments. The study was observational and used data already available in administrative and clinical databases, so that there is limited information available for thorough adjustments allowing to control for potential biases. Thus, we cannot confirm whether the observed increase is related or not to the pharmacological effect

of the antiviral agents, since treatments were not used simultaneously nor in the same types of patients, and results cannot be completely adjusted for indication biases, so that residual confounding may be still substantial. However, an increased cancer rate has yet been observed in patients once cured of their HCV infection, thus suggesting that after treatment completion they should not be discharged and lost to follow-up, but should undergo systematic follow-up screening for oncological diseases instead.

Conclusions: In general, treated HCV patients showed a slight increase in overall cancer incidence than matched controls without HCV infection and the risk was notably higher for HCC. Whether this increased risk is related to HCV infection, pharmacological treatment or any unidentified confounder requires further research, but in all cases continued monitoring after DAA treatment for early detection of cancer seems advisable, especially in cirrhotic patients.

5.2 Resum (Català)

Antecedents i objectius: La infecció crònica pel virus de l'hepatitis C (VHC) provoca cirrosi hepàtica i carcinoma hepatocel·lular (CHC). Els primers tractaments destinats a eradicar el VHC empraven interferó, sovint amb ribavirina (IFN); eren mal tolerats i reservats a pacients en bones condicions físiques. Els antivirals d'acció directa (DAA) van substituir l'IFN aportant una taxa elevada d'eradicació del VHC i bona tolerabilitat, emprant-se en tot tipus de pacients. En la pràctica clínica, en acabar el tractament els pacients solen rebre l'alta, i sovint se'n perd el seguiment. Es desconeix si malgrat l'eradicació del virus poden patir càncer després del tractament, i s'han plantejat dubtes sobre un possible augment del risc de càncer. S'ha dissenyat un estudi observacional retrospectiu amb l'objectiu de comparar la incidència de càncer entre pacients que reben tractament antiviral per infecció per VHC, i controls aparellats.

Mètodes: S'ha fet un estudi poblacional utilitzant dades de registres sanitaris del Servei Català de la Salut (ICS), incloent pacients amb VHC tractats entre el 2012 i el 2016 amb IFN, IFN i després DAA (IFN-DAA), o només amb DAA (DAA), i subjectes de control aparellats. Es van emprar puntuacions de propensió per a la selecció i emparellament dels controls (PSM) de cada grup de tractament (IFN, IFN-DAA i DAA) doncs els tractaments no eren contemporanis ni indicats al mateix tipus de pacients. Es van utilitzar models de regressió de Poisson per determinar la incidència anual del càncer i les raons de taxes (risc relatius, RR) entre pacients tractats per infecció de VHC i controls. Es van estimar les ràtios de risc (HR) amb models de risc proporcional de Cox. Per tenir en compte els possibles biaixos d'informació i selecció, es van realitzar diverses anàlisis de sensibilitat i subgrups.

Resultats: La incidència estimada de càncer per 100.000 persones-any (IC 95%) va ser de 596,1 (482,5-671,4) per IFN, 1255,3 (947,9-1662,2) per IFN-DAA, i 1552,0 (1380,1-1745,3) per DAA. Els RR (IC 95%) de càncer van ser discretament augmentats respecte els controls per a IFN-DAA (RR 1,77 (1,27-2,46)) i per a DAA (RR 1,90 (1,66-2,19)), però no per a IFN (RR 1,11, (0,92-1,32)). En pacients tractats amb DAA, el risc de càncer va augmentar sobretot en el subgrup de pacients amb cirrosi i atribuïble a CHC.

Discussió: S'ha observat un discret augment de la incidència de càncer en pacients tractats per infecció pel VHC després de finalitzar els seus tractaments. L'estudi va utilitzar les dades disponibles a les bases de dades de salut, pel que la informació disponible per a ajustos exhaustius de biaixos era limitada. Així, no es pot concloure si l'augment observat està relacionat o no amb l'efecte farmacològic dels antivirals, doncs els tractaments no es van utilitzar simultàniament ni en els mateixos tipus de pacients, no es pot ajustar completament per biaixos d'indicació, i la confusió residual pot ser substancial. No obstant, cal destacar l'augment de la taxa de càncer en pacients un cop eliminada la seva infecció pel VHC, de manera que no sembla aconsellable donar d'alta i perdre'n el seguiment en acabar el tractament antiviral, sinó que seria recomanable un seguiment sistemàtic per a la detecció precoç de càncers.

Conclusions: En general, els pacients tractats amb VHC van mostrar un lleuger augment de la incidència global del càncer respecte dels controls emparellats sense infecció per VHC, més evident per al CHC. Amb el disseny emprat no es pot concloure si aquest augment del risc està relacionat amb la infecció pel VHC, el tractament farmacològic o altres factors de confusió, però en qualsevol cas sembla aconsellable indicar un seguiment continuat dels pacients després del tractament amb DAA per a una detecció precoç del càncer, especialment en pacients cirròtics.

5.3 Resumen (Castellano)

Antecedentes y objetivos: La infección crónica por el virus de la hepatitis C (VHC) provoca cirrosis hepática y carcinoma hepatocelular (CHC). Los primeros tratamientos destinados a erradicar el VHC utilizaban interferón, a menudo con ribavirina (IFN); eran mal tolerados y reservados a pacientes en buenas condiciones físicas. Los antivirales de acción directa (DAA) sustituyeron al IFN aportando una tasa elevada de erradicación del VHC y buena tolerabilidad, empleándose en todo tipo de pacientes. En la práctica clínica, al terminar el tratamiento los pacientes suelen recibir el alta, y a menudo se pierde su seguimiento. Se desconoce si a pesar de la erradicación del virus pueden sufrir cáncer después del tratamiento, planteando dudas sobre un posible aumento del riesgo de cáncer. Se ha diseñado un estudio observacional retrospectivo con el objetivo de comparar la incidencia de cáncer entre pacientes que reciben tratamiento antiviral por infección por VHC, y controles emparejados.

Métodos: Se ha realizado un estudio poblacional utilizando datos de registros sanitarios del Servei Català de la Salut (ICS), incluyendo pacientes con VHC tratados entre 2012 y 2016 con IFN, IFN y después DAA (IFN-DAA), o sólo con DAA (DAA), y sujetos de control emparejados. Se emplearon puntuación de propensión para la selección y emparejamiento de los controles (PSM) de cada grupo de tratamiento (IFN, IFN-DAA y DAA) pues los tratamientos no eran contemporáneos ni indicados en el mismo tipo de pacientes. Se utilizaron modelos de regresión de Poisson para determinar la incidencia anual del cáncer y las razones de tasas (riesgos relativos, RR) entre pacientes tratados para la infección por VHC y controles. Se estimaron las razones de riesgo (HR) con modelos de riesgo proporcional de Cox. Para tener en cuenta los posibles sesgos de información y selección, se realizaron varios análisis de sensibilidad y subgrupos.

Resultados: La incidencia estimada de cáncer por 100.000 personas-año (IC 95%) fue de 596,1 (482,5-671,4) para IFN, 1255,3 (947,9-1662,2) para IFN- DAA, y 1552,0 (1380,1-1745,3) para DAA. Los riesgos relativos (IC 95%) de cáncer fueron discretamente mayores que en los controles para IFN-DAA (RR 1,77 (1,27-2,46)) y para DAA (RR 1,90 (1,66-2) ,19)), pero no para IFN (RR 1,11, (0,92-1,32)). En pacientes tratados con DAA, el riesgo de cáncer aumentó sobre todo en el subgrupo de pacientes con cirrosis, atribuible sobre todo a CHC.

Discusión: Se ha observado un discreto aumento de la incidencia de cáncer en pacientes tratados por infección por el VHC después de finalizar sus tratamientos. El estudio utilizó los datos disponibles en las bases de datos de salud, por lo que la información disponible para ajustes exhaustivos de sesgos era limitada. Así, no puede concluirse si el aumento observado está relacionado o no con el efecto farmacológico de los antivirales, pues los tratamientos no se utilizaron simultáneamente ni en los mismos tipos de pacientes, no se puede ajustar completamente por sesgos de indicación, y la confusión residual puede ser substancial. Sin embargo, cabe destacar el aumento de la tasa de cáncer en pacientes una vez eliminada su infección por el VHC, por lo que no parece aconsejable perder su seguimiento al finalizar el

tratamiento antiviral, sino que sería recomendable un seguimiento sistemático para la detección precoz de cánceres.

Conclusiones: En general, los pacientes tratados con VHC mostraron un ligero aumento de la incidencia global del cáncer respecto a los controles emparejados sin infección por VHC, más evidente para CHC. El diseño empleado no permite concluir si este riesgo está relacionado con la infección por el VHC, el tratamiento farmacológico u otros factores de confusión, pero en cualquier caso parece aconsejable indicar un seguimiento de los pacientes después del tratamiento con DAA para una detección precoz del cáncer, especialmente en pacientes cirróticos.

6 Table of Contents

6.1 Sections

1 TITLE PAGE 1

2 SIGNATURE PAGE: 3

3 ACKNOWLEDGEMENTS 7

4 ABBREVIATIONS 9

5 SUMMARIES 11

5.1 ABSTRACT (ENGLISH) 11

5.2 RESUM (CATALÀ) 13

5.3 RESUMEN (CASTELLANO) 15

6 TABLE OF CONTENTS 17

6.1 SECTIONS 17

6.2 TABLES AND FIGURES 21

7 INTRODUCTION 23

7.1 HEPATITIS C VIRUS INFECTION 23

7.1.1 Cause and impact 23

7.1.2 Description of virus 23

7.1.3 Infection and acute phase 23

7.1.4 Chronification and its effect on public health 24

7.1.5 Transmission mechanisms 25

7.1.6 Clinical characteristics of HCV infection 27

7.1.7 Diagnosis 30

7.2 TREATMENT OF HEPATITIS C VIRUS INFECTION 33

7.2.1 Therapeutic objective 33

7.2.2 Interferon and ribavirin-based treatments 34

7.2.3 Direct antiviral agents 35

7.2.3.1 NS3/4A protease inhibitors 38

7.2.3.2 NS5B polymerase inhibitors 38

7.2.3.3 NS5A polymerase inhibitors 39

7.2.4 Available treatments and current treatment recommendations 39

7.2.4.1 DAA treatments 39
7.2.4.2 Treatment guidelines 40
7.2.4.3 Availability of DAA in Spain 41
7.2.4.4 Use of DAA in clinical practice 45
7.3 HEPATOCELLULAR CARCINOMA (HCC) 46
7.4 CANCER RISK IN PATIENTS WITH HCV INFECTION 46
7.5 DESIGN AND ANALYSIS OF OBSERVATIONAL STUDIES 49
7.5.1 State of art 49
7.5.2 Definition, use and interpretation of propensity scores (PS) 54
7.5.3 PS-matching 55
7.5.4 Inverse probability of treatment weighting (IPTW) 56
7.6 PROJECT JUSTIFICATION 57
8 HYPOTHESIS 59
8.1 OBJECTIVES 59
8.1.1 Primary objectives 59
8.1.2 Secondary objectives 59
9 METHODOLOGY 61
9.1 DESIGN 61
9.2 DATA SOURCES 61
9.3 DATA FROM PADRIS PROGRAMME 61
9.4 DATA FROM SIDIAP 62
9.5 MERGER OF DATABASES. EXTRACTION AND EXPORTATION OF DATA TO DATASETS FOR STATISTICAL ANALYSES . 62
9.6 DEFINITION OF STUDY COHORTS 67
9.7 DEFINITION OF EVENT AND TIME OF FOLLOW-UP 69
9.8 DEFINITION OF COHORTS 69
9.9 STATISTICAL ANALYSIS 71
9.9.1 Main analysis 71
9.9.2 Sensitivity analyses 72
9.10 ETHICAL CONSIDERATIONS 74
10 RESULTS 75
10.1 PATIENT DISPOSITION AND ALLOCATION 75
10.2 CLINICAL AND SOCIODEMOGRAPHIC CHARACTERISTICS OF THE WHOLE COHORT 77
10.3 CANCER RISK ASSESSMENT IN TREATMENT-NAÏVE HCV PATIENTS WHO INITIATED THERAPY: GENERAL ANALYSES 89

10.3.1 Cancer incidence estimates ... 91
10.3.1.1 IFN-based treatment cohort ... 91
10.3.1.2 IFN+DAA cohort ... 91
10.3.1.3 DAA cohort ... 91
10.3.2 Role of HIV-HCV coinfection and cirrhosis ... 92
10.3.2.1 HIV and cirrhosis in controls ... 95
10.4 ESTIMATED INCIDENCE OF HEPATOCELLULAR CARCINOMA AND OTHER CANCER TYPES ... 98
10.5 ESTIMATED INCIDENCE OF HAEMATOLOGICAL MALIGNANCIES ... 100
10.6 EVALUATION OF INSTANT RISK OF CANCER DURING FOLLOW-UP ... 104
10.7 SENSITIVITY ANALYSIS EXCLUDING EXPOSURE TO ALCOHOL AND TOBACCO FROM PS-MATCHING ... 107
10.7.1 Clinical and sociodemographic characteristics ... 107
10.7.2 Cancer risk assessment ... 111
10.7.3 Incidence of cancer ... 113
10.7.4 Estimated incidence of hepatocellular carcinoma and other cancers ... 113
10.7.5 Role of HIV-HCV co-infection and cirrhosis ... 115
10.8 SENSITIVITY ANALYSIS EXCLUDING TOBACCO SMOKING FROM PS MATCHING ... 115
10.8.1 Clinical and sociodemographic characteristics ... 115
10.8.2 Cancer risk assessment ... 119
10.8.3 Incidence of cancer ... 121
10.8.4 Estimated incidence of hepatocellular carcinoma and other cancers ... 121
10.8.5 Role of HIV-HCV co-infection and cirrhosis ... 123
10.9 SENSITIVITY ANALYSIS EXCLUDING ALCOHOL FROM PS MATCHING ... 124
10.9.1 Clinical and sociodemographic characteristics ... 124
10.9.2 Cancer risk assessment ... 128
10.9.3 Incidence of cancer ... 130
10.9.4 Estimated incidence of hepatocellular carcinoma and other cancers ... 130
10.9.5 Role of HIV-HCV co-infection and cirrhosis ... 132
11 DISCUSSION ... 133
11.1 CONTEXT ... 133
11.2 METHODS AND STUDY DESIGN ... 134
11.3 COMPARABILITY OF CONTROL ARMS AND TREATED PATIENTS ... 136
11.4 ESTIMATION OF INCIDENCE AND MAIN RESULTS ... 136
11.5 SIMILARITY OF THE CONTROL GROUPS WITH THE GENERAL REFERENCE POPULATION ... 138
11.6 SENSITIVITY ANALYSES: CHANGES IN INCIDENCE OF MATCHED CONTROLS ... 140
11.7 INFLUENCE OF COINFECTION FOR HIV AND THE PRESENCE OF CIRRHOSIS. ... 142

11.8 Robustness and extension of main results 142
11.9 Study limitations 143

12 CONCLUSIONS 147

13 REFERENCES 149

14 ANNEXES 171

14.1 Annex 1: Ethics committee certificate 172
14.2 Annex 2: Agreement on good practices and security in the treatment of SIDIAP data 173
14.3 Annex 3: Manuscript for submission 178
14.4 Annex 4: Study Protocol 207

6.2 Tables and figures

TABLE 1. SUBJECTS AT HIGHER RISK OF HCV INFECTION 26

FIGURE 1. CLINICAL COURSE OF HCV INFECTION 29

FIGURE 2. DIAGNOSTIC STEPS IN SUSPECTED CASES OF HCV 31

TABLE 2. DIRECT-ACTING ANTIVIRAL AGENTS 37

FIGURE 3. CHRONOLOGY OF THE MARKETING OF DAA IN SPAIN 42

TABLE 3. DAAS AVAILABLE IN SPAIN 44

TABLE 4. SUMMARY CHARACTERISTICS OF THE MAIN METHODS* FOR HANDLING CONFOUNDING IN OBSERVATIONAL STUDIES$ 51

TABLE 5. ATC CODES USED AS TREATMENT FOR HCV 63

TABLE 6. CODES USED TO DETECT INCIDENT CASES 65

FIGURE 4. FLOW CHART OF CONTROL SUBJECTS 76

TABLE 7. MAIN CHARACTERISTICS OF HCV PATIENTS AND COMPARISON WITH CONTROLS 78

TABLE 8. SUPPLEMENTARY CLINICAL INFORMATION. 83

TABLE 9. CANCER INCIDENCE AND RATE RATIOS FOR THE THREE COHORTS OF HCV THERAPIES AND MATCHED CONTROLS 90

TABLE 10. CANCER INCIDENCE AND RATE RATIOS IN TREATED PATIENTS* FROM THE THREE COHORTS OF HCV THERAPIES FOR KEY COMORBIDITY FACTORS: HIV-COINFECTION AND DIAGNOSIS OF CIRRHOSIS 93

TABLE 11. PATIENTS IN CONTROL GROUPS WITH HIV OR CIRRHOSIS AND A DIAGNOSIS OF CANCER 96

FIGURE 5. DEGREE OF FIBROSIS BY TREATMENT GROUP AND YEAR. 97

TABLE 12. INCIDENCE AND RATE RATIOS OF HCC AND OTHER CANCER TYPES IN THE THREE COHORTS OF HCV THERAPIES AND MATCHED CONTROLS 99

TABLE 13. INCIDENCE AND RATE RATIOS OF HAEMATOLOGICAL MALIGNANCIES IN THE THREE COHORTS OF HCV THERAPIES AND MATCHED CONTROLS 101

TABLE 14. PATIENTS IN CONTROL GROUPS WITH HIV OR CIRRHOSIS AND A DIAGNOSIS OF CANCER 103

FIGURES 6A, 6B, 6C. KAPLAN-MEIER PLOTS AND RISK ESTIMATES OF CANCER FROM THE COX MODEL FOR THE THREE COHORTS OF HCV THERAPIES AND MATCHED CONTROLS 105

TABLE15. SENSITIVITY ANALYSIS EXCLUDING TOBACCO AND ALCOHOL: MAIN CHARACTERISTICS OF GROUPS 108

TABLE 16. SENSITIVITY ANALYSIS EXCLUDING ALCOHOL AND TOBACCO: CANCER INCIDENCE AND RATE RATIOS 112

TABLE 17. SENSITIVITY ANALYSIS EXCLUDING ALCOHOL AND TOBACCO: INCIDENCE AND RATE RATIOS OF HCC AND OTHER CANCER TYPES 114

TABLE 18. SENSITIVITY ANALYSIS EXCLUDING TOBACCO: MAIN CHARACTERISTICS OF GROUPS 116

TABLE 19. SENSITIVITY ANALYSIS EXCLUDING TOBACCO: CANCER INCIDENCE AND RATE RATIOS 120

TABLE 20. SENSITIVITY ANALYSIS EXCLUDING TOBACCO: INCIDENCE AND RATE RATIOS OF HCC AND OTHER CANCER TYPES. 122

TABLE 21. SENSITIVITY ANALYSIS EXCLUDING ALCOHOL: MAIN CHARACTERISTICS OF HCV PATIENTS AND COMPARISON WITH CONTROLS 125
TABLE 22. SENSITIVITY ANALYSIS EXCLUDING ALCOHOL: CANCER INCIDENCE AND RATE RATIOS 129

7 Introduction

7.1 Hepatitis C virus infection

7.1.1 Cause and impact

Hepatitis C virus infection(HCV) is a liver disease caused by an RNA virus of the *Flaviviridae* family, genus *Hepacivirus*, of which currently eight genotypes and more than 60 subtypes have been described (1,2). Genotype 1, the most frequent in Spain, represents 70% of all chronic hepatitis cases. The prevalence of the other most prevalent subtypes are genotype 3 (20%), genotype 4 (8%) and genotype 2 (3.1%). Genotypes 5 and 6 are infrequent in Europe and the United States of America (USA) but are more frequent in the south of Africa and south-east of Asia, respectively (1,2).

7.1.2 Description of virus

The HCV virus particle has an icosahedral capsid that contains the virus genome. The genome encodes a single polyprotein that, once translated, results in several proteins. Of these, core E1 and E2 are structural, and the rest (p7, NS2, NS3, NS4A, 51 NS4B, NS5A and NS5B) are not structural.

7.1.3 Infection and acute phase

When the virus infects a host cell, it adheres to a membrane receptor, is endocytosed, and the viral genome is released into the cell by fusion of the endosome. Once internalised, it behaves as messenger RNA (mRNA) that translates signals to synthetize the encoded polyprotein, using the host cell translational processes and enzymes. Then the polyprotein is processed and fragmented by several proteases, resulting in the different structural and non-structural viral proteins that assemble to form new viral particles in the sarcoplasmic reticulum and are exocytosed and released to infect further cells (3). The virus mainly infects, but also lymphocyte B and dendritic cells, and is not

directly cytopathic in immunocompetent hosts. Most clinical consequences of infection are derived from the host immune response (3)**.** Clinically, HCV infection is generally asymptomatic in the acute phase, but may evolve to chronicity to become a severe, life-long illness.

7.1.4 Chronification and its effect on public health

After infection, approximately 15-45% of infected people clear the virus spontaneously within six months without the need for treatment. However, the remaining 55-85% will develop chronic infection and, of these, 15-30% will develop liver cirrhosis within 20 years. Up to 25% of patients with cirrhosis will develop decompensated liver disease or hepatocellular carcinoma (4,5). Current estimates suggest that HCV infection affects 71 million people worldwide, of which up to 14 million cases are in the European Region (6).

According to data from the European Centre for Disease Prevention and Control (ECDC), which show the evolution of cases between 2015 and 2019, the incidence is heterogeneous among the participating European countries (7). The incidence (new cases per 100,000 persons-year) was between 0.1 in Greece and 99.9 in Latvia in 2015, for example. The temporal evolution between these years is also heterogeneous, and the causes of increased incidence in some countries explain the consequences of greater comprehensiveness in the detection of HCV for treatment, especially in the population groups at higher risk (7).

The World Health Organization (WHO) estimated that HCV caused up to 290 000 deaths in 2019, mostly due to cirrhosis and hepatocellular carcinoma. The WHO global hepatitis elimination strategy aims to reduce 90% of new HCV cases, 65% of deaths and treat at least 80% of patients by 2030 (8,9).

In Catalonia, the incidence of HCV in 2016 was estimated at 1.4 cases per 100,000 persons. In 2014 (year of implantation of DAA treatments), it was estimated that the

mortality attributable to HCV, excluding cases of alcohol abuse or malignant tumour, was 19.8 deaths per 100,000 persons (10).

7.1.5 Transmission mechanisms

The hepatitis C virus is transmitted mainly by percutaneous or mucosal exposure to blood and blood products infected with the virus. In the past, before the availability of virus detection, transmission occurred linked to the use of blood products and transplants from infected donor organs, and to inadequate sterilization of medical equipment, especially syringes and needles, in healthcare settings. Currently, most transmission is linked to the shared use of needles and other injection materials in intravenous drug users, biological accidents handling needles used in infected patients, tattooing and piercing in settings non-compliant with hygiene standards, sexual contacts and, to a lesser extent, to inadvertent family percutaneous contacts and vertical transmission from mother to offspring. Human immunodeficiency virus (HIV) and HCV share routes of transmission, and subjects coinfected by both viruses substantially increase the risk of HCV transmission to others (4) (Table 1).

Data currently comparable with the ECDC (11), indicate similar groups at higher risk for the presence of antibodies against HCV, which are higher in intravenous drug users, with an estimate of 66.6%. Other high-risk groups could be considered, such as the prison population, in which it was estimated in 2018 that the prevalence of HCV was 10.6% in Spain (12).

Table 1. Subjects at higher risk of HCV infection

Factors associated with an increased risk of HCV infection
Intravenous drug users
Receptors of infected blood products in health centres whose infection control practices are inappropriate
Patients undergoing procedures or invasive interventions in health centres with non-compliance with standard infection control precautions
Haemodialysis patients
Children born to mothers infected with HCV/coinfected with HCV and HIV
People with HIV infection.
People whose sexual partners are infected with HCV/coinfected with HCV and HIV
Men who have sex with men
People who share material when consuming drugs for intranasal administration
People who have had tattoos, piercings or procedures that use sharp instruments (acupuncture, mesotherapy) without adequate health controls
Healthcare workers exposed to procedures that pose a biological risk

Adapted from World Health Organization 2016 (4).

The incubation period after infectious contact varies between 2 weeks and 6 months, so the definition of acute infection is *the presence of HCV within six months after exposure to and subsequent infection with HCV* (13).

7.1.6 Clinical characteristics of HCV infection

Acute HCV infection is characterized by increased transaminases between weeks 6 and 8 after exposure, and only 20-30% of all acute infections are associated with noticeable clinical symptoms. Symptoms are generally nonspecific and mild, and may include fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, choluria, acholia, joint pain, and jaundice (14).

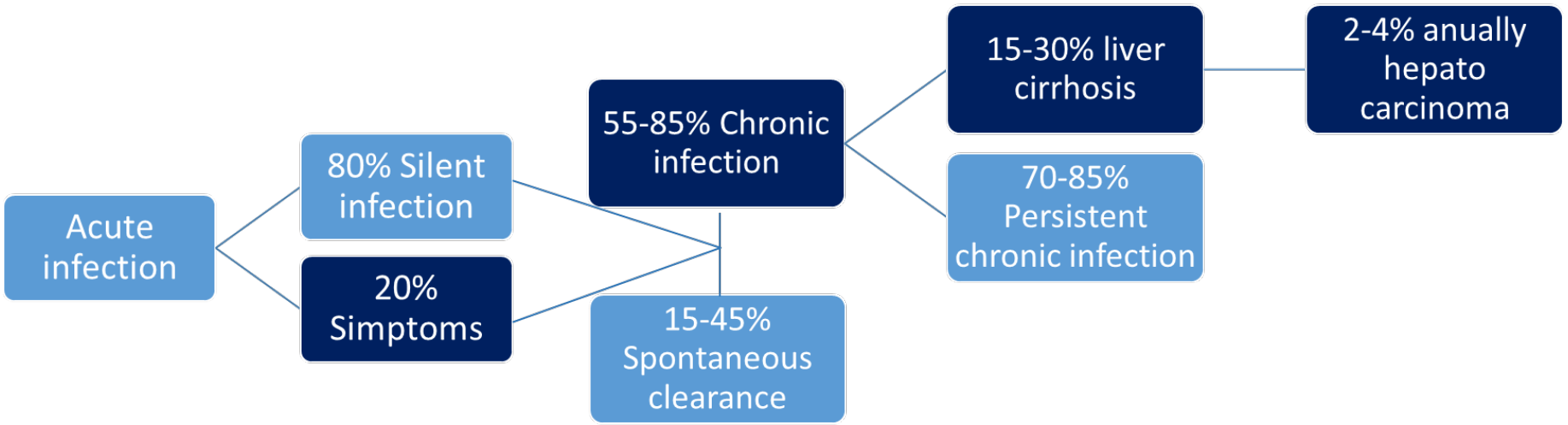
While HCV primarily affects the liver, extrahepatic manifestations can occur in up to 74% of patients. The most relevant extrahepatic involvement is mixed cryoglobulinemia, which is a small vessel vasculitis that is caused, in 80% of cases, by HCV infection, and that predominantly affects the skin, joints, peripheral nerves, and kidneys. Clinically, the symptoms may include mild purpura or arthralgia, and may also lead to glomerulonephritis or generalized vasculitis with a severe clinical expression (15,16). The incidence of B-cell non-Hodgkin's lymphoma has also been consistently described as increased in HCV infected patients (17), and a strong association between the two conditions has been observed in the Mediterranean countries, Japan and Brazil, as opposed to a weaker relationship observed in Northern Europe, United States and Canada(14). Other extrahepatic conditions may include autoimmune disorders, chronic kidney failure secondary to the onset of glomerulonephritis, cardiovascular diseases, thyroiditis and type 2 diabetes mellitus(14).

Without treatment, acute hepatitis may evolve to chronic infection in up to 85% of cases. Chronic infection is defined as the detection of anti-HCV immunoglobulins in the blood with persistence during 6 or more months of detectable HCV-RNA (14,17). During chronic hepatitis, transaminases may appear elevated in up to 70% of cases. Chronic

hepatitis may lead to cirrhosis in up to 30% of cases. Cirrhosis, over time, progresses to complications and decompensation with high morbidity and may lead to liver failure. Up to 4% of patients with cirrhosis will develop hepatocarcinoma, a severe form of cancer with a poor prognosis (Figure 1) (13).

Several risk factors may increase the risk of progression of hepatitis C infection to chronic hepatitis. These include the use of intravenous drugs, HIV coinfection, liver steatosis, alcohol abuse, advanced age, and genetic factors. Some are modifiable and permit the design of intervention strategies to prevent liver complications (18).

Figure 1. Clinical course of HCV infection



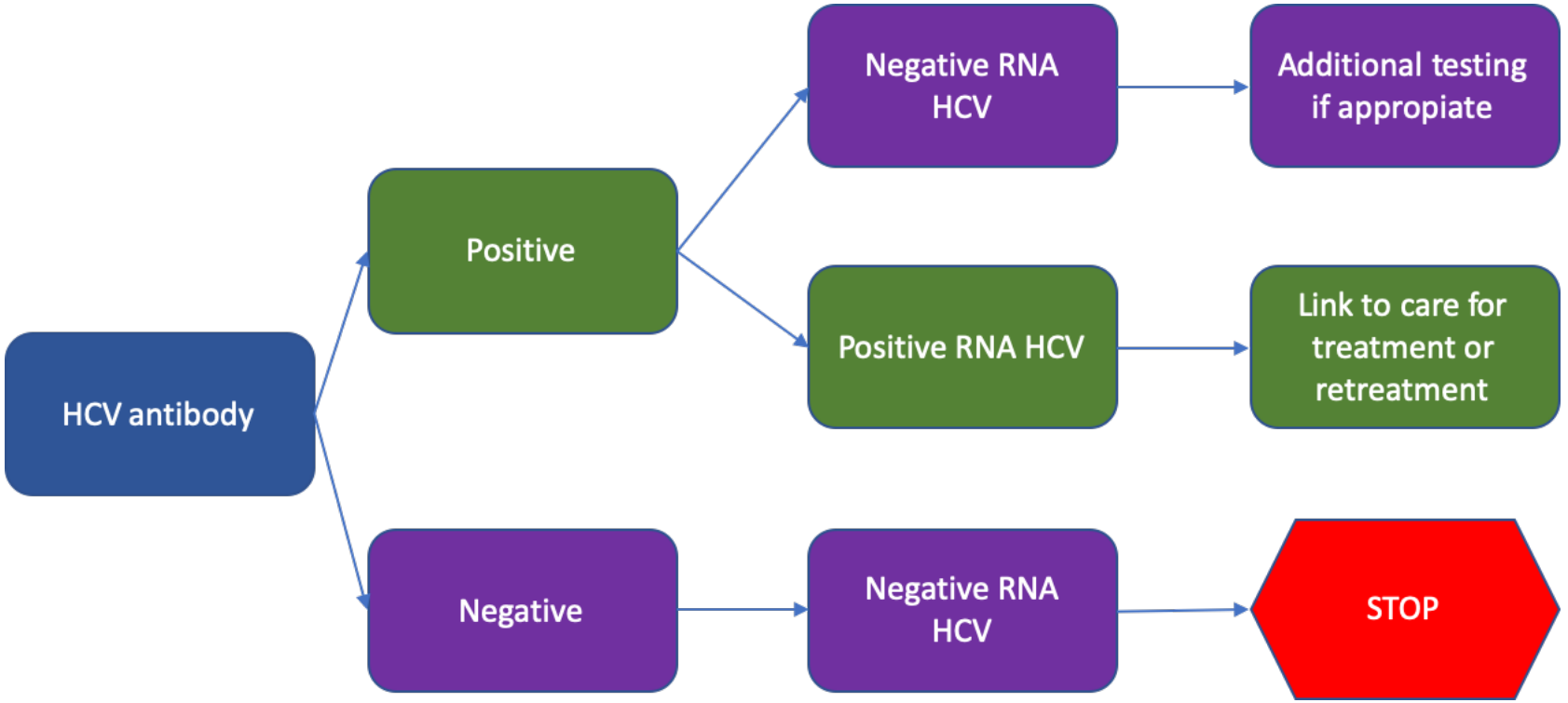
Adapted from "Secretaría General de Sanidad y Consumo. Ministerio de Sanidad y Asuntos Sociales 2015" (13).

7.1.7 Diagnosis

The diagnosis of HCV is not easy. The fact that most patients do not develop symptoms of primary HCV infection makes early diagnosis difficult, so often the first diagnosis is made when severe liver damage has already occurred. Screening of people at high risk of infection may help achieve early detection and treatment, as well as breaking transmission chains. Because of this, screening programs targeting high risk populations are often appropriate, and most policies consider focused screening and treatment as one of the key policies to advance HCV eradication objectives(4).

When HCV infection is suspected, a complete medical history and physical examination is mandatory, as is measurement of serum transaminase levels. Microbiological diagnoses should be made sequentially, so that HCV antibody testing is done first(13). Positive HCV antibody results indicate either an acute or chronic infection, current or past, so that positive results require further testing. A first positive serological result requires confirmation by a different assay, such as immunoblotting with recombinant antigens. Then, HCV RNA Polymerase Chain Reaction (PCR) testing can confirm active infection (positive) or past infection (non-detectable RNA). Alternatively, the detection of core antigens of the virus is a less frequent but also valid technique to confirm the diagnosis of active infection. Serology against other hepatotropic viruses and HIV must also be done as appropriate (Figure 2) (19).

Figure 2. Diagnostic steps in suspected cases of HCV



Adapted from Ghany and Morgan 2020 (19).

HCV RNA testing is indicated in persons who have had exposure to HCV in the last 6 months, even in the case of negative serology results, since they may still seroconvert. Additionally, HCV antibodies may be repeated to detect late positives in people with a history of exposure in last 6 months. Viral RNA should also be tested in persons with negative serology and immunosuppression. Before starting any pharmacological treatment, a quantitative measurement of RNA should be determined to determine the baseline viral load, a critical parameter in monitoring treatment efficacy. There are a number of indications for RNA PCR testing, such as acute infection in the so called "window period" when antibodies are still undetectable, to diagnose vertical mother-to-child transmission, to confirm chronic active hepatitis, in patients with a compromised humoral response, and to monitor antiviral treatment outcomes(13).

Genotyping of the virus is required for prognostic purposes, and to decide the choice of pharmacological antiviral treatment. Most methods detect the 6 main genotypes (1a, 1b, 2, 3, 4, 5 and 6), although not all can identify the virus subtype. Determination of certain polymorphisms of interleukin IL28B allow the prediction of the disease prognosis, as well as the efficacy of certain treatments, such as those based on pegylated interferon and ribavirin in the past. In addition, patients infected by certain genotypes may have a better chance of a treatment response than others, with a higher chance of spontaneous viral clearance and a lower chance of chronical infection(13).

After a person has been diagnosed with chronic HCV infection, the degree of liver damage (fibrosis and cirrhosis) should be determined. Liver biopsy permits semi-quantitative measurement of the degree and structure of collagen in the liver, and thus enables classification of the degree of liver fibrosis and damage. The METAVIR scoring system describes five 5 stages from 0 (no liver fibrosis) to 4 (cirrhosis) (20). The degree of liver damage is used to guide treatment decisions and management of the disease (8). Since biopsy is not always feasible, non-invasive tools such as hepatic transition elastography permit indirect measurement of the degree of liver fibrosis through liver

stiffness; patients can be then classified accordingly into high or low probability of advanced liver fibrosis, or cirrhosis (13).

7.2 Treatment of hepatitis C virus infection

7.2.1 Therapeutic objective

The goal of HCV therapy is to cure the infection in order to prevent the complications of chronic liver disease (necroinflammation, fibrosis, cirrhosis, decompensation of cirrhosis and hepatocarcinoma) and extra-hepatic diseases, some with severe forms leading to death. The goal is also to prevent onward transmission of HCV and to improve the quality of life and remove stigma. The antiviral treatment of HCV is aimed at eradicating chronic infection and achieve viral clearance, stopping sustained injury to the liver. The specific therapeutic objective is the so called “sustained viral response (SVR)” at 12 weeks (SVR12) or 24 weeks (SVR24) after the end of treatment. SVR is defined as the absence of detectable HCV RNA in serum or plasma by an assay with a lower limit of detection of ≤15 IU/ml. If RNA assays are not available, HCV core antigen in serum or plasma, 24 weeks after the end of treatment, is an alternative endpoint of effectivity in patients with detectable HCV core antigen prior to therapy. Currently, late relapse if SVR is achieved occurs in less than 0.2% of cases beyond 6 months of follow-up (21).

Transaminases normalise and necroinflammation and fibrosis regress in biopsies of patients achieving SVR; the prognosis improves even in patients with pre-treatment cirrhosis, with reduced liver complications, a lower incidence of hepatocarcinoma and improved survival. However, surveillance for HCC must be continued in patients with advanced fibrosis and cirrhosis, because an SVR will reduce, but not abolish, the risk of HCC (21,22)

7.2.2 Interferon and ribavirin-based treatments

Until 2014, the antiviral treatment of patients with HCV infection pivoted on two drugs, interferon and ribavirin.

Interferons (IFN) are a family of endogenous proteins that are naturally produced by cell of the immune system, including fibroblasts, epithelial cells, dendritic cells, and hepatocytes, amongst others. IFN-alpha has nonspecific antiviral, antiproliferative and immunomodulatory activity through the activation of specific genes via Janus kinase/signal transducers and activators of transcription (Jak/STAT), influencing cell growth and division, as well as modulating some immune system activities. Recombinant pegylated IFN-alpha consists of modified proteins that can be produced by biotechnology and typically have a longer half-life, allowing more convenient dosage/posology schedules, and they have longer effects (23).

Ribavirin is an analogue of guanosine that requires intracellular metabolization for its activation. It has been reported to directly inhibit HCV replication by inhibition of RNA polymerase, to inhibit the host inosine monophosphate dehydrogenase enzyme and thus limit the availability of guanosine for viral RNA synthesis, and to induce catastrophic mutagenesis of the virus, amongst other effects. The actual mechanism of action that is key to the antiviral effect is, however, unclear (24).

Ribavirin alone has not demonstrated significant efficacy in achieving SVR in HCV infection, in terms of mortality or the quality of life. When given together, ribavirin and IFN-alpha have a synergistic effect. Treatment schedules depend on the virus genotype, so that for genotype 2 and 3 patients, combination therapy duration is 24-weeks, while genotypes 1 and 4 require 48 weeks. Systematic reviews of the effectiveness of IFN or pegylated IFN combined with ribavirin compared with placebo concluded that treatment achieves a significant benefit in SVR, although the effects on hepatocarcinoma incidence, liver-related morbidity and all-cause mortality are inconsistent or statistically non-significant (4). When completed, treatments have an expected rate of SVR between 30 to 80%, depending on the viral genotype (24).

However, treatments based on IFN and ribavirin have serious safety issues and poor tolerability, which impair treatment adherence and compromise treatment completion and effectiveness. Interferon induces adverse effects in up to 95% of treated patients, including fever, fatigue and other constitutional symptoms, depression, anaemia and neutropenia, thyroid and dermatological reactions, amongst others. Ribavirin adverse events include haemolytic anaemia in roughly 30% of treated patients, which may limit the dose and may even require treatment interruption, and also nausea, pulmonary and dermatological effects. In addition, ribavirin is teratogenic (25). Because of these limitations, at the beginning of the 2010's there remained a huge need for better tolerated and more effective treatments.

7.2.3 Direct antiviral agents

Direct antiviral agents (DAA) are a group of antiviral drugs that target specific HCV enzymes.

The first generation of DAA included Boceprevir and Telaprevir, two protease inhibitors active mainly against the type 1 genotype. The new drugs were authorised in triple combination with interferon and ribavirin for 24 to 48 weeks. Pivotal trials showed SVR rates of about 75% in patients receiving their first course of antiviral treatment. Rates were lower in patients who had relapsed after previous interferon/ribavirin treatment and/or had cirrhosis. In addition, tolerability was not optimal, adding anaemia, dysgeusia and dermatological adverse reactions to the already poor safety profile of interferon/ribavirin (26).

The two drugs have rapidly been displaced by the second generation of DAAs, which include three classes of DAA, according to their mechanism of action: inhibition of polymerase NS5B (Sofosbuvir, Dasabuvir), inhibition of protease NS3/4A (Simiprevir, Paritaprevir, Grazoprevir, Voxilaprevir, Glecaprevir) or inhibition of polymerase NS5A (Ledipasvir, Ombitasvir, Daclatasvir, Elbasvir, Velpatasvir, Velpatasvir). The drug classes

differ not only in their mechanism of action, but also in on their antiviral potency, ability to act against different genotypes, and whether they are associated with induction of resistance by mutation (so called genetic barrier). Within a class, second generation drugs may provide wider genotype activity and greater antiviral potency/genetic barrier (Table 2) (27–29).

Table 2. Direct-acting antiviral agents

Class	Mechanism of action	Active principles	Activity and potency	Genetic barrier
NS3/4A protease inhibitors "previrs"	Translation and polyprotein processing	Boceprevir Telaprevir	Genotype 1, Low potency	Low
		Simiprevir Paritaprevir Grazoprevir	Genotypes 1, 4. High potency	Low Intermediate High
		Voxilaprevir Glecaprevir	Pangenotype, intermediate potency	High
NS5B polymerase inhibitors "buvirs"	Interference with replication	nucleotide analogue: Sofosbuvir	Pangenotype, high potency	Very high
		non-nucleotide analogue: Dasabuvir	Genotype 1, intermediate potency	Low
NS5A polymerase inhibitors "asvirs"	Mechanism unclear	Ledipasvir Ombitasvir Daclatasvir Elbasvir	Genotypes 1,4,6 +/- 2,3 High potency	Low Intermediate
		Velpatasvir	Genotypes 1 to 6 High potency	High

7.2.3.1 NS3/4A protease inhibitors

NS3/4A protease inhibitors disrupt the activity of the enzyme NS3/4A serine protease of HCV, which is necessary for post-translational processing and replication of HCV, by either blocking the NS3 catalytic site or the NS3/NS4A interaction. NS3/4A cleaves the viral polyprotein at four sites, releasing proteins that are necessary for viral maturation and infectivity. In addition, the NS3/4A protease also impairs viral elimination by host cells by cleaving immune signalling, such as TRIF-mediated Toll-like receptors and the Cardif-mediated retinoic acid–inducible gene 1 (RIG-1) and impairing the induction of interferons. Amongst this group, Boceprevir and Telaprevir were the first DAA to reach hospitals and were used in triple combination with peginterferon and ribavirin. They had a poor safety profile with potentially-serious dermatological and haematological reactions, and lower activity than upcoming improved DAAs, so that the marketing authorization holders voluntarily withdrew them from the market once better compounds became available. Other drugs are Glecaprevir and Voxilaprevir, which are pangenotypic inhibitors of NS3/4A, while Simiprevir, Paritaprevir and Grazoprevir do not offer satisfactory activity against genotypes 2,3, 5 and 6 (3,30).

7.2.3.2 NS5B polymerase inhibitors

HCV NS5B RNA-dependent RNA polymerase inhibitors may be nucleotide or non-nucleotide analogues.

Sofosbuvir is a prodrug nucleotide analogue that requires biotransformation to the active uridine analogue triphosphate form, which is incorporated into HCV RNA by NS5B polymerase, acting as a chain terminator. Sofosbuvir has been shown to be effective against different viral genotypes (1b, 2a, 3a and 4a). Because Sofosbuvir does not interfere with cytochrome metabolism, it has few metabolic interactions, although it is transported by P glycoprotein (PGP) and mainly excreted renally, so it may interact with competing drugs for excretion. The adverse effects of Sofosbuvir include interactions

with cardiovascular drugs at the PGP level, as well as to increased creatine kinase and lipase levels, as well as severe dermatological adverse reactions.

Dasabuvir is a non-nucleotide analogue active only against genotype 1, with intermediate potency and a low genetic barrier (3).

7.2.3.3 NS5A polymerase inhibitors

The mechanism of action of NS5A polymerase inhibitors is through interference with the NS5A protein, thus blocking the formation of a protein complex required to initiate viral replication – this interferes with virion assembly.

Daclatasvir, Ledipasvir, Elbasvir and Ombitpasvir have activity mainly against some viral genotypes (see summary table), while Pibrentasvir shows activity against the six major HCV genotypes and Velpatasvir has been reported to be a pangenotypic inhibitor; compared with the other agents, Velpatasvir has been reported to have a higher resistance barrier (31,32).

7.2.4 Available treatments and current treatment recommendations

7.2.4.1 DAA treatments

The availability of new DAA, which inhibit viral proteins and cellular processes that are essential for viral replication, has displaced all other alternatives to become the gold standard in treatment for HCV. All DAA have been studied with and without ribavirin, and with different treatment durations, and have demonstrated efficacy in schedules as short as 8 weeks of treatment, with the virus becoming undetectable roughly by week 4 and SVR rates consistently above 90%. Thus, currently, the treatment of HCV infection schedules are interferon-free and combine several DAA (21,33,34).

7.2.4.2 Treatment guidelines

Current European recommendations recommend that every patient with known HCV infection should be treated to eradicate the virus with DAA, as long as there are no specific contraindications. Pre-treatment testing may be limited to confirmation of active infection and checking of potential drug-drug interactions and the presence or absence of cirrhosis.

Treatment should be started as soon as possible in patients with advanced fibrosis (METAVIR score F2 or F3) or cirrhosis (METAVIR score F4), regardless of whether they have or not decompensated cirrhosis.

Immediate treatment is also recommended in patients with

- significant extrahepatic disease, such as symptomatic vasculitis in mixed cryoglobulinemia
- nephropathy
- non-Hodgkin B cell lymphoma
- patients with HCV recurrence after liver transplantation or at high risk of rapid evolution of liver disease due to concurrent morbidity.

Special attention should be paid to potential drug-drug interactions in patients receiving multiple medications, since many DAA may be either precipitators or victims of interactions by the induction or inhibition of metabolism or competing excretion. From inclusion to DAA treatments, there are reference tables with constant updating of potential interactions to support decisions on the best DAA selection considering interaction potentials. Currently, the only limitation to the use of these drugs is to the use of protease inhibitors in advanced cirrhosis and anticipated short life-expectancy (35).

Treatments should be free of IFN, including drugs from at least 2 (or 3) different mechanisms, one of which could be ribavirin, and when testing is difficult or not available, prioritising pangenotype DAA, which will be active regardless of the viral genotype. Simplified schedules are preferable to enhanced compliance, such as those with a duration limited to 8 weeks and using fixed dose combinations with fewer administrations per day (35). In Spain, the Spanish Association for the Study of the Liver (AEEH) recommends the use of combinations of either Elbasvir/Grazoprevir, Glecaprevir/Velpatasvir, Ledipasvir/Sofosbuvir or Sofosbuvir/Velpatasvir, depending on the genotype, degree of impairment of the liver, and previous treatment (36).

7.2.4.3 Availability of DAA in Spain

The availability of DAA has been progressive since 2011, as the products have completed their clinical development and marketing authorization applications. Rapid development of several compounds (as of September 2021, 15 active principles have been authorized) has been paralleled by a quick clinical uptake and changing therapeutic scenario, leading to the successful treatment and cure of thousands of infected persons (Figure 3).

Figure 3. Chronology of the marketing of DAA in Spain

Adapted from "Secretaría General de Sanidad. Ministerio de Sanidad 2020" (37). *DAA were marketed for use in combination, either as treatments to be used jointly or as fixed dose combinations.*

However, despite the fact of a rapid uptake, the availability of these drugs was perceived by citizens as an unnecessarily delayed process. This was because the first products reaching the market had huge price expectations: The budgetary impact of such prices represented a potential threaten to sustainability, considering the size of the population to be treated in countries with a relatively high prevalence of infection and universal health care coverage, like Spain. This led to difficulties in agreeing the price and reimbursement of the treatments and to several months' negotiation processes in most countries. In most places, negotiations occurred under the pressure of an intense communication campaign in the general media, and ended up with prices above the usual range of drugs providing a similar degree of benefits (38). The competition raised by the progressive availability of me-too drugs has normalised prices since then; prices now are 10-fold lower or less than those assigned to the first DAAs.

By September 2021, six treatments, including eight DAA active principles as monocomponents or as combinations of 2 or 3 drugs were available for use in Spain. Additionally, two more treatments including three DAA active principles have been authorised by the European Medicines Agency (EMA) but are awaiting price and reimbursement decisions. In the past, five treatments including four active principles as monotherapies were marketed and then withdrawn from the market. These are summarised in table 3.

Table 3. DAAs available in Spain

Brand name & description	Active principles	Marketing authorization holder	Status	Date	Marketed
Vosevi 400 mg/100 mg/100 mg film coated tablets	Sofosbuvir, Velpatasvir, Voxilaprevir	Gilead Sciences Ireland Uc	Authorized	13/08/2017	Yes
Maviret 100 mg/40 mg comprimidos recubiertos con película	Glecaprevir, Velpatasvir	Abbvie Deutschland Gmbh & Co. Kg	Authorized	03/08/2017	Yes
Epclusa 400 mg/100 mg comprimidos recubiertos con película	Sofosbuvir, Velpatasvir	Gilead Sciences Ireland Uc	Authorized	02/08/2016	Yes
Zepatier 50mg/100mg film coated tablets	Elbasvir, Grazoprevir monohydrate	Merck Sharp and Dohme B.V.	Authorized	01/08/2016	Yes
Harvoni 90 mg/400 mg film coated tablets	Ledipasvir, Sofosbuvir	Gilead Sciences Ireland Uc	Authorized	15/12/2014	Yes
Sovaldi 400mg film coated tablets	Sofosbuvir	Gilead Sciences Ireland Uc	Authorized	05/03/2014	Yes
Exviera 250 mg film coated tablets	Dasabuvir	Abbvie Deutschland Gmbh & Co. Kg	Authorized	03/02/2015	No
Viekirax 12,5 mg/ 75 mg/ 50 mg film coated tablets	Paritaprevir, Ombitasvir, ritonavir	Abbvie Deutschland Gmbh & Co. Kg	Authorized	03/02/2015	No
Daklinza 30mg film coated tablets	Daclatasvir	Bristol Myers Squibb Pharma Eeig	Revoked	26/08/2019	No
Daklinza 60mg film coated tablets	Daclatasvir	Bristol Myers Squibb Pharma Eeig	Revoked	26/08/2019	No
Olysio 150mg hard capsules	Simeprevir	Janssen-Cilag International N.V	Revoked	19/07/2018	No
Victrelis 200 mg hard capsules	Boceprevir	Merck Sharp and Dohme Ltd.	Revoked	29/10/2018	No
Incivo 375 mg film coated tablets	Telaprevir	Janssen-Cilag International N.V	Revoked	31/01/2017	No

Green: available for use; Blue: awaiting price and reimbursement decision; Red: previously available, revoked. Source: "Agencia Española de Medicamentos y Productos Sanitarios 2021" (39).

7.2.4.4 Use of DAA in clinical practice

There has been a wide uptake of DAAs since 2014, in the framework of the WHO global strategy for the eradication of HCV infection by 2030 (40), which has been widely accepted and deployed by countries. In Catalonia (41), the use of DAA is reported yearly by the Pharmacotherapeutic Harmonization Program. In 2018, 5,605 patients with HCV infection started 5,661 treatments with DAA. The mean age was 56 (SD: 13.1) years and 58.4% were male, with 42.1% of cases being due to genotype 1b and 24.7% type 1a. Most patients had mild fibrosis F0-F1 (64.1%), or F2 (15.4%). The Glecaprevir/Velpatasvir combination was the most frequently prescribed treatment (n=2,943; 52%), followed by Sofosbuvir/Velpatasvir (n=1,900; 32.6%) and Elbasvir/Grazoprevir (n=569; 10%), all with or without additional ribavirin. Half of the treatments used an 8-week schedule (51%) while most of the remaining treatments (48.5%) used a 12 week schedule. In treatments completed during 2018 (4,396), the reported SVR at 12 weeks was 96.4% (n=4,238), and slightly lower (about 94.5%) for genotypes 2 and 3; early discontinuations were 3.2% (n=140). The overall expense in DAAs for 2018 was € 38.3 million (41)**.**

The improvement in the effectiveness of the therapeutic options has led to the recommendation of HCV treatment in patients whose severity before not being treated with IFN, such as those with a history of cellular hepatocellular carcinoma (HCC) or awaiting liver transplantation(42). The substantial advances represented by DAAs has been accompanied by rapid introduction across the HCV clinical spectrum; first in patients with a high degree of severity and advanced fibrosis, and later in patients with less severity or in subpopulations poorly studied in clinical trials. The effectiveness and short-term safety of treatments in clinical practice has been shown to be similar to that described in clinical trials (43).

7.3 Hepatocellular carcinoma (HCC)

According to data from 2021, hepatocellular carcinoma (HCC) causes 700,000 deaths per year around the world. In the United States and Canada, at the beginning of the 19th century, it was the only cancer whose mortality increased, basically due to HCV (44). In Spain, according to data and estimates from the Spanish Society of Medical Oncology (SEOM)(45), the incidence has stabilized since 1993 and, in 2021, was 6,590 new cases (6,499 in 2020), less than 4.7% of worldwide cases according to GLOBOCAN 2020 data. The attributable mortality in Spain was 5,192 cases in 2017 and 5,555 in 2020, representing 4.5% and 4.9%, respectively, of cancer deaths, while globally it is 8.3% GLOBOCAN 2020. In Catalonia the cumulative incidence (new cases per 100,000 persons-year) were 24.89 and 25.07 for the years 2016 and 2020, respectively (46).

7.4 Cancer risk in patients with HCV infection

The development of DAA for the treatment of HCV infection is one of the most, if not the most, clinically relevant advancements in the field of hepatology. HCV eradication prevents the transition from chronic hepatitis to cirrhosis and, ultimately, to liver cancer. Consequently, the community benefit in terms of reducing liver related deaths is clear (47–50). When cirrhosis is already established at the time of treatment, the risk of liver cancer is not reduced during at least the first years of follow-up. This is related to the fact that oncogenic hits have already taken place and thus, malignant clones may emerge during the evolution of the patient (48). However, the progression of cirrhosis is stopped and the risk of decompensation is significantly reduced. As a consequence, liver related deaths in patients with cirrhosis are reduced (51).

Since the availability of DAAs, research has focused on liver related events, including improvement or deterioration in liver function and survival, but also in the development and recurrence of HCC after initial treatment (49,52–63).

These studies aimed to provide external validation of the seminal trials as well as to provide complementary information about the real-world clinical evolution. In this sense, in a previous study we reported that the risk of HCC development was associated with the imaging detection of non-characterised nodules prior to treatment initiation (64)**.** This relationship was validated in a study by Sangiovanni et al (65) in Italy and it is worth noting that in both investigations HCC emerged in a separate location from non-characterised lesions. Metanalysis of real-world data on the risk of HCC recurrence has been hampered by the heterogeneity of data, preventing definite conclusions on the risk (59,66).

Leaving aside the relevance of the impact of DAA therapy on liver disease progression and liver cancer, it is important to recall that extrahepatic cancer is a relevant comorbidity in patients with chronic HCV infection (67). It is known that B-cell non-Hodgkin lymphoma is associated with HCV infection and that it may regress after HCV eradication (68–71). In addition, the risk of non-hematologic neoplasms has been shown to be increased in this population due to HCV infection of non-hepatic cells and alteration of immune surveillance (67). Interestingly, Allaire et al(72) have shown that extrahepatic cancer is the most frequent cause of death in patients who have been cured from HCV.

However, the long-term safety or efficacy/safety balance in special populations, due to the short follow-up, just looking at SVR, and the limited and homogeneous population included, is not well quantified in clinical trials. Thus, some safety flags have been reported after marketing, including the risk of hepatic decompensation in patients with advanced fibrosis (73), reactivation of hepatitis B infection, and a possible increased risk of hepatocellular carcinoma recurrence after treatment with DAA (52,74).

Data on the occurrence of HCC in subjects without a history of tumour have also been reported, and preliminary data suggest that the pattern of tumour aggression in these cases is worse than expected (75)(76). These communications generated, in 2016, a

safety alert from the European Medicines Agency (EMA) (77), although these data are still pending confirmation by specific studies.

It has been proposed that the biological plausibility of the risk of HCC recurrence is based on the fact that the rapid disappearance of chronic HCV infection has a disruptive effect on common antiviral and antitumour immune surveillance, facilitating the emergence of pre-existing tumours. Therefore, it is theoretically possible to increase the risk of any cancer, and not just HCC.

This oncogenic hit leading to hepatic malignant transformation may have already taken place at the time of DAA therapy (61,78) and thus, liver cancer incidence may not be reduced at least during the first years after cure. However, since HCV eradication is associated with a disruption of immune surveillance, as exposed by the potential reactivation of Hepatitis B Virus (HBV) or herpes virus (79,80) such events may allow malignant clones at any site to emerge and accelerate their clinical recognition.

This suggests that there could be biological plausibility in the risk of recurrence of HCC, and this increase in risk is based on the fact that the rapid disappearance of chronic HCV infection has a disruptive effect on common antiviral and anti-tumour immune surveillance, facilitating the appearance of pre-existing tumours. Therefore, it is theoretically possible to increase the risk of any cancer, not just HCC.

7.5 Design and analysis of observational studies

7.5.1 State of art

Observational studies, as compared to experimental studies, collect information from routine clinical practice and do not interfere with the process of treatment. While experimental trials determine which treatment will be given to a subject by either randomization or other systematic assignment methods, in an observational context treatment assignment is decided by medical criteria, and based on an individual's baseline characteristics. Thus, when studying cohorts of patients that are defined by their exposure to a treatment, groups are generally not comparable for pre-treatment characteristics. Baseline differences impact the direct comparison of results between groups, giving biased estimates of effect. Thus, when assessing associations for causality between exposures and a dependent variable, methods are required to improve the comparability of the groups and partially control biases.

Methods include restriction, stratification, matching and adjusted analysis with multivariable methods. Instrumental variables analysis (IVA), propensity score-based methods can be applied to matching, weighting, stratification, or adjustment in order to improve bias control(81–83).

IVA has been proposed as a valid method for handling confounding, with the particularity of being able to cope with hidden biases compared with other methods used in observational studies (81,85–89). An instrumental variable requires three criteria:

a) correlation with the exposure of interest,
b) independence of confounders and
c) affects the outcome only through its relationship with the exposure of interest.

Table 4 shows summary characteristics, with advantages and limitations, of the main methods for handling confounding in observational studies.

Table 4. Summary characteristics of the main methods[*] for handling confounding in observational studies[$]

	Description of the approach	Main advantages	Main limitations
Traditional methods	Traditional methods in observational studies (matching, stratification, adjustment, restriction)	Experience of use Well-known and understood by non-statisticians	Presence of unknown or unmeasured factors may yield residual confounding and imbalance of key confounders
Matching	Individuals in both groups are matched with respect to observed key covariates	Produce balance in the covariates used No complex analyses are needed	Covariates should be categorical Limited number of confounders and strata at the same time
Stratification	Data are divided into strata according to levels of the confounder Then, stratum-specific estimates are calculated and aggregated to calculate an overall adjusted effect	Reliable estimates within strata and overall: individuals within each stratum have more similarity with each other, and therefore they can be compared directly, and the overall estimate is calculated	Covariates should be categorical Limited number of confounders and strata at the same time
Adjustment	Multivariable analyses may include several covariates to estimate the treatment effect	Permits inclusion of several types of covariates Experience of use Easier interpretation than other novel methods (PS & IVA)	Dependent on the accuracy of the model and the validity of the model assumptions May have statistical convergence issues, in particular for categorical outcomes when the number of covariates is high and the number of events is low
Restriction	Restriction to the group of interest in one of the categories of the confounder	Easy to conduct	Very limited extrapolation. Rarely used.

	Description of the approach	Main advantages	Main limitations
Propensity scores (PS)	PS is the probability that a patient will receive the treatment of interest is first estimated based on the covariates of interest	Considered better than traditional methods Can use more covariates than traditional methods	Presence of unknown or unmeasured factors may yield to residual confounding and imbalance of key confounders
Matching	Individuals in both groups are matched with respect to estimated PS	Conventional analyses are valid Easy to understand	Potential sample size losses due to lack of matching (even though less relevant than for the traditional matching)
IPTW	PS are used to calculate the statistical weight of each individual	Similar performance to PS matching and in addition may use all patients	The analysis is more complex and requires the use of weighting More difficult to be understood by non-statisticians
Stratification	Patients are classified according to a number of PS strata Then, stratum-specific estimates are calculated and aggregated to calculate an overall adjusted effect	Similar to traditional stratification but much more efficient since many more factors can be used (those used for PS estimates)	Less efficient than PS-matching and IPTW
Adjustment	The multivariate model is developed with the outcome as a dependent variable and with the treatment group and PS as predictive variables	Similar to traditional adjustment but much more efficient since many more factors can be used (those used for PS estimates)	Empirically, results are often very similar to traditional regression, but interpretation is less intuitive Discouraged because of several disadvantages including that it is less efficient that PS-matching and IPTW and PS-stratification

	Description of the approach	Main advantages	Main limitations
Instrumental variable analysis (IVA)	Uses an instrumental variable that matches 3 criteria: (1) is correlated with the exposure of interest, (2) is independent of the confounder, and (3) affects the outcome only through its relationship with the exposure of interest Group imbalances in the instrumental variable are corrected, and thus analyses are balanced for known and also hidden bias, someway mimicking randomization	Able to handle known and unknown confounders, as opposed to the other methods used in observational studies Adjustment for known and unknown confounders Mendelian randomization is a clear application, to be confirmed in the near future on practical grounds	Instrumental variables are difficult to be identified, basically because the validity of assumption (2) cannot be tested empirically Difficult to understand

*IVA instrumental variable analysis; IPTW: inverse probability of treatment weighting; PS: Propensity Score; PSM: Propensity Score Matching *: excluding the IVA approach. ; $: modified and adapted from Torres et al. 2017* (83).

PS was first introduced by Rosenbaum and Rubin in 1983 (90). PS is a probability which reflects the chance that a subject is exposed to the treatment of interest based on his/her pre-exposure characteristics, which are treated for analysis purposes as covariates (90,91). However, its usefulness will depend on the availability of parameters able to adjust by the potential confounders determining unbalance between groups due to indication bias; thus, covariates needed may ideally include information on the patient characteristics, medical practice and prescription uses of the physician, and clinical context. Because of that, it is often warned that the definition of the parameters to be included in a propensity score should include prospective planning based on clinical rationale, and not only on the statistical results of multivariable modelling.

7.5.2 Definition, use and interpretation of propensity scores (PS)

Some noteworthy revisions are available elsewhere (82,83,92–96). PS is normally estimated by means of logistic regression models, where the treatment variable is the outcome and the covariates are used to estimate PS (97). PS is then used with a balancing score (98) compensating for the distribution of covariates. This can be easily assessed by calculating the standardized difference (STD), by dividing the difference by the standard deviation, for each variable (92,93,99–101).

A STD of <|0.1| (i.e., 10%, and "| |" indicating absolute values with no + or - sign) numerically indicates a non-relevant difference (92,102), although for some authors values <|0.2| (20%) might also be considered as acceptable (101,103). If sufficient balance is not achieved, the model should be re-assessed by adding more variables, or transforming them, either as functions of the original variables, or by adding interaction terms until achieving a good balance (92).

There are two types of effects that may be estimated using PS techniques: the mean treatment effect (ATE) and the mean treatment effect for the treated (ATT) (92,104). ATE is interpreted as the mean effect of moving an entire population from control to treated, and ATT is the mean effect of the treatment in subjects finally treated with the treatment of interest, not as an effect of treatment in the whole population sample.

Matching using PS (PSM) allows the ATT to be estimated. This is because treated and untreated subjects are close due to the individual matching, but unmatched subjects are excluded from the analysis. Therefore, full PS-matching, including all subjects, treated and untreated (often unrealistic) or inverse probability of treatment weighting (IPTW) can estimate either the ATE or the ATT, depending on which weighting is used (93,104).

PS are used in several ways (91,92,94,97) as described in table 7. According to some authors, there is a hierarchy in terms of the effectiveness of balancing for these PS strategies: "matching or weighting above stratification above covariate adjustment"(83).

Both PSM and IPTW perform well in removing systematic differences and achieving balance, but in some cases PSM removed slightly more imbalance (105), but it excludes no-match cases, unlike using IPTW, which includes all cases.. The two most common forms of use for PS are explained in more detail below.

7.5.3 PS-matching

PS-matching (PSM) involves matching two (or more) groups of subjects having similar PS values. One group receives the treatment of interest and the other group(s) do not. Once the groups are individually matched, the difference in the PS is very small and, consequently, the STD should also be very small, less than |0.1| in ideal situations, but at least not larger than the |0.2| previously described.

There are several methods and criteria for matching: one-to-one (1:1) or one-to-several (1:k), where k is the number of extracted subjects in the untreated group. The subject is matched to one (or several) from the other group based on their similarity, and with or without a restriction on the maximum acceptable difference (92,94). There are two primary methods for this: nearest neighbour matching and nearest neighbour matching within a specified calliper distance, both without replacement. However these are not the only ones (106).

The latter has the restriction that the absolute difference in PS has to be less than the threshold (the calliper distance). There is no consensus on the general definition of a threshold of what constitutes a maximum acceptable distance (92) and different values have been used (92,99,107).

Therefore, the limitations are more a matter of data availability, i.e., reduction of unmatched subjects, mainly in the treatment of interest, and the optimization of the final STD of the comparison of key covariates between study groups. The analysis of the comparative effect between treatments is made by direct comparisons in this new matched sample.

The most attractive point of this method is that there is a plausible hypothesis that the analyses could be similar to a randomized process, with a restriction due to the distribution of the characteristics of patients included, which on average, are similar between the different treatment groups. Hence, depending on the ability to capture potential confounders, the final estimate could be considered an unbiased estimate of the comparative effect between treatment groups.

Randomized clinical trials do not have this problem, since randomization methodologically guarantees that the direct analysis between randomized groups is unbiased.

7.5.4 Inverse probability of treatment weighting (IPTW)

The inverse probability of treatment weighting (IPTW) was described by Rosenbaum (108). PS are used to calculate the statistical weight of each individual, and then each subject participates in the analysis with a different weighting than another based on the IPTW calculated. Then, this statistical weight creates a pseudo-population, so that groups are balanced across the covariates using individual weighting.

The application of these individual weights facilitates in the pseudo-population created, the distribution of potential confounding factors is independent of the exposure,

allowing an unbiased estimate of the relationship between treatment and outcome (109).

The weight of each subject is calculated using two variables: treatment status Z (0 if in the control arm and 1 if in the treatment arm) and PS (the propensity score of the subject). The weight (w) of the subject (w = Z/PS + (1-Z)/(1-PS)) is equal to the inverse probability of receiving the treatment the subject actually received (92,94), and it is recommended to stabilize the weighting by the treatment prevalence (92). Technically, this stabilization of the weighting is carried out by substituting the '1' of the numerator for the proportion of subjects who received one or the other treatment. As with PSM, when the balance of covariates is achieved, the estimate of the comparison between treatment groups will be carried out directly, but considering the IPTW as a statistical weighting in the procedure.

7.6 Project justification

Despite the benefits of treating HCV infection are unquestioned, the potential increase of any long term risks secondary to the use of antivirals is a relevant clinical question. There are suggestions that a rapid decline in HCV viral load observed with AAD treatments can change the immune environment in the liver. This rate of decrease in viral load may also alter systemic immune homeostasis, associated with an increase in the incidence and/or recurrence of cancer. The hypothesis is plausible, based on ceasing a sustained stimulus on common mechanisms between antiviral and antitumour immune surveillance. If confirmed, findings may have direct medical application, since specific interventions aimed to early detection and treatment could be useful to manage the risk and to treat tumours at an early and curable stage.

The lack of a systematic long-term follow-up of patients treated for HCV infection once cured makes it difficult to detect and establish suspicions of causality with subsequent tumours at the individual level. Besides, experimental designs are limited by the fact that clinical trials have consistently demonstrated that DAA is able to achieve eradication rates above 95% for most patients, reducing complications and death, thus

making it unacceptable to randomise subjects to receive any other treatment than DAA, which is currently considered an undisputed standard of care. Thus, it is neither feasible nor ethical to carry out randomized clinical trials to robustly assess this possible effect. Therefore, the most appropriate methodology for approaching the study of a potential association of increased cancer risk in patients treated with antivirals for HCV infection at this time is an observational analytical population study, which can be feasible using health data records.

Thus, the present study was designed, which aims to assess the cancer incidence in patients treated with DAAs, and to compare it with that of patients treated with prior antiviral therapies less able to induce quick HCV clearance, such as interferon-based regimens, in a specific time window that captures the moment of inclusion of DAA treatments in clinical practice. Also, the study aimed to compare it with the incidence in patients not infected with HCV, in order to obtain approaches to absolute risks that could guide further tailored medical interventions.

8 Hypothesis

The hypothesis of this work is that the treatment of HCV with DAAs may increase the cancer incidence as compared to the incidence observed in the period of treatment with interferon-based agents and with that of subjects without any HCV treatment.

8.1 Objectives

8.1.1 Primary objectives

To estimate the cumulative cancer incidence in patients treated with DAAs for HCV in Catalonia in clinical practice, and to compare it with a control population.

8.1.2 Secondary objectives

1. To estimate the cumulative cancer incidence in patients treated with interferon-based agents for HCV in Catalonia in the period of incorporation of DAA treatments in clinical practice, and its comparison with a control population.
2. To compare the cumulative cancer incidence in patients treated with DAA for HCV versus the cumulative incidence in patients treated with interferon-based antiviral regimens for HCV.
3. To estimate the cumulative cancer incidence, stratified by intra or extrahepatic, in patients treated with interferon-based agents and/or DAA for HCV in Catalonia, in the period of incorporation of DAAs treatments in clinical practice..
4. To estimate the cumulative cancer incidence, stratified by solid or haematologic, in patients treated with interferon-based agents and DAA for HCV in Catalonia in the period of incorporation of DAA treatments in clinical practice, and the effect of treatment on changes in this type of cancer.

5. To assess the temporal association between the diagnosis of cancer and the type of treatment of HCV infection.
6. To describe and analyse the recurrence pattern of HCC in the study period.

9 Methodology

9.1 Design

This was a retrospective population-based cohort study that included patients aged ≥18 years with clinical records in the population-based databases described below and without any initial record of a diagnosis of cancer or specific treatments for cancer. The analysis period included data from January 1st 2012 to December 31st 2016.

9.2 Data sources

Data were obtained from electronic clinical and administrative data sources from Catalonia, a region in Spain with >7.5 million persons that has public universal healthcare coverage. Electronic clinical records for primary care, administrative invoicing information of both hospital episodes and pharmacy dispensation, and a dedicated registry including drug-related clinical outcomes for hospital drugs for outpatient use were used, linked through a single patient ID code.

9.3 Data from PADRIS Programme

We used data provided by the Public Data Analysis for Health Research and Innovation Program (PADRIS)(110). PADRIS allows access to information from different clinical sources and pharmacy billing registry from hospitals linked at the patient level with the accomplishment of ethical principles. The Program depends on the Catalan Department of Health and may provide demographic information for all insured patients, diagnostic data for each episode of hospitalisation and pharmacy invoicing data for outpatient medications, both dispensed at community pharmacies and by hospital pharmacies. Also, data on clinical indication details and outcomes was provided for patients using treatments for HCV within the Registry of Patients and Treatments (RTP) of CatSalut, a

therapeutic registry created for longitudinal follow-up and assessment of clinical outcomes of hospital treatments for outpatients, including HCV.

9.4 Data From SIDIAP

We also obtained data from the Information System for the Development of Research in Primary Care (SIDIAP)(111) database, which contains curated data from longitudinal medical records of primary care practices managed by the Catalan Institute of Health (ICS) that use eCAP (electronic health records in primary care) since 2006, covering about 80% of the 7.5 million persons in Catalonia. The SIDIAP registry includes sociodemographic characteristics, health conditions registered as International Classification of Disease (ICD) version 10 codes, clinical parameters, laboratory data, and outpatient prescriptions. The corresponding pharmacy invoice data are available since 2005 and include information on all pharmaceutical products dispensed by community pharmacies for ICS prescriptions according to the Anatomic Therapeutic Chemical Classification System (ATC) codes. Lastly, the Minimum Basic Data Registry (MBDR) database includes also patient diagnoses at different healthcare levels, registered as ICD version 9 codes.

9.5 Merger of databases. Extraction and exportation of data to datasets for statistical analyses

As described, the main dataset of exposed patients was generated by selecting treatments under the diagnosis of HCV within the Registry of Patients and Treatments of CatSalut (RPT), and additionally completed with patients receiving specific treatments from ATC codes described in table 5. First date of antiviral treatment was defined as the index date.

Table 5. ATC codes used as treatment for HCV

(HIS) ATC	ATC
J05AB04	Ribavirin
J05AE11	Telaprevir
J05AE12	Boceprevir
J05AE14	Simeprevir
J05AP56	Sofosbuvir, Velpatasvir, Voxilaprevir
J05AX00	Glecaprevir, Pibrentasvir
J05AX14	Daclatasvir
J05AX15	Sofosbuvir
J05AX16	Dasabuvir
J05AX65	Sofosbuvir, Ledipasvir
J05AX67	Ombitasvir, Paritaprevir, ritonavir
J05AX68	Elbasvir, Grazoprevir
J05AX69	Sofosbuvir, Velpatasvir
L03AB10	Peginterferon alfa-2b
L03AB11	Peginterferon alfa-2a

To identify incident cancer cases, SIDIAP, MBDR and RPT were used as the main data source of information. The results were complemented using the CatSalut registry of oncological treatments and hospital pharmacy billing. Index dates were used for analysis of follow-up until the first agreed phenotype of malignancy appeared, regardless of whether they represent a diagnosis (ICD-9 or ICD-10 codes) or oncological treatment (ATC code). The date of event was the first data the qualifying phenotype of malignancy appeared. The codes used to identify incident cancer are shown in table 6.

Table 6. Codes used to detect incident cases

Pharmacy billing registry from hospitals or community pharmacies for Catalan Health System prescriptions from the PADRIS and SIDIAP registries for antineoplastic agents:
ATC codes group ‘L01’, ‘L02’ (with the exception of ‘L01BA01’, ‘L01XX33’, ‘L02AB01’, ‘L02AB02') and code 'L03AX91'.
SIDIAP registry from general practitioners (ICD-10), codes for malignancy:
C00, C00.0, C00.1, C00.2, C00.4, C00.8, C00.9, C01, C02, C02.0, C02.1, C02.4, C02.8, C02.9, C03, C03.1, C03.9, C04, C04.1, C04.8, C05, C05.0, C05.2, C05.8, C05.9, C06, C06.0, C06.2, C06.8, C06.9, C07, C08, C08.0, C08.1, C08.8, C08.9, C09, C09.1, C09.8, C09.9, C10, C10.0, C10.1, C10.2, C10.3, C10.4, C10.8, C10.9, C11, C11.0, C11.1, C11.2, C11.8, C11.9, C12, C13, C13.0, C13.1, C13.8, C13.9, C14, C14.0, C14.2, C14.8, C15, C15.0, C15.1, C15.2, C15.3, C15.4, C15.5, C15.8, C15.9, C16, C16.0, C16.1, C16.2, C16.3, C16.8, C16.9, C17, C17.0, C17.1, C17.2, C17.3, C17.8, C17.9, C18, C18.0, C18.1, C18.2, C18.3, C18.4, C18.6, C18.7, C18.8, C18.9, C19, C20, C21, C21.0, C21.1, C21.8, C22, C22.0, C22.1, C22.2, C22.3, C22.7, C22.9, C23, C24, C24.0, C24.1, C24.8, C24.9, C25, C25.0, C25.1, C25.2, C25.3, C25.4, C25.8, C25.9, C26, C26.0, C26.1, C26.8, C26.9, C30, C30.0, C30.1, C31, C31.0, C31.1, C31.8, C31.9, C32, C32.0, C32.1, C32.2, C32.3, C32.8, C32.9, C33, C34, C34.0, C34.1, C34.2, C34.3, C34.8, C34.9, C37, C38, C38.0, C38.1, C38.2, C38.3, C38.4, C38.8, C39, C39.0, C39.8, C39.9, C40, C40.0, C40.1, C40.2, C40.3, C40.8, C40.9, C41, C41.0, C41.1, C41.2, C41.3, C41.4, C41.8, C41.9, C43, C43.3, C43.4, C43.5, C43.6, C43.7, C43.9, C44, C44.0, C44.1, C44.2, C44.3, C44.4, C44.5, C44.6, C44.7, C44.8, C44.9, C45, C45.0, C45.2, C45.9, C46, C46.0, C46.1, C46.7, C46.8, C46.9, C47, C47.0, C47.8, C48, C48.0, C48.1, C48.2, C48.8, C49, C49.0, C49.1, C49.2, C49.4, C49.5, C49.8, C49.9, C50, C50.0, C50.1, C50.2, C50.3, C50.4, C50.5, C50.6, C50.8, C50.9, C51, C51.8, C51.9, C52, C53, C53.0, C53.1, C53.8, C53.9, C54, C54.0, C54.1, C54.2, C54.3, C54.8, C54.9, C55, C56, C57, C57.4, C57.7, C57.8, C57.9, C60, C60.1, C60.2, C60.8, C60.9, C61, C62, C62.0, C62.1, C62.9, C63, C63.1, C63.2, C63.7, C63.8, C63.9, C64, C65, C66, C67, C67.0, C67.1, C67.2, C67.3, C67.4, C67.5, C67.6, C67.7, C67.8, C67.9, C68, C68.0, C68.8, C68.9, C69, C69.0, C69.2, C69.3, C69.5, C69.6, C69.8, C69.9, C70, C70.0, C70.1, C70.9, C71, C71.0, C71.1, C71.2, C71.3, C71.4, C71.5, C71.6, C71.8, C71.9, C72, C72.0, C72.2, C72.4, C72.8, C72.9, C73, C74, C74.1, C74.9, C75, C75.0, C75.1, C75.2, C75.5, C75.9, C76, C76.0, C76.1, C76.2, C76.3, C76.4, C76.5, C76.7, C76.8, C77, C77.0, C77.1, C77.2, C77.4, C77.8, C77.9, C78, C78.0, C78.1, C78.2, C78.4, C78.5, C78.6, C78.7, C78.8, C79, C79.0, C79.1, C79.2, C79.3, C79.5, C79.6, C79.7, C79.8, C80, C81, C81.0, C81.1, C81.2, C81.3, C81.7, C81.9, C82, C82.0, C82.1, C82.2, C82.7, C82.9, C83, C83.3, C83.4, C83.5, C83.6, C83.7, C83.8, C83.9, C84, C84.0, C84.1, C84.2, C84.3, C84.4, C84.5, C85, C85.0, C85.1, C85.7, C85.9, C88, C88.0, C88.2, C88.7, C88.9, C90, C90.0, C90.1, C90.2, C91, C91.0, C91.1, C91.2, C91.3, C91.4, C91.7, C91.9, C92, C92.0, C92.1, C92.2, C92.3, C92.4, C92.7, C92.9, C93, C93.0, C93.1, C94,

C94.2, C94.4, C94.5, C94.7, C95, C95.0, C95.1, C95.7, C95.9, C96, C96.1, C96.2, C96.3, C96.7, C96.9, C97
MBDR databases from the SIDIAP and other registries (ICD-9), codes for malignancy:
1400, 1401, 1403, 1404, 1405, 1406, 1408, 1409, 1410, 1412, 1419, 1420, 1453, 1460, 1463, 1469, 1471, 1478, 1479, 1481, 1489, 1490, 1501, 1503, 1504, 1508, 1509, 1510, 1512, 1513, 1514, 1518, 1519, 1520, 1521, 1522, 1528, 1529, 1530, 1531, 1532, 1533, 1534, 1535, 1536, 1537, 1538, 1539, 1540, 1541, 1542, 1548, 1550, 1551, 1552, 1560, 1562, 1569, 1570, 1571, 1572, 1578, 1579, 1580, 1588, 1589, 1590, 1599, 1610, 1611, 1619, 1622, 1623, 1624, 1625, 1628, 1629, 1638, 1639, 1640, 1642, 1648, 1649, 1659, 1700, 1704, 1709, 1713, 1715, 17310, 1742, 1744, 1745, 1748, 1749, 179, 1800, 1809, 1820, 1828, 1830, 1844, 185, 1880, 1882, 1885, 1888, 1889, 1890, 1891, 1892, 1893, 1899, 1910, 1911, 1912, 1913, 1915, 1916, 1918, 1919, 193, 1940, 1950, 1953, 1960, 1961, 1962, 1963, 1965, 1966, 1968, 1969, 1970, 1971, 1972, 1974, 1975, 1976, 1977, 1978, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 19882, 19889, 1990, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2362, 2367, 23691, 2372, 2375, 2376, 2380, 2381, 2382, 2383, 2385, 2386, 2387, 23879, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 23989, 25801

In addition to the data necessary for the classification of patients, according to the presence of HCV infection at inclusion and the presence of malignancy and the type of cancer during follow-up, other relevant data on subject characteristics were included. Demographic and anthropometric data: age, sex, Body mass index (BMI) (Kg/m^2), toxic habits: consumption of alcohol or smoking, geographic zone and MEDEA index (used as a measure for socioeconomic deprivation), the presence of comorbidities: positive serology for HBV (IgG-HBV), diagnosis of HIV infection, diabetes mellitus, characterization of HCV infection: genotype, degree of cirrhosis, viral load, positive serology for HCV (IgG-HCV), and blood test results: Chronic Kidney Disease Epidemiology Collaboration estimation for glomerular filtration rate (CKD-EPI) ($mL/min^{-1}/1.73\ m^{-2}$), Gamma-Glutamil Transferase (GGT) (IU/L), Alanineamino Transferase (ALT) (IU/L), Platelets (10^9 count), Total bilirubin (mg/dL), Aspartateamino Trasnferase AST (IU/L), Prothrombin time (%), Albumin (mg/dL) and standardized prothrombin time expressed as International Normalised Ratio (INR).

The selection of the cohort that would source controls for patients treated for HCV infection identified from RPT registry and their linked data supplied by PADRIS, was done from the pool of uninfected patients included in SIDIAP database. Subjects were matched internally in the reference institution for research in primary care of the ICS (IDIAP Jordi Gol) by internal technicians independent from this project. The initial matching was done for exact restriction of sex and geographic location at a ratio of 1:20; the goal of this procedure was to provide a temporary selection of control patients without HCV infection for final statistical matching.

9.6 Definition of study cohorts

The exposed cohorts were defined from RPT according to the *de novo* pharmacy billing registry from hospitals in the study period (2012-2016) for the diagnosis of HCV, and the absence of previous diagnoses of cancer or billing for cancer drugs.

Exposed cases were divided into three cohorts: those who received only treatments based on IFN, patients who first received IFN-based therapy and later, or concomitantly, received DAA, and patients treated with DAA alone.

Since initially it was assumed that the proportion of patients who, during follow-up, would have received treatment based on IFN and DAA would be irrelevant, the initial design considered only two cohorts. However, 794 patients were treated initially with IFN and later on with DAA, representing 6.8% of the total, so an amendment to the protocol was implemented in order to analyse them separately.

The three cohorts were matched to controls in a 1 to 5 maximum rate, being controls subjects selected from the SIDIAP registry with no evidence of an HCV diagnosis or diagnosis of cancer or billing of cancer drugs before the index date. Control matching considered sex, year of birth, consumption of alcohol or smoking and a geographical code based on the administrative grouping of healthcare units (called DAP for their spelling in Catalan Direcció d'Atenció Primària) that groups the Catalan territory into 36 geographical sectors. DAPs are characterised for socio-economic aspects and access to health care, and are used for healthcare budgeting adjustment. Furthermore, the MEDEA index (112), which indicates the degree of deprivation index for urban census sectors, permits aggregation of DAPs with similar socioeconomic conditions categorized in quintiles, thus allowing secondary matching to similar DAPs of cases in low-density areas having few eligible control subjects or even none for a given DAP.

As a summary of the whole process of generation of the three analysis cohorts, matching was made using a two-step matching procedure executed on a sequential basis (113): first using exact sex and DAP matching by independent technicians from IDIAP Jordi Gol and then propensity score matching (PSM) using the logit calculation from a logistic regression model that included sex, year of birth, alcohol, smoking and DAP. The second step used greedy nearest neighbour matching (114) with a calliper distance of <0.06. This was decided based on the minimization of lost patients in treated HCV infected groups, with the aim of maximizing their representativeness.

9.7 Definition of event and time of follow-up

As previously described, we used the ICD-10 and ICD-9 coded data from eCAP and MBDR related to the diagnosis of cancer to identify incident events in the whole groups: exposed and controls for all three cohorts, IFN, IFN-AAD and AAD, which will be defined in the next section specifically. Additionally, we used ATC codes for oncological treatments from hospital pharmacy billing registry in order to detect potential missed incident cases of cancer.

The time of follow-up for patients treated for HCV infection (case group) was defined as the period between the index date and the date of the event.

For case groups, the index date was defined as the first date of prescription of HCV treatment. For patients in the control group the index date was that of the case they were paired with.

The date of the event was the date of recording of the cancer diagnosis or the first prescription of specific cancer treatment using the ATC codes previously described. The definition of censure was the absence of an event and, in this case, the last follow-up was the event date, defined as the last date available in the registry.

9.8 Definition of cohorts

Exposed cases were divided into three cohorts:

- IFN Cohort: Patients who received IFN treatment and their matched controls.
- IFN-DAA cohort: Patients who received IFN-based treatment, but HCV infection was also treated with DAA at some point during the study follow-up and their matched controls.
- DAA Cohort: Patients who received treatment with DAA alone and their matched controls.

The exposed groups, within each cohort, were defined using the RTP of CatSalut according to de novo pharmacy billing registry from hospitals in 2012-2016 for the

treatment of HCV infection. These cohorts had no previous records of cancer diagnosis or billing for cancer drugs.

The three exposed groups, within each cohort, were matched with patients without evidence, in previous population-base registries of a diagnosis of HCV infection or treatment for HCV infection, nor of a diagnosis of cancer or billing for cancer drugs. The matched control groups were extracted from the SIDIAP database and DMBD.

9.9 Statistical analysis

9.9.1 Main analysis

Descriptive results are shown as median and interquartile range (IQR: 25th and 75th percentiles) or absolute frequencies and percentages for quantitative and qualitative variables, respectively. All descriptive results were tabulated by cohort (IFN, IFN+DAA or DAA) and group (patients treated for HCV infection by any of the 3 strategies and their matched controls)

Homogeneity for baseline characteristics was assessed using standardized differences (STD, differences divided by pooled standard deviation) between each HCV group and their matched control group. The proper balance of all matching covariates was calculated by using a ±0.20 cut-off point for standardized differences (101); in this study all matching covariates were well balanced. Following the recommendations established (101) no inferential analysis was made to compare groups.

The main analyses, the estimate of cancer incidence, and the 95% confidence intervals (95% CI) were obtained using Poisson models with the natural logarithmic transformation of follow-up as an offset. Estimated incidence was calculated as new cancer diagnosis/100,000 patients-year of follow-up (Events/100k*PY) for HCV patients treated only with IFN (IFN group), for HCV patients sequentially treated with both types of HCV treatment (IFN+DAA group), for HCV patients treated only with DAA (DAA group), and for each matched control group.

Rate ratios (RR) and their 95% CI were estimated using the incidence of each matched control groups as a reference. A time-to-event analysis, for robustness purposes, was made using the Kaplan-Meier method to describe the instantaneous hazard with a maximum window of 3 years of follow up per patient. Additionally, the increased risk for each treatment group with respect to their matched control set were estimated using hazard ratios (HR) and their 95%CI from Cox proportional regression models.

A specific secondary objective, direct comparisons between treatment groups from the different cohorts were not made due to their inherent differences in cirrhosis and HIV infection, which are well-established risk factors for the development of cancer (115) and clinical limitations in prescribing IFN-based treatment. Also, estimation cancer incidences, for inferential purposes, stratified by solid and haematological malignancies, was not conducted either since the haematological cancer diagnoses was too low.

In order to describe cancer types, estimate of cumulative incidence were also made according to liver cancer, HCC mainly, or others. Finally, a descriptive approach for the type of tumour (solid organ or haematological) was carried out.

In all statistical analyses we applied a two-sided type I error of 5%. SAS v9.4 (Cary, NC, USA) statistical software was used for data management and the statistical analyses.

9.9.2 Sensitivity analyses

In the present study, cases treated de novo for HCV infection were compared with controls without evidence of HCV infection, as described above. This matching was carried out taking into account, among other variables described, the consumption of alcohol and smoking. These are the main cancer risk factors that are routinely collected in the records used in the analyses.

The objective of the three sensitivity analyses was to assess to what extent the results were sensitive to the inclusion of these known risk factors in the propensity models.

Three sensitivity analyses were proposed:

- Absence of these factors for the PS calculation
- Use of alcohol only for the calculation of the PS
- Use of smoking only for the calculation of the PS

For the matching, it was prioritized that the patients of the three groups, treated for HCV infection, was kept constant, so that only the composition of the matched control

groups was modified. In this way, the changes in the results and whether these would affect the conclusions of the main analysis could be assessed.

9.10 Ethical considerations

All datasets were pseudo-anonymised, in compliance with Regulation (EU) 2016/679 of the European Parliament and of the Council of April 27, 2016 on Data Protection (GDPR) and Organic Law 3/2018, of December 5, on the Protection of Personal Data and guarantee of digital rights, prior to the transfer to final data management and statistical analyses. The technicians had no access to clinical information, only to codes and IDs.

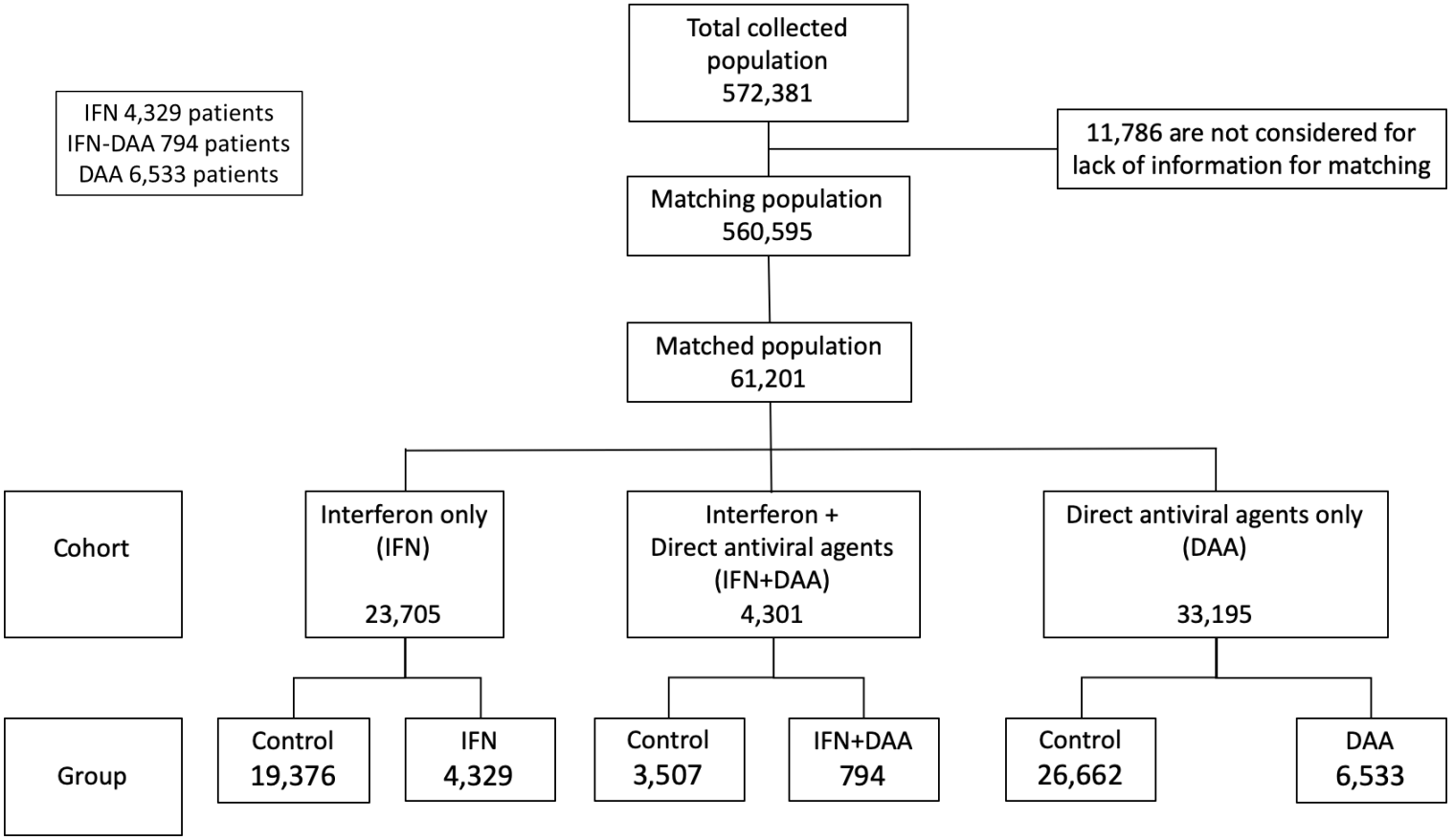
This study was made in accordance with the principles of Good Clinical Practice, the principles of the latest version of the Declaration of Helsinki and its appendices and national laws. Ethical approval for this research was obtained from the Ethics Committee for Clinical Research IDIAP Jordi Gol (Code CEI P17/061). The Committee waived the need for informed consent, since this was a retrospective study with pseudo-anonymized data from population register databases. This makes it unfeasible to re-identify patients due to the absence of identifiable variables, and thus to obtain informed consent is also impossible.

10 Results

10.1 Patient disposition and allocation

A total of 11,656 patients were identified in RPT that had a diagnosis of HCV with initiation of specific treatment within the study period for any of the three cohorts and registries in primary care. According to the treatments received, 4,329 patients were treated with IFN only, 794 patients had sequential/combination therapy treated with IFN and DAAs, and 6,533 patients were treated with DAAs only. The result of the initial data merger in IDIAP Jordi Gol in patients treated for HCV infection and candidates for control patients was 572,381 patients. Due to insufficient data for the effective PS estimate used for matching, 11,786 patients were excluded as candidates. Finally, data from 560,595 patients were obtained as potential candidates for the final matching procedure. This dataset includes treated HCV infected patients and candidates for control matching from primary care registries. The three groups of treated patients for their HCV infection were paired with 19,376, 3,507 and 26,662 controls respectively. This represents a mean of 4.25 controls for each patient treated because of an HCV diagnosis (Figure 4).

Figure 4. Flow chart of control subjects



10.2 Clinical and sociodemographic characteristics of the whole cohort

The variables that were used to calculate the Propensity Score, as well as the main characteristics that defined the patients treated for HCV infection and matched controls, are shown in Table 7.

Table 7. Main characteristics of HCV patients and comparison with controls

Cohort	**Interferon (IFN) only**			**IFN + direct-acting antivirals (DAA)**			**Direct-acting antivirals (DAA) only**		
Group*	**Control**	**IFN**	**STD**	**Controls**	**IFN + DAA**	**STD**	**Controls**	**DAA**	**STD**
No. of patients	**n = 19,376**	**n = 4,329**	**%**	**n = 3,507**	**n = 794**	**%**	**n = 26,662**	**n = 6,533**	**%**
Age, years	36.8 (29.6-44.1)	36.5 (29.1-43.8)	3	43.9 (39.2-49.3)	43.8 (39-48.9)	2	45 (39.5-55.8)	45.5 (39.5-57)	4
Sex, n (%)			1			0			0
Male	9,092 (46.9)	2,024 (46.8)		2,339 (66.7)	531 (66.9)		16,131 (60.5)	3,948 (60.4)	
Female	10,284 (53.1)	2,305 (53.2)		1,168 (33.3)	263 (33.1)		10,531 (39.5)	2,585 (39.6)	
Height, cm	165 (159-172)	166 (159.5-173)	5	167.7 (160-174)	168 (160-174)	6	166 (158-173)	166 (158-172.3)	3
Missing, n	5,648	1,072		903	159		5,744	1,194	
Weight, kg	72 (62-83.5)	69.7 (60.9-80)	17	77 (66.6-87.8)	73.8 (65.3-83.3)	17	76 (66.1-86.6)	72.5 (63.2-82)	25
Missing, n	5,132	905		804	127		4,858	930	
BMI, kg/m2	26.1 (23.2-29.5)	25 (22.4-28.2)	22	27.3 (24.5-30.6)	26.2 (23.9-29.1)	22	27.5 (24.7-30.8)	26.4 (23.6-29.4)	26
Missing, n	5,411	984		849	146		5,202	1,057	

Cohort	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
Group* No. of patients	Control n = 19,376	IFN n = 4,329	STD %	Controls n = 3,507	IFN + DAA n = 794	STD %	Controls n = 26,662	DAA n = 6,533	STD %
BMI, WHO categories, n (%)			21			21			24
Underweight (< 18.5)	269 (1.9)	85 (2.5)		29 (1.1)	1 (0.2)		170 (0.8)	74 (1.4)	
Normal weight (18.5-24.9)	5,401 (38.7)	1,590 (47.5)		745 (28)	240 (37)		5,742 (26.8)	1,960 (35.8)	
Pre-obesity (25.0-29.9)	5,141 (36.8)	1,147 (34.3)		1,121 (42.2)	273 (42.1)		9,092 (42.4)	2,258 (41.2)	
Obesity class I (30.0-34.9)	2,223 (15.9)	374 (11.2)		538 (20.2)	98 (15.1)		4,657 (21.7)	904 (16.5)	
Obesity class II (35.0-39.9)	666 (4.8)	113 (3.4)		161 (6.1)	30 (4.6)		1,343 (6.3)	223 (4.1)	
Obesity class III (> 40)	265 (1.9)	36 (1.1)		64 (2.4)	6 (0.9)		456 (2.1)	57 (1)	
Missing, n	5,411	984		849	146		5,202	1,057	
Smoking, n (%)			5			5			5
Non-Smoker	8,779 (45.3)	2,060 (47.6)		1,249 (35.6)	301 (37.9)		10,269 (38.5)	2,686 (41.1)	
Smoker or Ex-Smoker	10,597 (54.7)	2,269 (52.4)		2,258 (64.4)	493 (62.1)		16,393 (61.5)	3,847 (58.9)	
Alcohol consumption, n (%)			4			5			8
No	13,203 (68.1)	3,038 (70.2)		2,426 (69.2)	568 (71.5)		17,177 (64.4)	4,457 (68.2)	
Yes	6,173 (31.9)	1,291 (29.8)		1,081 (30.8)	226 (28.5)		9,485 (35.6)	2,076 (31.8)	

Cohort	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
Group* No. of patients	Control n = 19,376	IFN n = 4,329	STD %	Controls n = 3,507	IFN + DAA n = 794	STD %	Controls n = 26,662	DAA n = 6,533	STD %
MEDEA index, quintiles, n (%)			3			8			7
Q1	2,890 (20.5)	617 (19.5)		437 (16.8)	94 (16.2)		3,782 (18.8)	849 (17.8)	
Q2	2,783 (19.7)	632 (20)		473 (18.2)	96 (16.6)		3,894 (19.4)	867 (18.1)	
Q3	2,837 (20.1)	634 (20)		514 (19.8)	121 (20.9)		3,975 (19.8)	978 (20.5)	
Q4	2,768 (19.6)	659 (20.8)		584 (22.5)	118 (20.3)		4,150 (20.6)	964 (20.2)	
Q5	2,847 (20.2)	622 (19.7)		592 (22.8)	151 (26)		4,318 (21.5)	1,119 (23.4)	
Missing	5,251	1,165		907	214		6,543	1,756	
Diabetes mellitus, n (%)	1,213 (6.3)	285 (6.6)	1	379 (10.8)	132 (16.6)	17	3,579 (13.4)	1,251 (19.1)	16
HIV infection, n (%)	94 (0.5)	287 (6.6)	33	20 (0.6)	207 (26.1)	81	102 (0.4)	2,775 (42.5)	100
Cirrhosis, n (%)	47 (0.2)	12 (0.3)	1	17 (0.5)	367 (46.2)	100	115 (0.4)	2,824 (43.2)	100

Variables for matching by PS method: sex, age (calculated from year of birth to index date), consumption of alcohol, consumption of smoking and geographical code from DAP and evaluated with Medea index quintiles

BMI: body mass index, IFN: Interferon, DAA: Direct antiviral agents, HIV: human immunodeficiency virus, Q1-Q5: 1st to 5th quintiles

IQR: Interquartile range [25th-75th percentiles], |STD|: Absolute standardised differences (%), NA: Not applicable. Results shown as median (IQR) for quantitative variables and absolute frequencies with percentage otherwise.

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

According to the matching procedure, all three HCV groups of patients were evenly balanced with their respective controls for factors such as age, sex, alcohol and smoking and the MEDEA index.[31] However, we found standardized differences greater than 20% in other variables.

Regarding age and sex, the groups of the three cohorts were well balanced, with an STD below 5%. What stands out from these two variables is that the profile of the INF+DAA and DAA cohorts have closer characteristics between them than to the IFN cohort. The median age of the IFN cohort was slightly younger than that of the IFN+DAA and DAA cohorts. Regarding sex distribution, in the IFN cohort approximately 53% were women, while in the IFN+DAA and DAA cohorts the percentage of women was 33% and just over 39%, respectively.

The BMI and its categorization according to WHO categories showed standardized differences between the treated groups and matched controls of between 21% and 26%. In the case of the absolute value of the BMI, these differences, in medians, were always less than 1.5 kg/m2 between the treated patients and matched controls. For the classification of obesity, these differences were greater due to a higher proportion of patients with normal weight in the group of patients treated for HCV infection compared with their matched controls. Treated patients had the lowest BMI and a 10% higher proportion of normal weight than matched controls.

HIV prevalence was always higher in treated patients than in matched controls. The group of patients treated with IFN had a presence of coinfected patients of 6.6%, the IFN+DAA group of 26.1% and in the group treated only with DAA of 43.2%, which is consistent with the strategy of progressively introducing the DAA treatments to different patient profiles along time. In the case of the control groups, these proportions were 0.5%, 0.6% and 0.4% for the matched controls of the IFN, IFN+DAA and DAA cohorts, respectively, signalling the known fact that HIV

frequently accompanies HVC infection, but is relatively infrequent in the general population.

Similar effects were found for cirrhosis status. Prevalence of cirrhosis was balanced in the IFN cohort, but this was not the case in the other two cohorts where patients that prevalence was 46.2% in the IFN+DDA group and 43.2% in patients treated only with DAA, while controls have very low rates. Other clinical variables of interest in relation to patients treated for HCV infection are shown in table 8.

Table 8. Supplementary clinical information.

Cohort	**Interferon only**		**Interferon + Direct antiviral agents**		**Direct antiviral agents only**	
Group*	**Control**	**IFN**	**Control**	**IFN+DAA**	**Control**	**DAA**
No. of patients	**n=19,376**	**n=4,329**	**n=3,507**	**n=794**	**n=26,662**	**n=6,533**
Fibrosis degree, n (%)						
F0		14 (12)		13 (1.9)		126 (2)
F1		53 (45.3)		45 (6.5)		422 (6.7)
F2	NA	27 (23.1)	NA	123 (17.6)	NA	1,460 (23.3)
F3		11 (9.4)		149 (21.4)		1,431 (22.8)
F4		12 (10.3)		367 (52.7)		2,824 (45.1)
Missing/NA		4,212		97		270
Genotype, n (%)						
1		41 (35)		504 (64.9)		4,804 (74.3)
2		5 (4.3)		24 (3.1)		157 (2.4)
3	NA	40 (34.2)	NA	151 (19.4)	NA	717 (11.1)
4		31 (26.5)		98 (12.6)		784 (12.1)
5		0 (0)		0 (0)		5 (0.1)
Missing/NA		4212		17		66
Viral load (log, count)	NA	N=117 13.9 (11.7-15.2)	NA	N=777 14 (12.8-14.9)	NA	N=6,454 13.9 (12.6-14.9)

Cohort	**Interferon only**		**Interferon + Direct antiviral agents**		**Direct antiviral agents only**	
Group*	**Control**	**IFN**	**Control**	**IFN+DAA**	**Control**	**DAA**
No. of patients	**n=19,376**	**n=4,329**	**n=3,507**	**n=794**	**n=26,662**	**n=6,533**
IGG HCV		N=138 0.1 (0.1-10.5)		N=16 11.6 (10.3-26.3)		N=245 11.4 (10-26.7)
Exposure to, n (%):						
ribavirin						
No	NA	3,125 (72.2)	NA	37 (4.7)	NA	4,039 (61.8)
Yes		1,204 (27.8)		757 (95.3)		2,494 (38.2)
Telaprevir						
No	NA	4,329 (100)	NA	619 (78)	NA	6,533 (100)
Yes		0 (0)		175 (22)		0 (0)
Boceprevir						
No	NA	4,329 (100)	NA	725 (91.3)	NA	6,533 (100)
Yes		0 (0)		69 (8.7)		0 (0)
Simiprevir						
No	NA	4,329 (100)	NA	578 (72.8)	NA	5,589 (85.6)
Yes		0 (0)		216 (27.2)		944 (14.4)
Daclatasvir						
No	NA	4,329 (100)	NA	692 (87.2)	NA	5,779 (88.5)
Yes		0 (0)		102 (12.8)		754 (11.5)

Cohort	Interferon only		Interferon + Direct antiviral agents		Direct antiviral agents only	
Group*	**Control**	**IFN**	**Control**	**IFN+DAA**	**Control**	**DAA**
No. of patients	**n=19,376**	**n=4,329**	**n=3,507**	**n=794**	**n=26,662**	**n=6,533**
Sofosbuvir						
No	NA	4,329 (100)	NA	84 (10.6)	NA	1,736 (26.6)
Yes		0 (0)		710 (89.4)		4,797 (73.4)
Dasabuvir						
No	NA	4,329 (100)	NA	737 (92.8)	NA	5,056 (77.4)
Yes		0 (0)		57 (7.2)		1,477 (22.6)
Ledipasvir						
No	NA	4,329 (100)	NA	490 (61.7)	NA	3,598 (55.1)
Yes		0 (0)		304 (38.3)		2,935 (44.9)
Ombitasvir						
No	NA	4,329 (100)	NA	711 (89.5)	NA	4,812 (73.7)
Yes		0 (0)		83 (10.5)		1,721 (26.3)
peritaprevir						
No	NA	4,329 (100)	NA	711 (89.5)	NA	4,812 (73.7)
Yes		0 (0)		83 (10.5)		1,721 (26.3)
ritonavir						
No	NA	4,329 (100)	NA	711 (89.5)	NA	4,812 (73.7)
Yes		0 (0)		83 (10.5)		1,721 (26.3)

Cohort	Interferon only		Interferon + Direct antiviral agents		Direct antiviral agents only	
Group*	**Control**	**IFN**	**Control**	**IFN+DAA**	**Control**	**DAA**
No. of patients	**n=19,376**	**n=4,329**	**n=3,507**	**n=794**	**n=26,662**	**n=6,533**
CKD-EPI(mL/min^{-1}/1.73m^3)	N=14,903 90.1 (87.3-90.1)	N=3,839 90.1 (89.8-90.1)	N=2,856 90.1 (84.2-90.1)	N=717 90.1 (86.9-90.1)	N=22,434 90.1 (80.4-90.1)	N=5,891 90.1 (81.4-90.1)
GGT (IU/L)	N=10,760 21 (14-34)	N=3,331 24 (16-44)	N=2,154 26 (17-44)	N=648 79 (38.5-152.5)	N=17,643 25 (17-42)	N=5,402 63 (33-126)
ALT (IU/L)	N=12,683 19 (14-29)	N=3,331 24 (16-45)	N=2,472 22.5 (16-33)	N=632 64 (39-109)	N=20,029 21 (16-31)	N=5,368 61 (38-98)
Platelets (10^9 count)	N=13,111 245 (209-287)	N=3,569 225 (187-267)	N=2,520 239 (204-281)	N=665 170 (128-216)	N=20,414 237 (202-278)	N=5,539 173 (124-222)
Total bilirubin (mg/dL)	N=8,504 0.5 (0.4-0.7)	N=3,175 0.5 (0.4-0.7)	N=1,792 0.5 (0.4-0.7)	N=610 0.6 (0.5-0.9)	N=14,339 0.5 (0.4-0.7)	N=5,192 0.7 (0.5-0.9)
AST (IU/L)	N=6,862 21 (17-28)	N=3,018 24 (18-37)	N=1,437 23 (18-30)	N=608 58 (36-95)	N=11,545 22 (18-29)	N=5,212 54 (36-85)
Prothrombin time (%)	N=2,535 100 (95-107)	N=1,196 100 (92-103)	N=475 100 (93.1-106)	N=320 96 (87-100)	N=4,173 100 (93.5-104)	N=2,840 96 (85-100)
Albumin (mg/dL)	N=2,362 4.4 (4.1-4.6)	N=1,842 4.4 (4.1-4.6)	N=550 4.4 (4.1-4.6)	N=476 4.3 (4-4.5)	N=4,295 4.3 (4.1-4.6)	N=3,800 4.2 (3.9-4.5)
INR	N=1,824 1 (0.9-1.1)	N=927 1 (1-1.1)	N=376 1 (0.9-1)	N=278 1 (1-1.1)	N=3,053 1 (0.9-1.1)	N=2,436 1 (1-1.1)

Cohort	Interferon only		Interferon + Direct antiviral agents		Direct antiviral agents only	
Group*	**Control**	**IFN**	**Control**	**IFN+DAA**	**Control**	**DAA**
No. of patients	**n=19,376**	**n=4,329**	**n=3,507**	**n=794**	**n=26,662**	**n=6,533**
IGG HBV (mg/dL)	N=110 0.2 (0.2-0.3)	N=178 0.2 (0.1-0.4)	N=29 0.2 (0.1-2)	N=37 0.2 (0.1-2.6)	N=182 0.2 (0.1-0.3)	N=402 0.2 (0.2-1.5)

Results shown as median (IQR) for quantitative variables and absolute frequencies with percentage otherwise. IFN: Interferon, DAA: Direct antiviral agents, 100k/PY: 100,000 patient-years, NA. Not Applicable

NA: Not applicable

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

The evaluation of the degree of fibrosis was anecdotal in the groups of matched controls and also for the IFN group and it cannot be correctly evaluated. In the IFN+DAA group, fibrosis grades were F3-F4 for 74.1% of the cases and 67.9% in the DAA group.

Regarding the predominant genotype, in the case of the IFN group they were evenly distributed between 1, 3 and 4, while in the IFN+DAA and DAA groups it was clearly genotype 1, with 64.9% and 74.3% respectively the most frequent.

Both viral load and IgG titers assessment are not well characterized in the IFN group. In the IFN+DAA and DAA groups, the median viral load was 14 log-count (IQR: 12.8; 14.9) and 13.9 log-count (IQR: 12.6; 14.9), respectively. The number of HCV IgG titers were medians of 11.6 log-count (IQR: 10.3; 26.3) and 11.4 log-count (IQR: 10.0; 26.7) for the IFN+DAA and DAA groups, respectively.

Regarding treatment for HCV infection, in the IFN group, ribavirin was used in 27.8% of patients in combination with interferon. In the IFN+DAA group, the combination with ribavirin was used in 95.3% of cases and, as direct-acting antiviral treatment, sofosbuvir was the most frequent active principle, administered in 89.4% of patients, followed by ledipasvir, which was used in 38.3% of cases. In the group of patients who were only treated with DAA, the most frequent treatments were also sofosbuvir, administered in 73.4% of cases, and ledipasvir, which was prescribed in 44.9% of cases.

Regarding the possible changes in the DAA treatments used in the IFN+DAA groups compared to the DAA group, ribavirin was only used in 38.2% of the cases in the DAA group, telaprevir and boceprevir were no longer administered, and simiprevir decreased from 27.2% of cases in the IFN+DAA group to 14.4% in the DAA group. On the other hand, dasabuvir use clearly increased from 7.2% to 22.6%, as happened with ombitasvir, from 10.5% to 26.3%, peritaprevir, from 10.5% to 26.3%, and ritonavir, from 10.5% to 26.3%.

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In these cohorts, the median GGT (IU/L) and ALT (IU/L) were almost three times higher in the treated groups than in the matched controls. For AST (IU/L), the median values of the treated groups were slightly more than double that of the matched controls and the platelet count was 25% lower in the treated groups. The rest of the available parameters, such as DKD-EPI, total bilirubin, prothrombin time, albumin, and INR, were very similar between the three treated groups and matched controls.

10.3 Cancer risk assessment in treatment-naïve HCV patients who initiated therapy: general analyses

The main results regarding the cancer incidence between the treated groups: IFN, IFN+DAA and DAA, and matched controls in the three cohorts are shown in table 9.

Table 9. Cancer incidence and rate ratios for the three cohorts of HCV therapies and matched controls

Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
Interferon Only	Control	555	19,109	107,207	514.9 (472.3-561.3)	Ref.	
	IFN	141	4,329	24,774	569.1 (482.5-671.4)	1.11 (0.92-1.32)	0.2771
IFN + DAA	Control	123	3,507	17,163	710.8 (590.7-855.4)	Ref.	
	IFN+DAA	49	794	3,904	1,255.3 (947.9-1,662.2)	1.77 (1.27-2.46)	0.0008
DAA	Control	633	26,662	77,271	815.3 (752.9-882.9)	Ref.	
	DAA	283	6,533	18,170	1,552.0 (1,380.1-1,745.3)	1.90 (1.66-2.19)	< 0.0001

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

10.3.1 Cancer incidence estimates

The incidence in control groups was heterogeneous. In the IFN cohort, the control group had a lower cancer incidence, 514.9 cases /100kPY (95%CI: 472.3; 561.3) than the control group in the IFN+DAA cohort, 710.8 cases /100kPY (95%CI: 590.7; 855.4) and the control group in the DAA cohort, 815.3 cases /100kPY (95%CI: 752.9; 882.9).

10.3.1.1 IFN-based treatment cohort

Patients with HCV infection treated with an IFN-based regimen had a cancer incidence of 569.1 cases/100kPY (95% CI: 482.5; 671.4). This was not significantly different than in the matched controls with a rate ratio (RR) of 1.11 (95% CI: 0.92; 1.32), p-value = 0.2771. The estimated cumulative incidence of matched controls was 514.9 cases/100kPY (95% CI: 472.3; 561.3).

10.3.1.2 IFN+DAA cohort

The estimate of the cancer incidence of patients who received DAA and IFN-based regimens in the follow-up period was 1,255.3 cases/100kPY (95% CI: 947.9; 1662.2). This is significantly higher than in matched controls, who presented an estimated cancer incidence of 710.8 cases/100kPY (95% CI: 590.7; 855.4); RR: 1.77 (1.27; 2.46), p-value=0.0008.

10.3.1.3 DAA cohort

The estimated cancer incidence in the group of infected patients treated with DAA agents was 1,552.0 cases/100kPY (95% CI: 1,380.1; 1,745.3). This was significantly higher than the estimated incidence in the matched control group, which

presented an estimated incidence of 815.3 cases/100kPY (95% CI: 752.9; 882.9); RR: 1.90 (95% CI: 1.66; 2.19), p-value <0.0001.

10.3.2 Role of HIV-HCV coinfection and cirrhosis

Table 10 shows the results of the possible influence of HIV coinfection and the presence of cirrhosis at the time of starting treatment for HCV infection.

Overall, given the exceptionally low prevalence of HIV-HCV coinfection and cirrhosis in the control groups, the effect of these known cancer risk factors on therapies compared with the control arms could not be assessed.

Table 10. Cancer incidence and rate ratios in treated patients* from the three cohorts of HCV therapies for key comorbidity factors: HIV-coinfection and diagnosis of cirrhosis

Cohort	Group*	Stratum	Events	Patients at risk	Follow-up time (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
		HIV infection						
Interferon only	IFN	No	130	4,042	23,336	557.1 (469.1-661.6)	Ref.	
		Yes	11	287	1,439	764.6 (423.4-1,380.6)	1.37 (0.74-2.54)	0.3133
Interferon + Direct antiviral agents	IFN+DAA	No	43	587	2,855	1,506.4 (1,117.2-2,031.1)	Ref.	
		Yes	6	207	1,049	572.0 (257.0-1,273,1)	0.38 (0.16-0.89)	0.0263
Direct antiviral agents only	DAA	No	180	3,758	10,582	1,701.1 (1,469.9-1,968.2)	Ref.	
		Yes	103	2,775	7,589	1,344.1 (1,170.0-1,632.0)	0.79 (0.62-1.01)	0.0574
		Cirrhosis						
Interferon only	IFN	No	140	4,317	24,713	566.5 (480.0-668.6)	Ref.	
		Yes	1	12	62	1,620.4 (228.3-11,503.2)	2.86 (0.4-20.4)	0.2950
Interferon + Direct antiviral agents	IFN+DAA	No	24	427	2,106	1,139.6 (763.8-1,700.1)	Ref.	
		Yes	25	367	1,798	1,390.8 (939.8-2,058)	1.22 (0.7-2.14)	0.4856
Direct antiviral agents only	DAA	No	112	3,709	10,078	1,101.3 (914.4-1,326.5)	Ref.	
		Yes	171	2,824	8,092	2,113.3 (1,819.2-2,455)	1.92 (1.51-2.44)	< 0.0001

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, PY: person-years, 100k/PY: 100,000 patient-years

**Matched control groups are not included in this analysis due to extremely low figures (<0.5%) for HIV coinfection and cirrhosis*

In the treated arms of the three cohorts, the estimated cancer incidence excluding HIV coinfected patients was 557.1 cases/100kPY (95%CI: 469.1; 661.6) for the IFN group, 1,506.4 cases/100kPY (95%CI: 1,117.2; 2,031.1) in the IFN+DAA group and 1,701.1 cases/100kPY (95%CI: 1,469.9; 1,968.2) in the DAA group.

The number of treated patients with HIV coinfection was very limited in all groups except for the DAA treated group, with a prevalence of 42.5% (2,775 cases). In the group of HCV-HIV coinfected patients, the estimated cancer incidence was 1,344.1 cases/100kPY (95%CI: 1,170.0; 1,632.0). In this group of patients treated with DAAs the decrease was not statistically significant: RR 0.79 (95% CI: 0.62; 1.01), p-value = 0.0574.

Analysis of the influence of the presence of cirrhosis presents a similar methodological problem as HIV coinfection. The diagnosis of cirrhosis in the IFN group was 0.3%. However, the prevalence of cirrhosis was 46.2% (367 cases) and 43.2% (2,824 cases) in the IFN+DAA and DAA treated groups respectively. In these groups, the estimated cancer incidence in patients without a diagnosis of cirrhosis was 1,139.6 cases/100kPY (95CI: 763.8-1,700.1) and 1,101.3 cases/100kPY (95%CI: 914.4-1,326.5) for the IFN+DAA and DAA groups, respectively.

The cancer incidence in patients diagnosed with cirrhosis in the IFN+DAA group was 1,390.8 cases/100kPY (95%CI: 939.8; 2058), with an estimated RR of 1.22 (95%CI: 0.7; 2.14), which was not statistically significant. In the case of patients treated only with DAAs and diagnosed with cirrhosis, the incidence was 2,113.3 cases/100kPY (95%CI: 1,819.2; 2,455), higher than in patients without a diagnosis of cirrhosis, with an estimated RR of 1.92 (95%CI: 1 .51; 2.44), which was statistically significant.

10.3.2.1 HIV and cirrhosis in controls

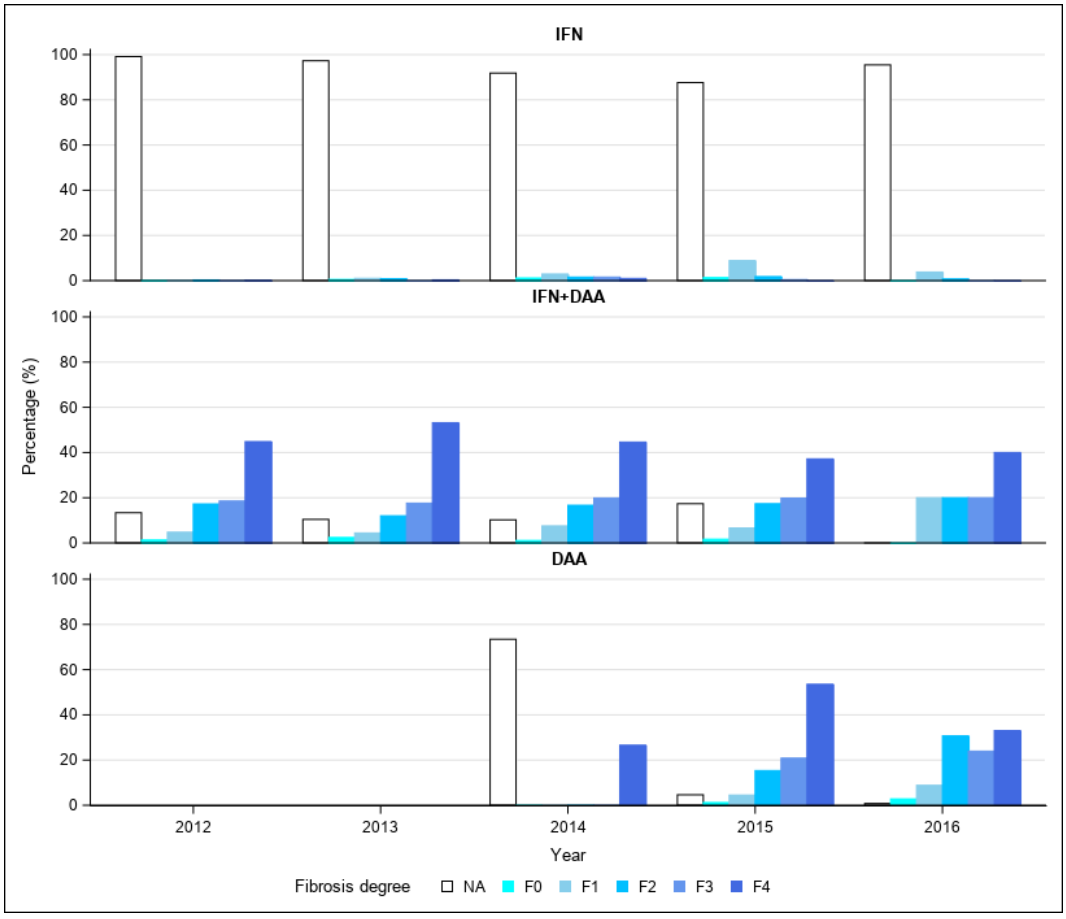
Table 11 represents the raw data of the controls in each cohort with a diagnosis of HIV or the presence of cirrhosis who had an event during the study period.

Table 11. Patients in control groups with HIV or cirrhosis and a diagnosis of cancer

Matched Control to	HIV	Cirrhosis	Liver cancer	Solid cancer	Follow-up after data index (months)
Interferon Only	No	Yes	No	Yes	71.00
	No	Yes	No	Yes	7.40
	No	Yes	Yes	Yes	12.04
	No	Yes	No	Yes	20.09
	No	Yes	Yes	Yes	51.20
Interferon + Direct antiviral agents	No	Yes	No	Yes	16.80
	Yes	No	No	Yes	30.25
Direct antiviral agents only	Yes	No	No	Yes	2.53
	No	Yes	No	Yes	5.20
	Yes	No	Yes	Yes	16.01
	Yes	No	No	No	6.54
	No	Yes	No	Yes	20.75
	No	Yes	No	No	22.46
	Yes	No	Yes	Yes	2.83
	Yes	No	No	Yes	19.73

Figure 5 shows the evolution of the degree of fibrosis according to the year of inclusion of the patients in the cohort.

Figure 5. **Degree of fibrosis by treatment group and year.**



In the temporal evolution of patients who started treatment with the three strategies, as indicated in the description of the baseline characteristics, in the group treated only with IFN there was a high percentage of patients who did not undergo tests for the classification of the degree of fibrosis. In the IFN+DAA treatment group, the most frequent category was F4 in all the years of inclusion in this study. In the group of patients treated with DAA, the degree of fibrosis F4 also predominated, most evidently in 2014 and 2015.

10.4 Estimated incidence of hepatocellular carcinoma and other cancer types

The incidence of HCC diagnosis in treated patients was significantly higher than in controls for all HCV treatments (Table 12) but not for extrahepatic new diagnoses of cancer.

Table 12. Incidence and rate ratios of HCC and other cancer types in the three cohorts of HCV therapies and matched controls

Cancer type Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
HCC							
Interferon only	Control	100	19,376	107,207	91.4 (75.0-111.5)	Ref.	
	IFN	34	4,329	24,774	137.2 (98.1-192.1)	1.50 (1.02-2.22)	0.0409
Interferon + Direct antiviral agents	Control	26	3,507	17,163	151.5 (102.3-224.3)	Ref.	
	IFN+DAA	23	794	3,904	589.2 (391.3-887.1)	3.89 (2.26-6.69)	< 0.0001
Direct antiviral agents only	Control	88	26,662	77,271	112.6 (90.9-139.4)	Ref.	
	DAA	132	6,533	18,170	726.5 (612.2-862.0)	6.45 (4.90-8.49)	< 0.0001
Other cancer types							
Interferon only	Control	455	19,376	107,207	423.5 (385.1-465.7)	Ref.	
	IFN	107	4,329	24,774	431.9 (357.3-522.1)	1.02 (0.83-1.25)	0.8521
Interferon + Direct antiviral agents	Control	97	3,507	17,163	559.3 (453.6-689.8)	Ref.	
	IFN+DAA	26	794	3,904	666.1 (453.2-978.9)	1.19 (0.77-1.84)	0.4325
Direct antiviral agents only	Control	545	26,662	77,271	702.7 (645.4-765.1)	Ref.	
	DAA	151	6,533	18,170	825.5 (703.1-969.2)	1.17 (0.98-1.41)	0.0793

HCC: Hepatocellular carcinoma, IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

The estimated cumulative incidence of HCC in the IFN-treated group was 137.2 cases/100kPY (95% CI: 98.1-192.1), higher than that of matched controls, with a statistically significant RR of 1.50 (95% CI: 1.02-2.22). In the IFN+DAA cohort, the incidence of HCC in the group treated for HCV infection was 589.2 cases/100kPY (95% CI: 391.3-887.1), statistically higher than matched controls, with a RR of 3.89 (95% CI: 2.26-6.69). Patients treated with DAA agents alone had an estimated incidence of 726.5 cases/100kPY (95% CI: 612.2-862.0), also higher than the matched controls, with a RR of 6.45 (95% CI: 4.90-8.49).

For new diagnoses of extrahepatic cancer, no increased incidence were observed between the treated groups and matched controls. In the case of the IFN cohort the RR was 1.02 (95% CI: 0.83-1.25), for the IFN+DAA cohort the RR was 1.19 (95% CI: 0.77-1.84) and for the DAA cohort the RR was 1.17 (95% CI: 0.98-1.41).

10.5 Estimated incidence of haematological malignancies

As described in the introduction, a relationship is known in patients diagnosed with HCV and the presence of haematological malignancies such as non-Hodgkin's lymphoma. A secondary objective was to study the incidence of hematologic malignancies among treated HCV patients and their controls. Table 13 shows the cumulative incidence for each group and the estimated RR with their 95%CI between patients treated for HCV and their controls within each cohort.

Table 13. Incidence and rate ratios of haematological malignancies in the three cohorts of HCV therapies and matched controls

Cohort	Group*	Events	Patients at risk	Follow-up (PY)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
Interferon Only	Control	22	19,376	107,207	20.5 (13.5 - 31.1)	Ref.	0.2726
	IFN	8	4,329	24,774	32.3 (16.2 - 64.6)	1.57 (0.70 - 3.54)	
IFN + DAA	Control	6	3,507	17,163	29.1 (12.2 - 69.9)	Ref.	0.9066
	IFN+DAA	1	794	3,904	25.6 (3.6 - 181.6)	0.88 (0.10 - 7.53)	
DAA	Control	31	26,662	77,271	40.1 (28.2 – 57.0)	Ref.	0.8147
	DAA	8	6,533	18,17	44.0 (22.0 - 88.1)	1.08 (0.50 - 2.39)	

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

Due to the low frequency of appearance of this type of malignant neoplasms, the effect size estimates are not very robust. No statistically significant change is observed between HCV-treated patients and their matched controls.

Table 14 shows the diagnosis of hematological malignancies diagnosed in the treated groups during the follow-up established by the study design.

Table 14. Patients in control groups with HIV or cirrhosis and a diagnosis of cancer

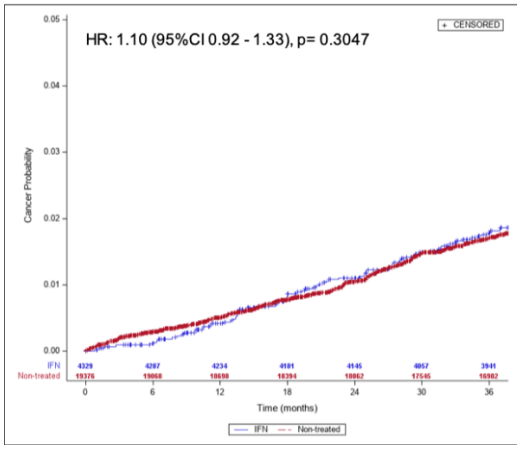
Group of treatment	Description of haematological malignancy	Number of cases
Interferon Only	Malignant Immunoproliferative Diseases	1
	Malignant Immunoproliferative Diseases	2
	Chronic Myeloid Leukemia	1
	Leukemia, unspecified	1
	Acute Myelofibrosis	1
	Multiple Myeloma	2
Interferon + Direct antiviral agents	Hodgkin's Disease, unspecified	1
Direct antiviral agents only	Hodgkin's Disease, unspecified	2
	Acute Myeloid Leukemia	1
	T-Cell Lymphoma, Peripheral and Cutaneous	1
	Multiple Myeloma	4

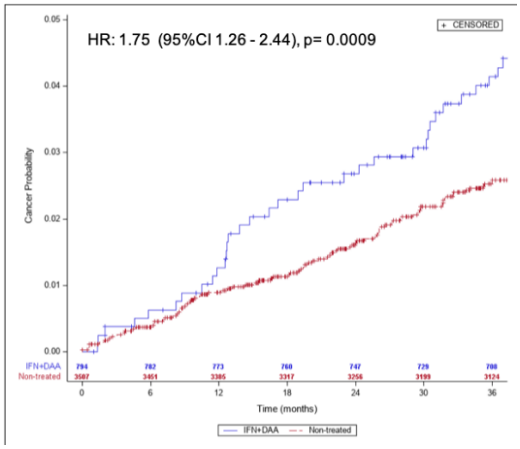
The most frequent diagnosis among treated patients was multiple myeloma, with 6 cases, followed by unspecified Hodgin's disease, with 3 cases.

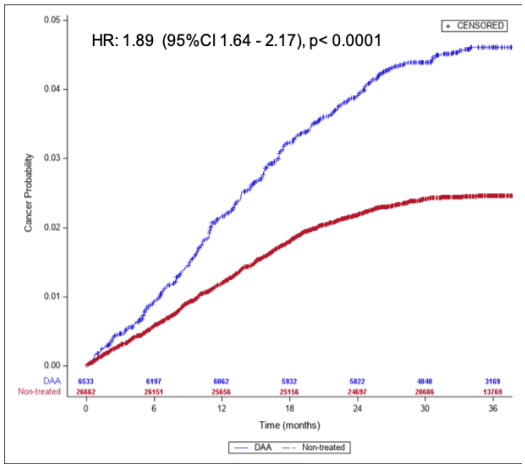
10.6 Evaluation of instant risk of cancer during follow-up

In order to evaluate a description of the instantaneous risk of cancer in time after the start of treatment of patients with HCV infection in relation to matched controls, graphical assessments were made following the Kaplan-Meier methodology, which can be observed in figures 6a, 6b and 6c. Additionally, the relative risk between groups was measured by estimating the Hazard Ratio (HR) from Cox proportional hazards regression models, as described in the methods section, in order to carry out a robustness analysis of the main results with Poisson regression models. In the three cohorts, the follow-up of patients was truncated to the first three years after the start of treatment for HCV infection.

Figures 6a, 6b, 6c. Kaplan-Meier plots and risk estimates of cancer from the Cox model for the three cohorts of HCV therapies and matched controls







In the case of the IFN cohort, the instantaneous risk curves of cancer overlapped and intertwined throughout the follow-up period, and the result of the comparison using HR was 1.1 (95% CI: 0.92; 1.33) without statistical significance. In the analysis of the IFN+DAA cohort, the instantaneous risk curve of cancer of the group of treated patients was always above that of the group of matched controls, and the separation was more evident from one year of follow-up onwards, with an estimated HR of 1.75 (95%CI: 1.26; 2.44) indicating a statistically significant increase in cancer risk. In the DAA cohort, treated patients had an instantaneous risk curve of cancer that was always higher than in the group of matched controls, with an estimated HR 1.89 (95% CI: 1.64; 2.17), which was statistically significant.

10.7 Sensitivity analysis excluding exposure to alcohol and tobacco from PS-matching

10.7.1 Clinical and sociodemographic characteristics

Matching was made using a two-step matching procedure executed on a sequential basis (113), first using an exact restriction for sex and DAP, and then propensity score (PS) matching using the logit calculation from a logistic regression model, previously described, excluding alcohol consumption or smoking as covariables.

As in the main PS-matching, all variables used to calculate the Propensity Score were balanced. Alcohol consumption and smoking were not well balanced in any of the three study cohorts: the standardized differences were 4.7%, 23.8%, and 19.1% for the INF, IFN+DAA, and DAA cohorts, respectively, for alcohol consumption and 26.1%, 38.0%, and 35.5% for the INF, IFN+DAA, and DAA cohorts, respectively for smoking. The remaining results are shown in table 15, where no notable changes in the descriptive results in relation to the cohorts of the main analysis were found.

Table15. Sensitivity analysis excluding tobacco and alcohol: Main characteristics of groups

Cohort	**Interferon (IFN) only**			**IFN + direct-acting antivirals (DAA)**			**Direct-acting antivirals (DAA) only**		
Group*	**controls**	**IFN**	**STD%**	**controls**	**IFN + DAA**	**STD%**	**controls**	**DAA**	**STD %**
No. of patients	**n = 19,109**	**n = 4,329**		**n = 3,425**	**n = 794**		**n = 26,328**	**n = 6,533**	
Age, years	37.1 (29.8-44.4)	36.5 (29.1-43.8)	5	44 (39.3-49.5)	43.8 (39-48.9)	4	45.3 (39.7-56.1)	45.5 (39.5-57)	2
Sex, n (%)			1			2			1
Men	8,867 (46.4)	2,024 (46.8)		2,266 (66.2)	531 (66.9)		15,780 (59.9)	3,948 (60.4)	
Women	10,242 (53.6)	2,305 (53.2)		1,159 (33.8)	263 (33.1)		10,548 (40.1)	2,585 (39.6)	
Height, cm	165 (158.5-172)	166 (159.5-173)	7	168 (160-174)	168 (160-174)	5	166,5 (158-173)	166 (158-172.3)	0
Missing, n	6,147	1,072		1,008	159		6,498	1,194	
Weight, kg	72 (62-83)	69.7 (60.9-80)	15	77 (67.7-87.4)	73.8 (65.3-83.3)	17	75.9 (66-85.9)	72,5 (63.2-82)	23
Missing, n	5,665	905		893	127		5,685	930	
BMI, kg/m^2	26.1 (23.2-29.5)	25 (22.4-28.2)	22	27.3 (24.8-30.6)	26.2 (23.9-29.1)	24	27.5 (24.8-30.7)	26.4 (23.6-29.4)	25
Missing, n	5,943	984		936	146		6,015	1,057	

Cohort	**Interferon (IFN) only**			**IFN + direct-acting antivirals (DAA)**			**Direct-acting antivirals (DAA) only**		
Group*	**controls**	**IFN**	**STD%**	**controls**	**IFN + DAA**	**STD%**	**controls**	**DAA**	**STD %**
No. of patients	**n = 19,109**	**n = 4,329**		**n = 3,425**	**n = 794**		**n = 26,328**	**n = 6,533**	
BMI, WHO categories, n (%)			21			23			24
Underweight (< 18.5)	270 (2.1)	85 (2.5)		14 (0.6)	1 (0.2)		153 (0.8)	74 (1.4)	
Normal weight (18.5-24.9)	5,055 (38.4)	1,590 (47.5)		664 (26.7)	240 (37)		5,285 (26)	1,960 (35.8)	
Pre-obesity (25.0-29.9)	4,888 (37.1)	1,147 (34.3)		1,116 (44.8)	273 (42.1)		8,859 (43.6)	2,258 (41.2)	
Obesity class I (30.0-34.9)	2,082 (15.8)	374 (11.2)		481 (19.3)	98 (15.1)		4,424 (21.8)	904 (16.5)	
Obesity class II (35.0-39.9)	620 (4.7)	113 (3.4)		165 (6.6)	30 (4.6)		1,188 (5.8)	223 (4.1)	
Obesity class III (> 40)	251 (1.9)	36(1.1)		49 (2)	6 (0.9)		404 (2)	57 (1)	
Missing, n	5,943	984		936	146		6,015	1,057	
Smoking, n (%)			26.1%			38.0%			35.3%
Non-Smoker	11,562 (60.5)	2,060 (47.6)		1,937 (56.6)	301 (37.9)		15,394 (58.5)	2,686 (41.1)	
Smoker or Ex-Smoker	7,547 (39.5)	2,269 (52.4)		1,488 (43.3)	493 (62.1)		10,934 (41.5)	3,847 (58.9)	
Alcohol Consumption, n (%)			4.7%			23.8%			19.1%
No	12,999 (68.0)	3,038 (70.2)		2,067 (60.4)	568 (71.5)		15,549 (59,2)	4,457 (68.2)	
Yes	6,110 (32.0)	1,291 (29.8)		1,358 (39.6)	226 (28.5)		10,779 (40.9)	2,076 (31.8)	

Cohort	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
Group* No. of patients	controls n = 19,109	IFN n = 4,329	STD%	controls n = 3,425	IFN + DAA n = 794	STD%	controls n = 26,328	DAA n = 6,533	STD %
MEDEA index, quintiles, n (%)			5			12			10.7
Q1	2,895 (20.6)	617 (19.5)		457 (17.8)	94 (16.2)		3,972 (20.1)	849 (17.8)	
Q2	2,866 (20.4)	632 (20)		513 (19.9)	96 (16.6)		3,856 (19.6)	867 (18.1)	
Q3	2,847 (20.2)	634 (20)		519 (20.2)	121 (20.9)		3,912 (19.8)	978 (20.5)	
Q4	2,705 (19.2)	659 (20.8)		517 (20.1)	118 (20.3)		3,922 (19.9)	964 (20.2)	
Q5	2,766 (19.6)	622 (19.7)		567 (22)	151 (26)		4,054 (20.6)	1,119 (23.4)	
Missing	5,030	1,165		852	214		6,612	1,756	
Diabetes mellitus, n (%)	1,107 (5.8)	285 (6.6)	3	355 (10.4)	132 (16.6)	18	3,242 (12.3)	1,251(19.1)	19
HIV infection, n (%)	78 (0.4)	287 (6.6)	34	18 (0.5)	207 (26.1)	81	98 (0.4)	2,775 (42.5)	100
Cirrhosis, n (%)	27 (0.1)	12 (0.3)	3	8 (0.2)	367 (46.2)	100	70 (0.3)	2,824 (43.2)	100

Variables for matching by PS method: sex, age (calculated from year of birth to index date), consumption of alcohol, consumption of smoking and geographical code from DAP and evaluated with Medea index quintiles

BMI: body mass index, IFN: Interferon, DAA: Direct antiviral agents, HIV: human immunodeficiency virus, Q1-Q5: 1 to 5 quintiles

IQR: Interquartile range [25-75 percentiles], |STD|: Absolute standardised differences (%), NA: Not applicable. Results shown as median (IQR) for quantitative variables and absolute frequencies with percentage otherwise.

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

10.7.2 Cancer risk assessment

The main results regarding cancer incidence, globally and stratified by site (hepatic and extrahepatic), among the treated groups in the three cohorts with this matching approach, using a PS calculation for matching excluding alcohol or smoking are shown in table 16.

Table 16. Sensitivity analysis excluding alcohol and tobacco: Cancer incidence and rate ratios

Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
Interferon only	Control	555	19,109	105,473.2	526.2 (482.1-574.3)	Ref.	
	IFN	141	4,329	24,776.0	596.1 (482.5-671.3)	1.08 (0.90-1.30)	0.004
IFN + DAA	Control	112	3,425	16,811,8	666.2 (554.1-800.9)	Ref.	
	IFN+DAA	49	764	3,903.4	1,255.3 (948.7-1,660.9)	1.88 (1.34-2.64)	0.0002
DAA	Control	591	26,328	76,346.7	774.1 (713.1-840.3)	Ref.	
	DAA	283	6,533	18,234.5	1,552.0 (1,380.1-1,744.1)	2.00 (1.74-2.31)	< 0.0001

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

10.7.3 Incidence of cancer

Since the groups of treated patients were the same, the estimated incidence of cancer for treated patients with HCV infection were identical to the main statistical analysis approach. For matched controls, the IFN group had a cancer incidence of 526.2 cases /100kPY (95%CI: 482.1; 574.3), the IFN+DAA had an incidence of 662.2 cases /100kPY (95%CI: 554.2; 800.9) and the DAA control group had an incidence of 774.1 cases /100kPY (95%CI: 713.1; 840.3), very close to the estimated incidence in the main analysis. This was comparable with the main analysis, but the results showed slightly lower point estimates for controls in the DAA+IFN and DAA cohorts.

The comparison of cumulative incidence between groups showed differences in the three cohorts in the sense of a slight increase in risk in patients treated for HCV infection. The Rate Ratios were 1.08 (95%CI: 0.90-1.30) for the IFN cohort, 1.88 (95%CI: 1.34-2.64) for the IFN+DAA cohort and 2.00 (95%CI: 1.74-2.31) for the DAA cohort. In this analysis, RR showed statistical significance for all three cohorts analysed.

Analysis of instantaneous risk, using Cox proportional hazards models, was close to the main analysis: HR 1.04 (95% CI 0.8-1.33, p-value = 0.776) in the IFN group, HR 1.81 (95% CI 1.20-2.73, p-value = 0.005) in the IFN+DAA group and HR 1.99 (95% CI 1.73-2.29, p-value < 0.0001) in the DAA group.

10.7.4 Estimated incidence of hepatocellular carcinoma and other cancers

The incidence of HCC diagnoses in matched controls was similar to that of the main analysis. For the diagnosis of extrahepatic cancer, some differences were observed (Table 17).

Table 17. Sensitivity analysis excluding alcohol and tobacco: Incidence and rate ratios of HCC and other cancer types

Cancer type Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
HCC							
Interferon only	Control	89	19,109	106,078.7	83.9 (67.9-103.7)	Ref.	
	IFN	34	4,329	24,781,3	137,2 (98.1-192.1)	1.64 (1.10-2.43)	0.020
Interferon + Direct antiviral agents	Control	24	3,425	16,806,7	142.8 (94.6-215.5)	Ref.	
	IFN+DAA	23	794	3,903.6	589.2 (391.5-886.6)	4.12 (2.32-7.35)	< 0.0001
Direct antiviral agents only	Control	74	26,328	77,244.3	95.8 (75.7-121.1)	Ref.	
	DAA	132	6,533	18,169.3	726.5 (612.5-861.6)	7.59 (5.67-10.15)	< 0.0001
Other cancer types							
Interferon only	Control	466	19,109	105,358.4	442.3 (402.0-486.7)	Ref.	
	IFN	107	4,329	24,774.3	431.9 (357.4-522)	0.97 (0.79-1.20)	0.800
Interferon + Direct antiviral agents	Control	88	3,425	16,813.1	523.4 (424.8-644.9)	Ref.	
	IFN+DAA	26	794	3,903.3	666.1 (453.5-978.2)	1.27 (0.82-1.97)	0.300
Direct antiviral agents only	Control	517	26,328	76,220.0	678.3 (621.3-740.5)	Ref.	
	DAA	151	6,533	18,291,9	825.5 (703.4-968.8)	1.21 (1.02-1.46)	0.030

HCC: Hepatocellular carcinoma, IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

Rate Ratios estimations and their 95% CI for HCC diagnosis are slightly higher than in the main analysis, but comparable and statistically significant, as in the main analysis. In the case of the Rate Ratios estimates for extrahepatic cancer, results were very close to the main analysis, but the estimate for the DAA cohort was statistically significant, with a RR of 1.21 (95%CI: 1.02-1.46).

10.7.5 Role of HIV-HCV co-infection and cirrhosis

Given the objective of the main analysis, focused on the possible changes in the conclusions due to not considering the two toxic habits collected in the registries (alcohol and smoking), the patients treated for the infection are the same, meaning that the analysis of the influence of co-infection by HIV or the presence of cirrhosis as a comorbidity did not change.

10.8 Sensitivity analysis excluding tobacco smoking from PS matching

10.8.1 Clinical and sociodemographic characteristics

Matching was made using a two-step matching procedure executed on a sequential basis (113), first using an exact restriction for sex and DAP, and then propensity score (PS) matching using the logit calculation from a previously-described logistic regression model including alcohol consumption as a risk factor, but not tobacco smoking (Table 18)

Table 18. Sensitivity analysis excluding tobacco: Main characteristics of groups

Cohort	**Interferon (IFN) only**			**IFN + direct-acting antivirals (DAA)**			**Direct-acting antivirals (DAA) only**		
Group*	**controls**	**IFN**	**STD%**	**controls**	**IFN + DAA**	**STD%**	**controls**	**DAA**	**STD %**
No. of patients	**n = 19,899**	**n = 4,329**		**n = 3,621**	**n = 794**		**n = 27,548**	**n = 6,533**	
Age, years	36.8 (29.6-44.1)	36.5 (29.1-43.8)	3	44 (39.2-49.5)	43.8 (39-48.9)	2	45.3 (39.6-56.3)	45.5 (39.5-57)	2
Sex, n (%)			1			0			0
Male	9,207 (46.3)	2,024 (46.8)		2,418 (66.8)	531 (66.9)		16,521 (60)	3,948 (60.4)	
Female	10,692 (53.7)	2,305 (53.2)		1,203 (33.2)	263 (33.1)		11,027 (40)	2,585 (39.6)	
Height, cm	165 (158.7-172)	166 (159.5-173)	6	168 (160-174)	168 (160-174)	5	166 (158-173)	166 (158-172.3)	2
Missing, n	4,939	1,072		780	159		5,464	1,194	
Weight, kg	72.5 (62.2-83.5)	69.7 (60.9-80)	18	77.1 (67.4-87)	73.8 (65.3-83.3)	18	75.9 (66.3-86)	72.5 (63.2-82)	24
Missing, n	4,357	905		650	127		4,530	930	
BMI, kg/m^2	26.1 (23.3-29.6)	25 (22.4-28.2)	24	27.3 (24.6-30.6)	26.2 (23.9-29.1)	23	27.5 (24.8-30.8)	26.4 (23.6-29.4)	27
Missing, n	4,656	984		707	146		4,894	1,057	

Cohort	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
Group* No. of patients	controls n = 19,899	IFN n = 4,329	STD%	controls n = 3,621	IFN + DAA n = 794	STD%	controls n = 27,548	DAA n = 6,533	STD %
BMI, WHO categories, n (%)			23			23			25
Underweight (< 18.5)	306 (2)	85 (2.5)		20 (0.7)	1 (0.2)		161 (0.7)	74 (1.4)	
Normal weight (18.5-24.9)	5,763 (37.8)	1,590 (47.5)		794 (27.2)	240 (37)		5,961 (26.3)	1,960 (35.8)	
Pre-obesity (25.0-29.9)	5,660 (37.1)	1,147 (34.3)		1,246 (42.8)	273 (42.1)		9,804 (43.3)	2,258 (41.2)	
Obesity class I (30.0-34.9)	2,405 (15.8)	374 (11.2)		605 (20.8)	98 (15.1)		4,761 (21)	904 (16.5)	
Obesity class II (35.0-39.9)	792 (5.2)	113 (3.4)		180 (6.2)	30 (4.6)		1,470 (6.5)	223 (4.1)	
Obesity class III (> 40)	317 (2.1)	36 (1.1)		69 (2.4)	6 (0.9)		497 (2.2)	57 (1)	
Missing, n	4,656	984		707	146		4,894	1,057	
Smoking, n (%)			23			34			34
Non-Smoker	11,731 (59)	2,060 (47.6)		1,984 (54.8)	301 (37.9)		15,999 (58.1)	2,686 (41.1)	
Smoker or Ex-Smoker	8,168 (41)	2,269 (52.4)		1,637 (45.2)	493 (62.1)		11,549 (41.9)	3,847 (58.9)	
Alcohol Consumption, n (%)			9			10			10
No	9,142 (45.9)	1,886 (43.6)		1,889 (52.2)	395 (49.7)		13,572 (49.3)	3,090 (47.3)	
Yes	6,288 (31.6)	1,291 (29.8)		1,091 (30.1)	226 (28.5)		9,487 (34.4)	2,076 (31.8)	

Cohort	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
Group*	**controls**	**IFN**	**STD%**	**controls**	**IFN + DAA**	**STD%**	**controls**	**DAA**	**STD %**
No. of patients	**n = 19,899**	**n = 4,329**		**n = 3,621**	**n = 794**		**n = 27,548**	**n = 6,533**	
MEDEA index, quintiles, n (%)			6			10			8
Q1	2,903 (19.6)	617 (19.5)		488 (17.8)	94 (16.2)		4,022 (19.3)	849 (17.8)	
Q2	2,973 (20.1)	632 (20)		477 (17.4)	96 (16.6)		4,117 (19.7)	867 (18.1)	
Q3	2,948 (19.9)	634 (20)		564 (20.6)	121 (20.9)		4,104 (19.7)	978 (20.5)	
Q4	2,864 (19.4)	659 (20.8)		599 (21.8)	118 (20.3)		4,249 (20.4)	964 (20.2)	
Q5	3,111 (21)	622 (19.7)		615 (22.4)	151 (26)		4,369 (20.9)	1,119 (23.4)	
Missing	5,100	1,165		878	214		6,687	1,756	
Diabetes mellitus, n (%)	1,298 (6.5)	285 (6.6)	0	420 (11.6)	132 (16.6)	14	3,716 (13.5)	1,251 (19.1)	15
HIV infection, n (%)	100 (0.5)	287 (6.6)	34	19 (0.5)	207 (26.1)	81	95 (0.3)	2,775 (42.5)	100
Cirrhosis, n (%)	36 (0.2)	12 (0.3)	2	11 (0.3)	367 (46.2)	100	114 (0.4)	2,824 (43.2)	100

Variables for matching by PS method: sex, age (calculated from year of birth to index date), consumption of alcohol, smoking and geographical code from DAP and evaluated with Medea index quintiles

BMI: body mass index, IFN: Interferon, DAA: Direct antiviral agents, HIV: human immunodeficiency virus, Q1-Q5: 1 to 5 quintiles

IQR: Interquartile range [25-75 percentiles], |STD|: Absolute standardised differences (%), NA: Not applicable. Results showed as median (IQR) for quantitative variables and absolute frequencies with percentage otherwise.

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

The variables used to calculate the Propensity Score, as well as the main characteristics of the patients treated for HCV infection and their corresponding controls, are depicted in Table 18 above.

Since exposure to smoking was not included in the logistic regression model, this key factor was not well balanced. The standardized differences for smoking habit were 23%, 34%, and 34% for the INF, IFN+DAA, and DAA cohorts, respectively.

10.8.2 Cancer risk assessment

The main results regarding cancer incidence among the treated groups for the three cohorts with this matching approach, using only alcohol consumption as a toxic risk, are shown in table 19.

Table 19. Sensitivity analysis excluding tobacco: Cancer incidence and rate ratios

Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
Interferon only	Control	571	19,899	110,816	514.4 (472.3-560.1)	Ref.	
	IFN	141	4,329	24,774	569.1 (482.5-671.4)	1.11 (0.92-1.33)	0.2735
IFN + DAA	Control	142	3,621	17,893	782.5 (656.2-933.0)	Ref.	
	IFN+DAA	49	794	3,904	1,255.3 (947.9-1,662.2)	1.60 (1.16-2.22)	0.0046
DAA	Control	636	27,548	80,116	785,1 (724,3-851,0)	Ref.	
	DAA	283	6,533	18,170	1,552.0 (1,380.1-1,745.3)	1.98 (1.72-2.28)	< 0.0001

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

10.8.3 Incidence of cancer

This sensitivity analysis, in terms of patients included, only showed changes in the matched control groups. Therefore, the estimated incidence in the groups of treated patients were the same as in the main analysis.

In the IFN cohort, the control group had a lower cancer incidence of 514.4 cases/100kPY (95%CI: 472.3; 560.1) than the control group in the IFN+DAA cohort, 782.5 cases/100kPY (95%CI: 656.2; 933.0) and the control group in the DAA cohort, 785.1 cases/100kPY (95%CI: 724.3; 851.0), very close to the estimated incidence in the main analysis.

In view of the above data, the Rate Ratio estimates were also comparable with the main analysis. As for the estimates of change in the relative risk, these were RR: 1.11 (95%CI: 0.92-1.33), RR: 1.60 (95%CI: 1.16-2.22) and RR: 1.98 (95%CI: 1.72-2.28) for the IFN, IFN+DAA and DAA cohorts, maintaining the statistical significance of the increased risk, in the groups of patients treated with the IFN+DAA or DAA strategy.

The analysis using Cox proportional hazards models was led to similar risk estimates to those of the Poisson model, with HR: 1.11 (95% CI: 0.92 - 1.33) for the IFN cohort, HR: 1.58 (1.14 - 2.18) for the INF+DAA cohort and 1.94 (95% CI: 1.69 - 2.24) for the DAA cohort.

10.8.4 Estimated incidence of hepatocellular carcinoma and other cancers

In this sensitivity analysis, the incidence of HCC diagnosis in matched control patients was comparable with the main analysis (Table 20).

Table 20. Sensitivity analysis excluding tobacco: Incidence and rate ratios of HCC and other cancer types

Cancer type Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
HCC							
Interferon only	Control	91	19,899	110,816	82.1 (66.9-100.9)	Ref.	
	IFN	34	4,329	24,774	137.2 (98.1-192.1)	1.67 (1.14-2.44)	0.0082
Interferon + Direct antiviral agents	Control	27	3,621	17,893	145.3 (99.5-212.2)	Ref.	
	IFN+DAA	23	794	3,904	589.2 (391.3-887.1)	4.05 (2.30-7.15)	< 0.0001
Direct antiviral agents only	Control	83	27,548	80,116	103.6 (83.6-128.3)	Ref.	
	DAA	132	6,533	18,170	726.5 (612.2-862.0)	7.01 (5.33-9.22)	< 0.0001
Other cancer types							
Interferon only	Control	480	19,899	110,816	432.3 (394.4-473.7)	Ref.	
	IFN	107	4,329	24,774	431.9 (357.3-522.1)	1.00 (0.81-1.23)	0.9939
Interferon + Direct antiviral agents	Control	115	3,621	17,893	637.1 (524.9-773.3)	Ref.	
	IFN+DAA	26	794	3,904	666.1 (453.2-978.9)	1.05 (0.68-1.60)	0.8377
Direct antiviral agents only	Control	553	27,548	80,116	681.5 (625.1-743.0)	Ref.	
	DAA	151	6,533	18,170	825.5 (703.1-969.2)	1.21 (1.01-1.45)	0.0374

HCC: Hepatocellular carcinoma, IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups were control patients matched for each hepatitis C virus therapy regimen*

The estimates of the Rate Ratio and their 95% CI for the diagnosis of HCC are comparable and statistically significant in the three cohorts, as in the main analysis.

In the case of the Rate Ratio estimates for extrahepatic cancer, it was also numerically comparable with the main analysis, but the estimate for the DAA cohort was statistically significant, with a RR of 1.21 (95%CI: 1.01-1.45).

10.8.5 Role of HIV-HCV co-infection and cirrhosis

Since the groups of patients treated for HCV infection in this sensitivity analysis is the same as in the main analysis, the results of the stratified analysis for the presence of co-HIV infection or diagnoses of cirrhosis are the same.

10.9 Sensitivity analysis excluding alcohol from PS matching

10.9.1 Clinical and sociodemographic characteristics

Matching was made using a two-step matching procedure executed on a sequential basis (113), first using an exact restriction for sex and DAP, and then propensity score (PS) matching using the logit calculation from a previously-described logistic regression model including smoking as a toxic risk factor but not alcohol consumption (Table 21).

Table 21. Sensitivity analysis excluding alcohol: Main characteristics of HCV patients and comparison with controls

Cohort	**Interferon (IFN) only**			**IFN + direct-acting antivirals (DAA)**			**Direct-acting antivirals (DAA) only**		
Group* No. of patients	**Controls n = 19,432**	**IFN n = 4,329**	**STD %**	**Controls n = 3,540**	**IFN + DAA n = 794**	**STD %**	**Controls n = 29,953**	**DAA n = 6,533**	**STD %**
Age, years	36.8 (29.5-44.2)	36.5 (29.1-43.8)	3	43.9 (39.3-49.1)	43.8 (39-48.9)	2	45.1 (39.5-55.6)	45.5 (39.5-57)	5
Sex, n (%)			1			1			0
Male	9,030 (46.5)	2,024 (46.8)		2,385 (67.4)	531 (66.9)		16,305 (60.5)	3,948 (60.4)	
Female	10,402 (53.5)	2,305 (53.2)		1,155 (32.6)	263 (33.1)		10,648 (39.5)	2,585 (39.6)	
Height, cm	165 (158.8-172)	166 (159.5-173)	6	168 (160-174)	168 (160-174)	4	166 (158-173)	166 (158-172.3)	4
Missing, n	5,464	1,072		848	159		5,358	1,194	
Weight, kg	71.8 (61.8-83.2)	69.7 (60.9-80)	15	77.8 (67.5-87.7)	73.8 (65.3-83.3)	19	76.2 (66.2-86.5)	72.5 (63.2-82)	25
Missing, n	4,976	905		717	127		4,472	930	
BMI, kg/m^2	26 (23.1-29.4)	25 (22.4-28.2)	20	27.4 (24.7-30.7)	26.2 (23.9-29.1)	22	27.5 (24.7-30.7)	26.4 (23.6-29.4)	25
Missing, n	5,232	984		773	146		4,782	1,057	

			20			22		24
BMI, WHO categories, n (%)								
Underweight (< 18.5)	310 (2.2)	85 (2.5)		728 (26.3)	1 (0.2)		191 (0.9)	74 (1.4)
Normal weight (18.5-24.9)	5,560 (39.2)	1,590 (47.5)		31 (1.1)	240 (37)		5,926 (26.7)	1,960 (35.8)
Pre-obesity (25.0-29.9)	5,180 (36.5)	1,147 (34.3)		1,206 (43.6)	273 (42.1)		9,489 (42.8)	2,258 (41.2)
Obesity class I (30.0-34.9)	2,180 (15.4)	374 (11.2)		585 (21.1)	98 (15.1)		4,732 (21.3)	904 (16.5)
Obesity class II (35.0-39.9)	715 (5)	113 (3.4)		156 (5.6)	30 (4.6)		1,394 (6.3)	223 (4.1)
Obesity class III (> 40)	255 (1.8)	36 (1.1)		61 (2.2)	6 (0.9)		439 (2)	57 (1)
Missing, n	5,232	984		773	146		4,782	1,057
Smoking, n (%)			5			4		6
Non-Smoker	8,780 (45.2)	2,060 (47.6)		1,269 (35.8)	301 (37.9)		10,309 (38.2)	2,686 (41.1)
Smoker or Ex-Smoker	10,652 (54.8)	2,269 (52.4)		2,271 (64.2)	493 (62.1)		16,644 (61.8)	3,847 (58.9)
Alcohol Consumption, n (%)			30			47		35
No	5,885 (30.3)	1,886 (43.6)		999 (28.2)	395 (49.7)		8,351 (31)	3,090 (47.3)
Yes	6,888 (35.4)	1,291 (29.8)		1,550 (43.8)	226 (28.5)		12,297 (45.6)	2,076 (31.8)
MEDEA index, quintiles, n (%)			5			14		9
Q1	2,848 (19.9)	617 (19.5)		456 (17.2)	94 (16.2)		4,014 (19.9)	849 (17.8)
Q2	2,840 (19.8)	632 (20)		543 (20.5)	96 (16.6)		3,924 (19.4)	867 (18.1)
Q3	2,945 (20.5)	634 (20)		532 (20.1)	121 (20.9)		3,888 (19.3)	978 (20.5)
Q4	2,754 (19.2)	659 (20.8)		575 (21.7)	118 (20.3)		4,190 (20.8)	964 (20.2)
Q5	2,944 (20.5)	622 (19.7)		545 (20.6)	151 (26)		4,161 (20.6)	1,119 (23.4)

Missing	5,101	1,165		889	214		6,776	1,756	
Diabetes mellitus, n (%)	1,190 (6.1)	285 (6.6)	2	396 (11.2)	132 (16.6)	16	3,589 (13.3)	1,251 (19.1)	16
HIV infection, n (%)	79 (0.4)	287 (6.6)	34	17 (0.5)	207 (26.1)	81	106 (0.4)	2,775 (42.5)	100
Cirrhosis, n (%)	43 (0.2)	12 (0.3)	1	20 (0.6)	367 (46.2)	100	93 (0.3)	2,824 (43.2)	100

Variables for matching by PS method: sex, age (calculated from year of birth to index date), consumption of alcohol, consumption of smoking and geographical code from DAP and evaluated with Medea index quintiles

BMI: body mass index, IFN: Interferon, DAA: Direct antiviral agents, HIV: human immunodeficiency virus, Q1-Q5: 1 to 5 quintiles

IQR: Interquartile range [25-75 percentiles], |STD|: Absolute standardised differences (%), NA: Not applicable. Results shown as median (IQR) for quantitative variables and absolute frequencies with percentage otherwise.

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

Alcohol consumption presents a high imbalance, with an STD% > 30 in all three cohorts. Results are shown in table 21 above.

10.9.2 Cancer risk assessment

The main results regarding cancer incidence among the treated groups for the three cohorts with this matching approach, using only the tobacco as the toxic risk habit, are shown in table 22.

Table 22. Sensitivity analysis excluding alcohol: Cancer incidence and rate ratios

Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
Interferon Only	Control	499	19,432	108,199	460.3 (420.6-503.7)	Ref.	
	IFN	141	4,329	24,774	596.1 (482.5-671.4)	1.24 (1.03-1.49)	0.0236
IFN + DAA	Control	133	3,540	17,525	758.9 (636.9-904.3)	Ref.	
	IFN+DAA	49	794	3,904	1,255.3 (947.9-1,662.2)	1.65 (1.20-2.28)	0.0022
DAA	Control	643	26,953	78,462	818.7 (753.1-883.4)	Ref.	
	DAA	283	6,533	18,170	1,552.0 (1,380.1-1,745.3)	1.90 (1.66-2.19)	< 0.0001

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

10.9.3 Incidence of cancer

In the IFN cohort, the control group had a lower cancer incidence of 460.3 cases/100kPY (95%CI: 420.6-503.7) than the control group in the IFN+DAA cohort, 758.9 cases/100kPY (95%CI:636.9-904.3) and the control group in the DAA cohort, 818.7 cases/100kPY (95%CI:753.1-883.4), an estimated incidence similar to that of the main analysis. As expected, groups of patients treated for HCV infection did not change.

Given this slight change in the patients included in the control groups, substantial changes were not expected. In this context the Rate Ratio calculations were RR 1.24 (95%CI: 1.03-1.49, RR 1.65 (95%CI: 1.20-2.28) and RR 1.90 (95%CI: 1.66-2.19) for the IFN, IFN+DAA and DAA cohorts, respectively. There was statistical significance for increased risk in the whole cohort.

The robustness analysis using Cox proportional hazards models was consistent with estimates of RR, in terms of feasible conclusions, with those obtained in the main analysis, with risk estimates of HR: 1.23 (95%CI: 1.02 - 1.49) for the IFN cohort, HR: 1.65 (95%CI: 1.19 - 2.29) for the INF+DAA cohort and 1.89 (95%CI: 1.64 - 2.17) for the DAA cohort, which were statistically significant for each cohort.

10.9.4 Estimated incidence of hepatocellular carcinoma and other cancers

In this sensitivity analysis, the incidence of an HCC diagnosis in the matched control patients was comparable with the main analysis (Table 23).

Table 23. Incidence and rate ratios of HCC and other cancer types for the three cohorts of HCV therapies and matched controls

Cancer type Cohort	Group*	Events	Patients at risk	Follow-up (person-years)	Incidence per 100k/PY (95%CI)	Rate Ratio (95%CI)	p-value
HCC							
Interferon only	Control	94	19,432	108,199	86.0 (70.3-105.1)	Ref.	
	IFN	34	4,329	24,774	137.2 (98.1-192.1)	1.60 (1.09-2.35)	0.0171
Interferon + Direct antiviral agents	Control	25	3,540	17,525	142.7 (95.5-213.2)	Ref.	
	IFN+DAA	23	794	3,904	589.2 (391.3-887.1)	4.13 (2.31-7.40)	< 0.0001
Direct antiviral agents only	Control	91	26,953	78,462	113.4 (92.0-139.8)	Ref.	
	DAA	132	6,533	18,170	726.5 (612.2-862.0)	6.40 (4.89-8.3)	< 0.0001
Other cancer types							
Interferon only	Control	405	19,432	108,199	374.3 (338.8-413.6)	Ref.	
	IFN	107	4,329	24,774	431.9 (357.3-522.1)	1.16 (0.93-1.42)	0.1833
Interferon + Direct antiviral agents	Control	108	3,540	17,525	616.3 (508.2-747.3)	Ref.	
	IFN+DAA	26	794	3,904	666.1 (453.2-978.9)	1.08 (0.71-1.64)	0.7141
Direct antiviral agents only	Control	552	26,953	78,462	702.3 (645.0-764.6)	Ref.	
	DAA	151	6,533	18,170	825.5 (703.1-969.2)	1.18 (0.98-1.41)	0.0783

HCC: Hepatocellular carcinoma, IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, PY: person-years, 100k/PY: 100,000 patient-years

**Control groups are control patients matched for each hepatitis C virus therapy regimen*

The estimates of the Rate Ratio and their 95% CI for the diagnosis of HCC are comparable and statistically significant in the three cohorts, as in the main analysis.

In the case of the Rate Ratio estimates for extrahepatic cancer, it is also numerically comparable with the main analysis, but the estimate for the DAA cohort was statistically significant, with a RR of 1.21 (95%CI: 1.01-1.45).

10.9.5 Role of HIV-HCV co-infection and cirrhosis

As previously described, patients in the groups treated for HCV infection were the same as in the main analysis, so this analysis was not applicable.

11 Discussion

11.1 Context

Follow-up investigations in patients with HCV infection who have received DAA treatment focused on the evolution of liver function and the impact on the development of liver cancer. The benefits of DAA therapy are known in these domains, as viral eradication prevents progression from chronic hepatitis to cirrhosis. In fact, if cirrhosis has not reached a point of no return, patients can avoid further hepatic decompensation or bleeding due to portal hypertension.

The risk of liver cancer is reduced when patients have been treated at a pre-cirrhotic stage, while the cancer risk seems to stay stable despite viral eradication when cirrhosis is already present (64–66).

However, data on extrahepatic cancer, solid or haematologic, is still very limited, and it has been suggested there is a need to ascertain whether the cancer incidence is the same as in the general population or whether there is an increased cancer risk in HCV patients. A French study found that extrahepatic cancer had become the major cause of death in this cohort of HCV patients (72), pointing to the need to ascertain whether cancer incidence is the same as that of the global population, or whether there is an increased cancer risk in HCV patients.

DAA has demonstrated benefits, and accordingly, the life expectancy of HCV treated patients has increased. Thus, their clinical follow-up should no longer focus intensely on the evolution of liver disease, and attention needs to be paid to events with a low incidence. These were not previously seen as a consequence of the competing risk of death, or regarded as of enough importance as compared with progressive liver function impairment, transition to end-stage disease, need for consideration for transplant or just palliative care. As mentioned, the risk of

liver cancer does not diminish despite an HCV cure, and data about the development of extrahepatic cancer are very limited.

It is known that HCV infection is associated with an increased risk of B-cell non-Hodgkin lymphoma as well as several cancer types with the most frequent locations being the upper aerodigestive tract, such as the oesophagus and lung, or haematological tumours such as non-Hodgkin's lymphoma (68–71). The oncogenic hit leading to hepatic malignant transformation may have already taken place at the time of DAA therapy (61,78), and therefore the incidence of liver cancer may not be reduced at least during the first years after a plausible viral eradication.

However, since HCV eradication is associated with a disruption of immune surveillance, as shown by the potential reactivation of hepatitis B virus (HBV) or herpes virus (79,80), such events may allow malignant clones at any site to emerge and accelerate their clinical recognition. This mechanism cannot be excluded as a partial explanation of our findings in this large population-based investigation.

11.2 Methods and study design

The lack of applicability of experimental designs to the study of long-term complications of DAA has been already exposed before. Therefore, the most appropriate methodology for approaching the study of a potential association of increased cancer risk in patients treated with antivirals for HCV infection at this time is an observational analytical population study. A retrospective cohort study was designed, using already available health data records. Other alternative designs could have considered case-control designs, by identifying cancer cases and ascertaining HCV antiviral exposure retrospectively, or transversal studies; however, both case-control and transversal designs are less efficient when exposures are relatively infrequent in the general population, as it is the case for HCV antivirals, with an overall number of exposed subjects below 12,000 in an

overall population of 7.5 million inhabitants. Other alternatives requiring prospective collection of data would make no sense, in the context of Public Health campaigns aimed to treat most people infected with HCV in the shortest possible period of time: time to obtain answers would be a loss of opportunity to act. Thus, a retrospective cohort seemed to be the most feasible choice.

A number of difficulties raised. The most prominent and complex to handle was the fact that the treatment strategy varied along the study period, so that IFN treatments, which were used first, were not fully effective in eradication, were poorly tolerated, and thus used only for young and very fit subjects, who due to their disease status, they required to be treated. Later, DAA appeared with excellent results and good tolerability, changing the scope of patients that could be treated. However, treatments were initially extremely expensive, and as such a limited amount was available for use. Prioritization led to a progressive deployment of the treatment strategy that started by patients with poor condition, co-infections for HIV, and severe disease that were not fit for IFN treatments; as these were completed, more fit patients and with less severe disease were progressively up taken.

Besides of potential biases derived from indication of treatments, it is likely that the baseline risk of cancer of the cohorts is far from stable, posing difficulties to statistical approaches, but also – and more importantly – to clinical interpretation and extrapolation of results to clinical practice. For the changing risks along time, the selection of contemporary controls for each cohort was aimed to partially account for external exposures and imbalances. Regarding the changes in clinical practice, a number of measures aimed to account for indication biases were implemented. Of all the potential options, IVA was ruled at the first stage due to the difficulties in identifying an appropriate instrumental variable in a retrospective design based on registries. Traditional methods were ruled out because of a number of critical limitations (83) (Table 7) and PS methodology appeared to be the most suitable to cope with the observational design.

11.3 Comparability of control arms and treated patients

As described in the clinical-demographic variables, patients treated with IFN might have had a different profile from the rest of the groups of patients treated for HCV infection. They were somewhat younger, there were more women, a lower proportion of patients who were smokers and, clearly, a lower proportion of HIV coinfection and diagnosed of cirrhosis. This fact is also appreciated in the description of the laboratory variables: the group of patients treated with IFN had laboratory values related to their liver function that were more similar to matched controls than to the patients treated in the IFN+DAA and DAA groups.

In the temporal evolution of patients who started treatment with the three strategies, as indicated in the description of the baseline characteristics, in the group treated only with IFN there was a high percentage of patients who did not undergo tests for the classification of the degree of fibrosis. In the IFN+DAA treatment group, the most frequent category was F4 in all the years of inclusion in this study. In the group of patients treated with DAA, the degree of fibrosis F4 also predominated, most evidently in 2014 and 2015.

Overall, due to the known limitation of the use of IFN, there was almost certainly a strong indication bias, so a comparative analysis between IFN and groups treated with the presence of DAA agents, such as IFN+DAA or DAA, is also not suitable for matching or other techniques such as the IPTW method

11.4 Estimation of incidence and main results

In general terms, in the groups of patients treated for HCV infection in this large population-based study, cancer incidence was higher in groups treated totally or partially with DAAs (IFN+DAA or DAA) than in groups treated with combinations based on IFN, with a magnitude of more than double. Also, the incidence in control

groups was heterogeneous with a lower cancer incidence in the IFN cohort than in the IFN+DAA cohort and in DAA cohort. This is consistent with the differences previously described in the pattern of use and the selection of patients without or with comorbidities and exposure to toxic habits such as smoking and consumption of alcohol for treatment with IFN or DAA in clinical practice.

This comparison cannot be considered valid due to the wide clinical differences found between patients in the IFN group in relation to the IFN+DAA and DAA groups. A consequence of these wide differences is that we could not make valid comparisons with techniques such as those explained in the methodology, such as PS-matching or the use of weighting (IPTW). These differences affect factors known to be modifiers of cancer incidence, such as smoking, co-HIV infection and cirrhosis, in addition to the fact that patients treated with IFN were somewhat younger and with a predominance of women compared with the other two treated groups.

With respect to the relation to the comparison between patients treated for HCV infection and matched controls, these differences could be assessed. The increased incidence was statistically significant in the DAA and the IFN+DAA cohorts when the treated groups were compared with their matched control cohorts formed by individuals without either HCV therapy or known HCV carriage.

As expected, the increased cancer risk in patients with HCV included both hepatic and extrahepatic malignancies, but mainly hepatic cancer. This is also the case in patients with chronic liver disease related to alcohol consumption (116) and to non-alcoholic steatohepatitis (117) or just fatty liver disease. Chronic inflammation in these conditions is responsible for an increased risk that is further intensified by coexisting oncogenic factors such as smoking, specific dietary habits or the environment (118–123).

From a methodological standpoint, the ideal control groups would have included untreated HCV-infected patients, but these were not identified in administrative

databases. In addition, HCV carriers were actively sought during the study period in order to be treated, so that probable cross-over between cohorts occurred. The chosen approach of selecting healthy subjects provides pragmatic information, in that it identifies a population with a higher cancer risk, deserving active surveillance regardless of treatment causality of the observed risks.

Since such factors may vary across the country, the selection of controls took into account the most relevant factors, such as the geographical code (DAP), and confounders and concentrated on the factors related to HCV management. The stratification of patients according to the treatment received permitted identification of an increased cancer incidence in the cohorts treated with DAA, either after initial IFN-based therapies or as the only treatment approach. While environmental contaminants may be regarded as controlled by the geographical matching, and smoking and alcohol consumption were adjusted in the propensity model, no reliable data for other toxic exposures were systematically available for adjustment and this is a limitation of the present study.

Finally, the analyses of the Poisson and the Cox proportional hazards models were very closely similar risk estimates, indicating robustness and model independency to the study conclusions.

11.5 Similarity of the control groups with the general reference population

HIV prevalence was higher in treated patients than in matched controls, signalling the known fact that HIV frequently accompanies HVC infection, but is relatively infrequent in the general population. Cirrhosis prevalence was also higher in some group of treated patients compared to their matched controls; since only fit patients could be treated with IFN, cirrhosis was well balanced in the IFN cohort, but this was not the case in the other two cohorts. The strategy of treatment with DAA prioritised initially to treat severely affected patients with advanced fibrosis,

leading to a prevalence of cirrhosis of 46.2% in the IFN+DDA group, and 43.2% in patients treated only with DAA, while controls have very low rates.

The evaluation of the degree of fibrosis was anecdotal in the groups of matched controls and also for the IFN group. Considering that most of the IFN cohort was treated before in time, and fibroscan data was not yet systematically collected, in the group of patients treated with IFN, this classification was only available in 117 cases, so it could not be correctly evaluated. Both viral load and IgG titers assessment are not well characterized in the IFN group, due to lack of systematic collection of information at the time these treatments were mostly used.

Overall, the results of the analyses that indicate, among other aspects, the degree of liver function impairment show how the IFN group was closer to the matched controls than in the IFN+DAA and DAA cohorts. This effect can be explained by the same reasons as the differences between groups of the same cohort for HIV coinfection and the presence of cirrhosis.

This kind of population-based research in a subgroup of patients with a defined entity (HCV infection in this case) requires that the control group against which it should be compared should be representative of the global population and not be skewed. The validity of our control population is supported by the fact that the cancer incidence in the control population reproduces the figures of the registry maintained by the *Asociación Española contra en Cáncer* (Spanish Association Against Cancer, AECC) (124). This splits Spain into separate areas and reports cancer incidence in citizens over time, and we selected citizens aged > 15 years.

In 2012, the registry reported that 39,237 new cases of cancer were diagnosed in Catalonia, corresponding to an incidence of 614 cases per 100,000 person-years. Stratified by sex, the incidence was 713 cases per 100,000 person-years in males and 519 cases in females. We found an incidence in the control population of 514.9, 710.8 and 815.3 cases per 100,000 person-years for IFN-based treatment, IFN+DAA and DAA, respectively. These incidences are higher than those described

in AECC registries. This increase may be explained, at least partially by the selection of controls for the main analysis, which was conditioned by smoking and alcohol consumption in the population with HCV infection. In another epidemiological evaluation in Catalonia (125), the percentage of smokers was around 40% in 2012 and was similar to Spain (126) for daily consumption in the last 30 days, between 12% to 29% less than for our selected controls. In relation to alcohol consumption, the proportion of subjects with daily consumption in the last 30 days (126) was close to 10% and binge drinking around 20% of subjects in the last year. Notably, in the selected controls, consumption of alcohol was just under 30%.

11.6 Sensitivity analyses: Changes in incidence of matched controls

After discussing the ideal control groups and how we tried to control confounding factors such as environmental ones, access to health systems or the presence of risk factors for toxic habits available in population registries, the similarity of the controls was assessed and compared with the reference population.

All this leads to the question of the possible influence of the selection criteria of the control groups on the results. For this, three sensitivity analyses were considered in relation to the selection of the control groups matched with the treated patients.

The sensitivity analyses were made to assess the effect of changing the selection criteria of matched controls, taking into account or not the distribution of the two known risk toxic factors.

Not considering smoking or alcohol consumption in the selection of controls had almost no influence on the control group of the IFN cohort which rose from 514 to 526 cases 100k/PY, but in the controls of the IFN+DAA groups it fell from 710 to 666 cases 100K/PY and in the DAA controls from 815 to 774 cases 100K/PY. This

analysis shows how the increase in cancer is now significant in the IFN cohort and for extrahepatic cancer in the DAA cohort.

The second sensitivity analysis consisted of only taking into account alcohol consumption in the selection of control groups. The incidence in the control group of the IFN cohort can be considered the same at 514 100K/PK. In the control group of the IFN+DAA cohort it increased from 710 to 782 100K /PY and in the control group of the DAA cohort it decreased from 815 to 785 100K /PY. These changes only affected the statistical evaluation of extrahepatic cancer, which was statistically significant in the DAA cohort.

The third and final sensitivity analysis consisted of taking into account smoking as the only toxic risk factor in the selection of controls. In this case, the incidence of controls in the IFN cohort decreased to 460 100K/PA, and increased to 758 100K/PA in the IFN+DAA cohort, and was virtually unchanged in the control group of the AAD cohort at 818 100K/PA. This decreased incidence in the control group of the IFN cohort made the change in risk statistically significant. This was the only change observed.

However, these changes in statistical significance were not related to a large change in the effect size. Thus, in the IFN cohort, for the estimate of the effect on general cancer incidence, the main result was RR=1.11 with no statistically significant relationship (p-value=0.277). In the case of not considering alcohol nor smoking, the RR was 1.08 (p-value=0.004), considering only alcohol consumption, the RR was 1.11 (p-value=0.277) and considering only smoking, the RR was 1.24 (p-value=0.0236). For the rest of the cohorts, IFN+DAA and DAA, the statistical significance is always maintained, with very similar RR estimates. For the effect on intrahepatic cancer, the conclusions remain unchanged in terms of statistical significance and effect size estimated by mean RR between the main analysis and the sensitivity analyses. In the DAA cohort, for extrahepatic cancer, the main result was RR=1.17 (p-value=0.079), while in the pairing without considering alcohol nor

smoking was RR=1.21 (p-value=0.030) while, when considering alcohol consumption, it was RR=1.21 (p-value=0.037).

11.7 Influence of coinfection for HIV and the presence of cirrhosis.

The analysis of the influence of HIV coinfection or the presence of cirrhosis at the time of starting treatment is not conclusive, despite presenting a trend in the expected direction: HIV coinfection as a possible protective factor and cirrhosis as a risk factor. This might be partially explained by the small number of cases with coinfection or cirrhosis in the group of patients treated with IFN, an aspect that is inherent to the patient profile, in which the use of this therapeutic approach is discouraged. This high prevalence of HIV coinfection and the diagnosis of cirrhosis was comparable to the contemporary figures observed in Spain (127), with a percentage of grade 3-4 fibrosis of 62.6% and cirrhosis of 40.8% of treated patients. The prevalence of grade 3-4 fibrosis (cirrhosis) in our treated DAA or INF+DAA groups was of 67.9% and 74.9% (45.1% and 52.7%), and the HIV coinfection prevalence was 42.5% and 26.1%, respectively. In 2013 in Catalonia, the percentage of patients with active HCV and a diagnosis of HIV was 69% (128).

11.8 Robustness and extension of main results

The central finding of the study is that there was a higher cancer incidence in HCV-infected patient groups in the DAA and INF-DAA HCV treatment groups, compared with matched control subjects. This increased incidence is mainly, but not exclusively, due to the incidence of liver cancer.

It should be noted that this conclusion is established in large cohorts of patients and with a relatively short follow-up. The Kaplan-Meier curves suggest that the effect could be observed early at the beginning of treatment for HCV in the IFN+DAA and DAA treated groups of patients. In the main analysis, and in the three

sensitivity analyses, the statistical conclusion is the same: no increased risk was observed in the IFN cohort, while in the IFN+DAA and DAA cohorts there was a statistically significant effect, in the sense of an increased risk in groups of patients treated for HCV infection.

11.9 Study limitations

It may be argued that the increased cancer incidence in DAA-treated patients reflect the fact that they had undergone a more intense follow-up after initiation of DAA therapy, priming the clinical suspicion of cancer and its diagnosis and treatment. This would mean that all other cohorts have not been regularly followed after treatment. This hypothesis is highly unlikely, as countrywide screening programs for breast and colon cancer are in place under the public healthcare system, while symptom-related diagnosis is unlikely to be affected by the recommended follow-up after HCV eradication. Indeed, it may be suggested that patients cured of HCV may assume that they have no health problems and are thus less prone to demand medical visits and to be concerned by minor symptoms. Again, the duration of DAA treatment is shorter than that of IFN-based regimens, and with less adverse complications, thus requiring less intensive clinical care, which would be opposed to increased cancer detection due to frequent visiting and testing.

This potential heterogeneity is common to registries in any country that are maintained by different persons, including primary care physicians, institution administrations and pharmacy accounts. However, the large numbers and consistency of data across several assessments, as applied in our study, provide assurance of the validity of the data generated by this and all other epidemiology studies in large population registries for general estimates (129) or for specific risk factors for toxic habits (116) and collateral pharmacological effects (130) that, in

this sense, show an incidence of HCC similar to patients treated sequentially with IFN+DAA or only with DAA in this observational population-based registry study.

This study shows the inherent limitations of the analysis of population registries, such as the occurrence of missing data, imputed to the absence of a factor as a general consensus, and the paucity of variables to adjust for potential differences in risk factors between treated cohorts.

The expected low counts of patients with HIV coinfection or cirrhosis in several groups did not allow a robust estimate of the stratified cancer incidence. Nor was it possible to estimate the incidence by type of cancer or grouped by haematological type.

In addition, the inclusion of a treatment regimen with some contraindications in patients with worse liver function or coinfected patients, as is the case of treatment based on combinations with IFN, has made direct comparison between treatment groups impossible, since the good tolerance of DAAs has given the opportunity to treat at the beginning of the availability of these drugs, a high proportion of patients who could not be treated with an IFN-based regimen due to contraindications.

In this sense, this study describes the effect of the inclusion of a new treatment, DAA, for the treatment of HCV, so the data are not contemporary. IFN-based treatments were used up to 2015, and DAA treatments were used thereafter. Since we cannot exclude the possibility that cancer risk or cancer detection has changed over the study period, the incidence across treatment cohorts should be interpreted with caution. In fact, while the general incidence figure is consistent with epidemiological data, we observed significant differences in cancer incidence between the control cohorts for IFN (514.9 cases/100kPY) and the DAA (815.3 cases/100kPY), which was 58% higher in the DAA group. This may have been influenced by unknown external factors, but also may reflect that, because of the different tolerability profile of the drugs, the characteristics of the patients treated

with each drug was not alike, so the DAA cohort apparently included so the DAA cohort apparently included, in addition to patients with poorer liver function and higher frequency of cirrhosis, older patients with more known risk factors for cancer, such as obesity, diabetes, cirrhosis and HIV infection. In any case, despite PS matching, considering the paucity of available data to adjust for indication bias or other confounders, we cannot exclude the possibility that residual confounding remains, limiting the comparability between exposed and control cohorts for subgroup analyses, and mainly, across treated cohorts.

In summary, this population-based study of cancer outcomes in HCV patients treated with different regimes found that the cancer incidence in Catalonia was significantly higher in DAA treated patients, both when used as the only therapy or following a previous IFN-based treatment, in comparison with matched control patients without an HCV diagnosis or treatment. While the absolute risk remains low and the benefits of treating HCV are not questioned, increased awareness or the potential occurrence of rare malignant events in the general population, especially of HCC, in order to guarantee early detection and treatment, seems appropriate after DAA treatment, regardless of the achievement of a sustained viral response.

Similar studies in different geographic settings should confirm or refute these findings and eventually prime the research of the mechanisms leading to this apparently increased risk.

12 Conclusions

1. This population-based study of cancer outcomes in HCV patients treated with different regimes found that cancer incidence in Catalonia was significantly higher in DAA treated patients, both when used as the only therapy or following a previous IFN-based treatment, in comparison with matched control patients without an HCV diagnosis or treatment.

2. The cumulative cancer incidence in patients treated with DAA could not be formally compared with that in patients treated with interferon-based antiviral regimens for HCV, due to heterogeneity of groups and lack of covariates to apply reliable methods for adjustment.

3. Cumulative incidences of intra or extrahepatic cancer and of solid and haematological cancers in patients treated with interferon-based agents and/or DAA for HCV could not be ascertained due to low number of incident cases, limiting statistical feasibility of methods.

4. The temporal association between the diagnosis of cancer and the type of treatment of HCV infection could not be established, due to scarcity of cases with complete information.

5. While the absolute risk of cancer in patients treated with antivirals for HCV infection remains low, and the benefits of treating HCV are not questioned, there is a need for increased awareness of the potential occurrence of rare malignant events, especially of HCC, in order to guarantee early detection and treatment, seems appropriate after DAA treatment.

6. Similar studies in different geographic settings should confirm or refute these findings; if confirmed, research on the mechanisms that lead to such an increased risk should be primed.

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14 Annexes

14.1 Annex 1: Ethics committee certificate

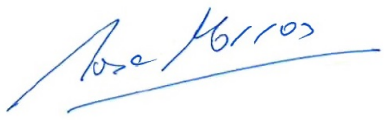
INFORME DEL COMITÈ ÈTIC D'INVESTIGACIÓ CLÍNICA

Rosa Morros Pedrós, Presidenta del Comitè Ètic d'Investigació Clínica de l'IDIAP Jordi Gol.

CERTIFICA:

Que aquest Comitè en la reunió del dia 29/03/2017, ha avaluat el projecte ***Incidencia de cáncer en relación con el tratamiento farmacológico de la infección por virus de la hepatitis C*** amb el codi **P17/061** presentat per l'investigador/a **Rosa Morros Pedrós.**

Considera que respecta els requisits ètics de confidencialitat i de bona pràctica clínica vigents.



Barcelona, a 03/04/2017

14.2 Annex 2: Agreement on good practices and security in the treatment of SIDIAP data

SIDIAP

ACORD DE BONES PRÀCTIQUES I SEGURETAT EN EL TRACTAMENT DEL FITXER DE DADES SIDIAP

263

Barcelona, a 11 de julio de 2018

Nom i Cognoms: Jose Rios Guillermo
NIF: 46729266-M
Centre de treball i Institució: Unitat Bioestadística. Univ. Aut. Bcn (UAB)
Correu electrònic: jose.rios@uab.cat

Que actua com Investigador Principal (IP) i/o encarregat extern de tractament de les dades del projecte: Hepatitis C

CLÀUSULES

PRIMERA.- OBJECTE

1.1. L'investigador principal, amb la condició d'encarregat extern de tractament ha sol·licitat la col·laboració de la unitat del SIDIAP per el desenvolupament del projecte de recerca mencionat prèviament.

1.2. Si en relació a aquest projecte de recerca existís un acord de col·laboració amb organització externa, s'haurien de respectar els acords marcats per aquest acord.

SEGON.- TITULARITAT I MANEIG DE LES DADES

2.1. El maneig de les dades es realitzarà a la unitat del SIDIAP de l'IDIAP Jordi Gol. El seu objectiu és garantir que la qualitat de les dades que s'obtinguin sigui la millor possible a partir de les dades disponibles.

2.2. Los dades són propietat de l'IDIAP Jordi Gol el qual encarrega el tractament de les dades a l'equip investigador per a la seva utilització exclusivament pel projecte mencionat en aquest acord per al seu posterior anàlisis. Per tant, només les anàlisis esmentades al protocol aprovat pel Comitè Científic SIDIAP són permeses d'acord amb aquest contracte.

2.3. Si fos necessària la seva utilització per un fi diferent a l'exposat en el present acord, s'haurà de demanar autorització prèvia al propietari de les dades (IDIAP Jordi Gol), mitjançant una sol·licitud d'esmena al Comitè Científic SIDIAP amb l'imprès dissenyat a tal efecte, tot exposant clarament els motius que justifiquen aquesta petició.

TERCER.- SEGURETAT DE LES DADES

3.1. l'IP del projecte es declara coneixedor de la part que afecta a la seva tasca del document de seguretat del SIDIAP en el que s'explicita com s'han de declarar les incidències en el tractament de les dades i quin es el procediment per la destrucció dels fitxers de dades de l'estudi.

3.2. El present document autoritza la cessió de dades per un període de 8 anys des del moment del lliurament de la BD.

3.4 El fitxer s'eliminarà una vegada finalitzat el període de cessió sent responsabilitat de l'investigador i l'entitat que ho sol·licita la seva destrucció que la tindrà que comunicar al responsable del Fitxer de recerca del IDIAP Jordi Gol (SIDIAP).

3.5 L'investigador es compromet a tractar les dades en un dispositiu (ordinador) al qual es te accés a través de un codi d'encriptació (usuari i contrasenya) i està sota el domini de la institució que pertany l'investigador i que figura al principi del document.

3.6. Amb la signatura d'aquest conveni, l'IP permet les auditories preceptives de Protecció i Seguretat de Dades respecte a les dades del SIDIAP.

3.7. En cas d'auditoria, l'IDIAP Jordi Gol es compromet a no envair la intimitat de l'usuari referida a la part privada del Dispositiu.

3.8. L'IDIAP Jordi Gol no es responsabilitza dels Continguts i Aplicacions que l'investigador pugui allotjar a la seva zona privada.

3.9.- En cas d'eliminació del suport informàtic (ordinador) utilitzat pel tractament de les dades, aquest haurà de ser prèviament sotmès a un procediment de destrucció del seu contingut de manera que no sigui possible en cap cas l'accés o recuperació de la informació continguda prèviament.

QUART.- MANTENIMENT DE LA INFORMACIÓ DEL GIR

4.1. L'investigador principal és responsable de **mantenir la seva informació personal actualitzada al GIR**.

4.2. Tanmateix, haurà d'**actualitzar aquella informació relativa al projecte que sigui de la seva competència**.

CINQUÈ.- TITULARITAT DELS DRETS SOBRE ELS RESULTATS DE LA INVESTIGACIÓ I PUBLICACIONS

5.1. De cara a poder retre comptes de l'ús de dades públiques, l'equip investigador haurà d'informar a l'IDIAP Jordi Gol mitjançant un informe final o les publicacions corresponents que es facin utilitzant les dades del SIDIAP. Aquests, només seran utilitzats per l'IDIAP Jordi Gol per a difusió interna. Només és podrà utilitzar per a difusió externa (presentacions, pàgina web, etcètera) el material no protegit dels mateixos (autors, títol, lloc de difusió i resum).

5.2. En qualsevol mitjà de difusió dels resultats obtinguts a l'apartat de *material y mètodes* **s'haurà d'especificar la font d'obtenció de les dades**.
En cursiva i negreta, es detalla el text tal i com s'ha de citar a l'article.

Font d'obtenció de dades: ***SIDIAP (Sistema d'informació per al Desenvolupament de Investigació en Atenció Primària)***.
Per a ser citat en anglès: ***SIDIAP (Information System for Rersearch in Primary Care).***

En cas que el projecte hagués utilitzat altres fonts addicionals d'informació es farà constar la font utilitzada i la institució responsable de la mateixa.

A continuació de la cita de les fonts cal posar aquest paràgraf:
Aquest manuscrit no ha estat preparat en col·laboració amb aquest/s registres i, per tat no reflecteix necessàriament les seves opinions o punts de vista. La qualitat i exactitud és responsabilitat exclusiva de l'autor del manuscrit.

5.3. A l'apartat ***agraïments*** també **haurà de constar el SIDIAP**. En el cas que s'haguessin utilitzat altres fonts de dades, també haurà d'aparèixer en aquesta secció l'entitat propietària d'aquestes bases de dades.

5.4. **En cap cas s'utilitzarà la imatge o nom del SIDIAP, ICS o IDIAP Jordi Gol** per a la difusió de resultats sense el consentiment d'aquestes institucions.

5.5. L'Investigador principal del projecte, **haurà de preparar un resum de l'informe final** que serà enviat a la Direcció Assistencial de l'ICS juntament amb l'**informe o memòria final** o el corresponent article amb els resultats de l'estudi.

SISÈ.- CANVIS I MODIFICACIONS

6.1. **Les dades es donaran d'acord al protocol operatiu acordat**. Qualsevol modificació al respecte requerirà d'una nova aprovació del Comitè Científic seguint el formulari establert per aquests casos.

SETÈ.- CONFIDENCIALITAT

7.1. L'IP o l'equip col·laborador del projecte, **s'abstindran de cedir o prestar a tercers les dades** resultants de l'estudi.

7.2. Qualsevol informació **serà utilitzada exclusivament per a la realització de la investigació indicada** en el present acord.

7.3. L'investigador principal evitarà anàlisis que permetin la identificació d'algun pacient de la base de dades lliurada, així, en el cas que en l'anàlisi de les dades, s'arribés a categories/grups de pacients amb 5 o menys persones (per exemple, després d'encreuar diverses variables/categories), l'investigador principal es compromet a aturar el projecte immediatament, no fer cap comunicació científica de l'estudi i a notificar-ho de forma URGENT a SIDIAP, qui estudiarà el cas i prendrà la decisió oportuna.

VUITÈ.- PRESSUPOST

8.1. El projecte mencionat en el present acord té un **pressupost** de € per a l'extracció de dades de SIDIAP.
Aquesta quantitat serà facturada per l'IDIAP Jordi Gol a l'entitat gestora de la subvenció, en funció dels acords institucionals signats entre ambdues entitats.

Amb la signatura d'aquest document faig constar que he rebut i accepto la informació relativa a les normes de seguretat i ús de dades que apliquen al fitxer de dades SIDIAP que em serà lliurat.

_____

En compliment de la Llei Orgànica 15/1999 de 14 de desembre, de Protecció de Dades de Caràcter Personal, s'informa a la persona interessada que les dades de caràcter personal que facilita, inclosa l'adreça electrònica i que resulten necessàries per a la gestió administrativa, així com a l'execució i el desenvolupament de tota activitat institucional pròpia de l'IDIAP Jordi Gol, seran incorporades al fitxer automatitzat GIR, la titularitat i responsabilitat del qual és ostentada per l'IDIAP.

La persona interessada, n'autoritza expressament la utilització a efectes de comunicacions, incloent expressament les que es puguin realitzar entre l'IDIAP i l'ICS sempre amb finalitats relacionades amb l'activitat institucional que s'hi desenvolupa.
La persona interessada es **compromet a**: Respectar i complir les normes ètiques, així com vetllar per la confidencialitat de les dades a les que tingui accés. I a respectar l'autoria i propietat intel·lectual de les idees i projectes als que tingui accés.

La persona interessada podrà exercir els drets d'accés, rectificació, cancel·lació i oposició sobre les seves dades a l'adreça electrònica gir@idiapjgol.org.

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14.3 Annex 3: Manuscript for submission

Journal of Hepatology

Incidence of liver and non-liver cancer risk after hepatitis C virus eradication: a population-based cohort study

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original Article
Section/Category:	Hepatic and Biliary Cancer
Keywords:	hepatitis C virus; direct antiviral agents; interferon; hepatocellular carcinoma; solid cancer; propensity score matching analysis
First Author:	José Ríos, BSc, MSc
Corresponding Author:	Ferran Torres Universitat Autonoma de Barcelona Facultat de Medicina Barcelona, Barcelona SPAIN
Order of Authors:	José Ríos, BSc, MSc
	Víctor Sapena, BSc, MSc
	Zoe Mariño, MD, PhD
	Jordi Bruix, MD, PhD
	Xavier Forns, MD, PhD
	Rosa Morros, MD, PhD
	María Reig, MD, PhD
	Ferran Torres, MD, PhD
	Caridad Pontes, MD, PhD
Abstract:	Background & Aims: Direct-acting antivirals (DAA) offer a high rate of hepatitis C virus (HCV) eradication but their effect on cancer risk remains unclear. Methods: We performed a population-based study using real-world data sources of linked healthcare registries of the public healthcare system of Catalonia (Spain) between 2012 and 2016. Propensity score matching of HCV patients treated with interferon-based therapy (IFN), sequential IFN and DAA, and DAA only with concurrent comparable controls was done. Poisson regression models were used to determine the annual incidence of cancer and the rate ratios between HCV-treated patients and controls. Results: The estimated incidence of cancer was 596.1 cases per 100,000 person-years (95% confidence interval [CI] 482.5-671.4) for IFN monotherapy, 1255.3 cases per 100,000 person-years (95% CI 947.9-1662.2) for sequential IFN and DAA, and 1552.0 cases per 100,000 person-years (95% CI 1380.1-1745.3) for DAA only. An estimated increased cancer risk was found for IFN (rate ratio [RR] 1.11, 95% CI 0.92-1.32), sequential IFN and DAA (RR 1.77, 95% CI 1.27-2.46) and DAA only (RR 1.90, 95% CI 1.66-2.19). In DAA-treated patients, risk for cancer was increased in the presence of cirrhosis. Conclusions: In general, treated HCV patients showed slight increase for overall cancer incidences than matched controls without HCV infection and the risk was particularly higher for hepatocellular carcinoma (HCC). Whether this increased risk is related to the HCV infection, the pharmacological treatment or any non-identified confounder requires further research.
Opposed Reviewers:	

Cover Letter

Barcelona, April 07, 2022

Professor Paolo Angeli
Editor-In-Chief
Journal of Hepatology

Dear Professor Angeli,

We are pleased to submit our manuscript entitled "*Incidence of liver and non-liver cancer risk after hepatitis C virus eradication: a population-based cohort study*" to the Journal of Hepatology.

This manuscript reports our study that assessed the effect of the interferon-based (IFN)-based combination therapies and the acting antiviral (DAA) therapies for HCV infection on potential changes in cancer incidence.

We carried out a population-based study using real data sources from the medical records linked to the public health system of Catalonia (Spain) between 2012 and 2016.

This real word data study includes health electronic records from different sources covering about 80% of the total of 7.7 million inhabitants of Catalonia (a region in the north-west of Spain), hosted by the Catalan Health Service (CatSalut). Data were extracted from 572,381 patients who served to draw the conclusions of our study.

The main finding is that we found a slight increase in the incidence of cancer in treated patients shortly after the treatment prescription. Due to the design of our study, we cannot confirm that this increase is related or not to the pharmacological effect of the antiviral agents, but we conclude that patients, once cured of their HCV infection, should undergo a follow-up screening for oncological diseases.

We hope that the manuscript will be felt suitable for publication in Journal of Hepatology. All authors have approved the final version and we ensure that the manuscript has not been submitted elsewhere.

Yours sincerely,

Ferran Torres and Caridad Pontes, on behalf of the authors and collaborators.

Category: Research article

Incidence of liver and non-liver cancer risk after hepatitis C virus eradication: a population-based cohort study

José Ríos[1,2*], Víctor Sapena[1,3*], Zoe Mariño,[4] Jordi Bruix[3], Xavier Forns[4], Rosa Morros[5], María Reig[3], Ferran Torres[2], Caridad Pontes[6]

1. *Medical Statistics core facility, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain.*
2. *Biostatistics Unit, Faculty of Medicine, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.*
3. *Barcelona Clinic Liver Cancer (BCLC), Liver Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBERHED), Barcelona, Spain.*
4. *Liver Unit, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBERHED), Barcelona, Spain*
5. *Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol), Barcelona, Spain. Departament de Farmacologia, Terapeutica i Toxicologia Universidad Autónoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain.*
6. *Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.*

Corresponding author: Ferran Torres, PhD, Biostatistics Unit, Faculty of Medicine, Universitat Autònoma de Barcelona (UAB), Campus, 08193 Bellaterra (Barcelona), Spain. e-mail: Ferran.Torres@uab.cat

*José Ríos and Víctor Sapena share first authorship credit.

Keywords: hepatitis C virus, direct antiviral agents, interferon, hepatocellular carcinoma, solid cancer, propensity score matching analysis.

Electronic word count: 4,385.

Number of figures and tables: 5.

Conflict of interest statement

JR has received educational/training fees from Amgen, AstraZeneca, Boehringer Ingelheim, Janssen-Cilag, Novartis, and Lilly. ZM has received consultancy fees from Gilead, Abbvie, Alexion, Orphalan, and Deep Genomics, speaker fees from Gilead and Abbvie, and research grants from Gilead. VS has received travel grants from Bayer and consultancy fees from Leo Pharma. JB has been consultant for Arqule, Bayer-Shering Pharma, Novartis, BMS, BTG- Biocompatibles, Eisai, Kowa, Terumo, Gilead, Bio-Alliance, Roche, AbbVie, MSD, Sirtex, Ipsen, Astra-Medimmune, Incyte, Quirem, Adaptimmune, Lilly, Basilea, Nerviano, and Sanofi, has received research/educational grants from Bayer, and lecture fees from Bayer-Shering Pharma, BTG-Biocompatibles, Eisai, Terumo, Sirtex, and Ipsen. XF acted as advisor for Gilead and Abbvie. MR has received consultancy fees from Bayer, BMS, Roche, Ipsen, AstraZeneca and Lilly, lecture fees from Bayer, BMS, Gilead, and Lilly, and research grants from Bayer and Ipsen. FT: has received fees for Data and Safety Monitoring Board (DSMB) from Basilea Pharmaceutica International and ROVI, and educational/training fees from Janssen and Ferrer. RM and CP have no conflict of interest to disclose.

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Authors contributions

JR, JB, RM, MR, FT, and CP: concept and design of the study.
ZM, VS, XF, RM, MR, and CP: data collection.
JR, VS, and FT: statistical analysis.
JR, JB, and FT: interpretation of results.

JR: writing of the manuscript.

ZM, VS, JB, XF, RM, MR, FT, and CP: draft review for important intellectual content.

All authors have approved the final draft.

Abstract

Background & Aims: Direct-acting antivirals (DAA) offer a high rate of hepatitis C virus (HCV) eradication but their effect on cancer risk remains unclear.

Methods: We performed a population-based study using real-world data sources of linked healthcare registries of the public healthcare system of Catalonia (Spain) between 2012 and 2016. Propensity score matching of HCV patients treated with interferon-based therapy (IFN), sequential IFN and DAA, and DAA only with concurrent comparable controls was done. Poisson regression models were used to determine the annual incidence of cancer and the rate ratios between HCV-treated patients and controls.

Results: The estimated incidence of cancer was 596.1 cases per 100,000 person-years (95% confidence interval [CI] 482.5-671.4) for IFN monotherapy, 1255.3 cases per 100,000 person-years (95% CI 947.9-1662.2) for sequential IFN and DAA, and 1552.0 cases per 100,000 person-years (95% CI 1380.1-1745.3) for DAA only. An estimated increased cancer risk was found for IFN (rate ratio [RR] 1.11, 95% CI 0.92-1.32), sequential IFN and DAA (RR 1.77, 95% CI 1.27-2.46) and DAA only (RR 1.90, 95% CI 1.66-2.19). In DAA-treated patients, risk for cancer was increased in the presence of cirrhosis.

Conclusions: In general, treated HCV patients showed slight increase for overall cancer incidences than matched controls without HCV infection and the risk was particularly higher for hepatocellular carcinoma (HCC). Whether this increased risk is related to the HCV infection, the pharmacological treatment or any non-identified confounder requires further research.

Lay summary

After eradication of hepatitis C virus infection with three different treatment strategies based on effective drugs, there was a small increase in the risk of cancer in treated patients. Whether this risk is due to the disease, the treatment or any non-identified confounder requires further research. Close monitoring of patients with HCV DAA treatment for cancer detection seems reasonable, especially for HCC.

Highlights

- Patients treated for their HCV infection have slightly incidence of cancer.
- HIV coinfection does not appear to increase the risk of cancer after viral eradication.
- Cirrhosis is associated to an increased risk of cancer after direct-acting antivirals.
- Despite causal relationship with drugs cannot be concluded, patients with HCV treated with DAA should be monitored for cancer detection.

Introduction

The development and recurrence of hepatocellular carcinoma (HCC) after curative treatment of HCV infection has been a focus of increasing interest.[1-14] We previously reported that the risk of HCC development was associated to the imaging detection of non-characterized nodules prior to HCV treatment initiation.[15] This relationship was confirmed in the Italian study of Sangiovanni et al.,[16] and it is worthy of remark that in both studies HCC could emerge in a separate location of the non-characterized lesions.[15,16] However, meta-analyses on the risk of HCC recurrence have been hampered by heterogeneity of data and definite conclusions are lacking.[6,17] Also, in relation to extrahepatic cancer,[18] it is well known that B-cell non-Hodgkin lymphoma is associated to HCV infection and may regress after HCV eradication.[19-22] The risk of non-hematological neoplasms has been suggested to be increased because of HCV infection of non-hepatic cells and alteration of the immune surveillance system.[18] In a multicenter French study, extrahepatic cancer was the most frequent cause of death in patients with sustained viral response/HCV eradication.[23]

The present study was conducted to assess the incidence of cancer in patients treated with direct-acting antivirals (DAA) for HCV infection at the population level in comparison with properly matched control subjects infected with HCV who received interferon (INF)-based treatment mainly before 2014 (the year at which DAA began to be available in Catalonia) and non-infected HCV patients. We performed a population-based study using real-world data sources of linked healthcare registries of the public healthcare system of Catalonia (Spain) between 2012 and 2016.

Patients and methods

Design

This was a population-based cohort study which included the resident population in Catalonia (Spain) aged 18 years or older, the clinical records of which were available from existing national sources and without any previous record of diagnosis of cancer or specific treatment for cancer. The period of analysis was from January 1, 2012 to December 31, 2016. The study protocol was approved by the Ethics Committee for Clinical Research of the Institute of Research in Primary Care (IDIAP Jordi Gol, code CEI P17/061). Informed consent was waived because of the retrospective design and the analysis of pseudo-anonymized data collected from electronic databases.

Data sources

Data sources were clinical and administrative databases from Catalonia, which is a Spanish region with approximately 7.7 million inhabitants (2021 census) that has a universal, public and free of charge healthcare system. To access the system, an individual health card (TSI) is necessary. Electronic clinical records for primary care, administrative invoicing information of hospital and pharmacy dispensation episodes, and a specific registry including drug-related clinical outcomes for certain drugs, are linkable through the TSI code of each citizen.

We used data provided by the Public Data Analysis for Health Research and Innovation Program (PADRIS).[24] PADRIS allows access to information from different clinical sources and pharmacy billing registry from hospitals linked at the patient level with accomplishment of ethical principles. The sources databases belong to the Catalan Health Service (CatSalut) and includes demographic information for all insured patients, diagnostic data for each episode of hospitalization and pharmacy invoicing data for outpatient medications (dispensed at pharmacies of the community and hospital settings). Also, a specific subset of data was generated by diagnosis of HCV within the Registry of Patients and Treatments (RPT)[25] of CatSalut, which is a therapeutic registry created for longitudinal follow-up and assessment of clinical outcomes of specific treatments such as HCV. Moreover, data from the Information System for the Development of Research in Primary Care (SIDIAP)[26] database was obtained. This database contains longitudinal medical records of primary care practices managed by the Catalan Institute of Health (ICS) that uses electronic health records in primary care (eCAP) since 2006, covering about 80% of the total of 7.7 million inhabitants of Catalonia. The SIDIAP registry includes sociodemographic characteristics, health conditions registered as International Classification of Diseases (ICD) 10th revision (ICD-10) codes, clinical parameters, laboratory data, and outpatient prescriptions. Their corresponding pharmacy invoice data are available since 2005 and include information on all pharmaceutical products dispensed by community pharmacies for ICS prescriptions using the Anatomic Therapeutic Chemical (ATC) Classification System codes. Finally, the database corresponding to the Primary Care Minimum Basic Data Set (PC-MBDS) that includes diagnoses made in the primary care setting and registered as ICD 9th revision (ICD-9) codes was also used.

These population-based data sources were matched by two independent technicians who were unaware of the characteristics of the project and had no access to clinical information. All datasets were pseudo-anonymized in compliance with Regulation (EU) 2016/679 of the European Parliament and of the Council of April 27, 2016 on General Data Protection Regulation (GDPR) and Organic Law 3/2018, of December 5,

on Protection of Personal Data and guarantee of digital rights, prior to the transfer to final data management system and statistical analyses.

Study cohorts

The exposed cohorts were defined from RPT of CatSalut according to *de novo* pharmacy billing registry from hospitals within the study period (2012-2016) for the diagnosis of HCV infection, and the absence of previous diagnosis of cancer or billing of cancer drugs. Exposed cases were divided into three different cohorts: a) patients treated with interferon (IFN) with or without ribavirin (IFN-based regimens); b) patients who received an IFN-based regimen first and later (or concomitantly) DAA (sequential INF and DAA regimen); and c) patients treated with DAA only.

These three cohorts were matched 1 to 5 (as a maximum rate) with patients who should lack evidence of HCV diagnosis or diagnosis of cancer or billing of cancer drugs before January 1, 2012, collected from the SIDIAP database. Control matching was performed according to gender, year of birth, smoking habit and a geographical code grouping of healthcare units (DAP) into 36 areas covering the entire territory of Catalonia. DAP is characterized by sociocultural aspects and access to healthcare services, primarily used for healthcare budgeting adjustment processes. Additionally, the MEDEA index,[27] which is a deprivation index based on urban census data was used to aggregate DAPs with similar socioeconomic conditions and to categorize them into quintiles (Q1 to Q5), allowing check matching for DAPs in low-density inhabited areas that may have few eligible subjects or even none. Comorbidities, as diabetes, HIV, cirrhosis and toxic habits (tobacco and alcohol consumption), was assessed throughout all follow-up. According to clinical practice, absence of reporting was considered as absence those factors. Matching was performed following a two-step procedure executed on a sequential basis, firstly using an exact restriction for gender and DAP, and then a propensity score (PS) procedure based on the logit from a logistic regression model that included gender, age (calculated from the year of birth to index date), smoking habit, alcohol consumption and geographical code from DAP,[28] and evaluated with MEDEA index quintiles. This second step used the greedy[29] nearest neighbors matching with a caliper of < 0.06 of distance.

Definition of event and length of follow-up

In order to identify all incident events related to the diagnosis of cancer in the study cohorts, the ICD-10 codes from eCAP and ICD-9 codes from PC-MBDR were used. In addition, ATC codes of oncological treatments recorded at pharmacy billing registries from hospitals were checked to detect potentially missed incident cases of cancer (Supplementary material, Table S1). The length of follow-up for HCV patients (case group) was defined as the period between the index date, defined as first date of

prescription of HCV treatment, to the date of event or last available record. For patients in the control group the index date was that of the case they were paired with, while the last follow-up date was the date of the event or the last date available in the registry.

Statistical analysis

Categorical data are expressed as frequencies and percentages, and continuous data as median and interquartile range (IQR) (25th-75th percentile). Homogeneity for baseline characteristics was assessed using standardized differences (STD), i.e. differences divided by pooled standard deviation [SD]) between each HCV group and its matched control group. Proper balance of all matching covariates was pursued by using an after ± 0.20 cut-off point for STD,[30] in this study all matching covariates were well balanced and following recommendations,[30] no inferential analysis was performed to compare groups.

The estimation of the cumulative incidence of cancer and the 95% confidence intervals (CI) were performed using Poisson models with the natural logarithmic transformation of follow-up as offset. Estimation of incidences were calculated as new cancer diagnosis per 100,000 person-years of follow-up for patients treated with IFN only (IFN group), patients treated with sequential IFN and DAA (IFN+DAA group), patients treated with DAA only (DAA group), and for each matched control groups. Rate ratios (RR) and the 95% CI were estimated using the incidence for each matched control group as reference. The same analysis was performed to assess the incidence of HCC, non-liver cancer, and in the presence of comorbidities including HIV coinfection and cirrhosis. In the case of HIV coinfection and cirrhosis a comparison between treated patients and controls was not made due to the low prevalence of these conditions in controls. A complementary time-to-event analysis for sensitivity purposes was performed using the Kaplan-Meier method to describe the instantaneous hazard in a 36-month follow-up window. Additionally, the risk increases for each treatment group respect to their matched control set were estimated through hazard ratios (HR) and their 95% CI from Cox proportional regression models. A direct comparison between groups from the different cohorts were not planned due to inherent differences in prevalence of cirrhosis and HIV infection, which are well-established risk factors for the development of a neoplasm,[31] as well as clinical limitations for prescribing IFN-based treatment. However, the effect of HIV coinfection or cirrhosis within each group of HCV-treated patients was compared. In analyses, a two-sided type I error of 5% was applied. The Statistical Analysis Systems (SAS) v9.4 (SAS Institute, Cary, NC, USA) was used for data management and analysis.

Results

Disposition of patients and allocation

A total of 11,656 patients diagnosed with HCV infection were identified from RTP as starting anti-HCV treatment during the study period. They were divided into the groups of IFN-based therapy with 4,329 patients, IFN+DAA with 794 patients, and DAA with 6,533 patients. The screened population for control sampling included 572,381 patients, of whose 11,786 were excluded because of insufficient data for matching. Of the remaining 560,595 patients potentially eligible for the matching procedure, 19,376, 3,507, and 26,662 paired controls were selected, respectively, with an average of 4.25 controls per patient (Figure 1).

Clinical characteristics of patients and controls

Demographic and clinical characteristics of patients and controls are shown in Table 1. Patients in the three groups of antiviral treatment were evenly balanced to their controls for age, gender, smoking habit and MEDEA index. STDs > 20% were shown when comparing the treated groups versus their matched controls in HIV coinfection (for all three cohorts), and in cirrhosis (for the IFN+DAA and DAA cohorts), which accounts for the higher prevalence of HIV coinfection (in all treated arms) and cirrhosis (in the IFN+DAA or DAA treated arms). Also, STD > 20% for body mass index (BMI) with approximately a mean of 1 kg/m^2 of lower BMI in treated patients vs. matching controls was found. In the IFN+DAA and DAA groups as compared with controls, STD > 15% for the presence of diabetes was observed, with a prevalence of diabetes of 16.6% and 19.1% in the IFN+DAA and DAA groups, respectively, representing around a 6% higher prevalence of diabetes as compared to controls. Age and the prevalence of diabetes, HIV and cirrhosis was higher in the IFN-DAA and DAA groups in comparison with the IFN group (Table 1). Data of other clinical variables are shown in the Supplementary material Table S2.

Cancer risk and incidence of HCC and non-liver cancer

As shown in Table 2, in the IFN group, the estimated incidence of cancer was 569.1 cases per 100,000 person-years (95% CI 482.5-671.4) as compared with 514.9 (95% CI 472.3-561.3) in controls, with a RR of 1.11 (95% CI 0.92-1.32) (p = 0.2771). In the IFN+DAA group, the incidence of cancer was 1.255.3 cases per 100,000 person-years (95% CI 947.9-1,662.2) as compared with 710.8 (95% CI 590.7-855.4) in controls, with an RR of 1.77 (95% CI 1.27-2.46) (p = 0.0008). In the DAA group, there was an incidence of cancer of 1.552 cases per 100,000 person-years (95% CI 1,380.1-1,745.3), which was

also significantly higher than in controls (815.3 cases per 100,000 person-years, 95% CI 752.9-882.9) with a RR of 1.90 (95% CI 1.66-2.19) ($p < 0.0001$).

The incidence of HCC diagnosis was significantly higher in treated patients than in controls irrespective of the treatment modality (Table 2). The IFN group showed the lowest increase of risk (RR 1.50, 95% CI 1.02-2.22), the IFN+DAA group an intermediate risk (RR 3.89, 95% CI 2.26-6.69), and the DAA group the highest risk (RR 6.45, 95% CI 4.90-8.49). The incidence of non-liver cancer was non-significant in any of matched comparisons, but with a trend in the DAA group, with 825.5 cases per 100,000 person-years as compared with controls (702.7 cases per 100,000 person-years) ($p = 0.0793$). In the other two treatment groups, differences between patients and controls were not significant (Table 2).

Cancer risk in patients with HIV coinfection or cirrhosis

The number of patients with HIV coinfection was limited in most groups, except for the DAA group (prevalence of 42.5%). In this group, the estimated incidence of cancer was 1,344.1 cases per 100,000 person-years, with a RR of 0.79 (95% CI 0.62-1.01) ($p = 0.0574$) (Table 3). In relation to cirrhosis, the prevalence of this comorbidity was 46.2% in the IFN+DAA group, and 43.2% in the DAA group. The estimated incidence of cancer was 1,620.4 cases per 100,000 person-years in the IFN group, 1,390.8 cases per 100,000 person-years in the IFN+DAA group and 2,113.3 cases per 100,000 person-years in the DAA group (Table 3). The presence of cirrhosis at the beginning of follow-up was associated with an increased risk of cancer in the DAA group only (RR 1.92, 95% CI 1.52-2.44) ($p < 0.0001$).

Instant risk of cancer at follow-up

At follow-up, a lower proportion of events were consistently observed in the IFN group than in the IFN+DAA or DAA group. As compared with controls, the HR for cancer was 1.10 (95% CI 0.92-1.33; $p = 0.3047$) in the IFN group, increasing to 1.75 (95% CI 1.26-2.44; $p = 0.0009$) in the IFN+DAA group and 1.89 (95% CI 1.64-2.17; $p < 0.0001$) in the DAA group (Figure 2).

Discussion

Follow-up studies in HCV-infected patients treated with DAA have been focused on liver function and the impact on the development of liver cancer. However, data on extrahepatic cancer is very limited, and it has been suggested the need to ascertain whether the incidence of cancer is the same than in the general population or if there is an increased cancer risk in HCV patients.[23] It is well known that HCV infection is

associated with an increased risk of B-cell non-Hodgkin lymphoma as well as several cancer types in different sites, such as the upper digestive tract, non-Hodgkin's lymphoma, esophagus and lung.[19-22] The oncogenic hit leading to hepatic malignant transformation may have already taken place at the time of DAA therapy,[8,32] and thus, the incidence of liver cancer may not be reduced at least during the first years after viral eradication. However, since HCV eradication is associated with a disruption of immune surveillance as exposed by the potential reactivation of hepatitis B virus (HBV) or herpes virus,[33,34] such event may allow malignant clones at any site to emerge and accelerate its clinical recognition. This mechanism cannot be excluded as partly explaining our findings in this large population-based investigation.

We found that the incidence of cancer in patients with HCV infection treated with DAA was higher than in patients treated with IFN-based monotherapy or with sequential IFN and DAA, but comparisons between the HCV treatment groups were not feasible due to relevant differences among them. This increased incidence was statistically significant in the DAA and the IFN+DAA cohorts when the treated arms were compared to their matched control cohorts formed by individuals without either HCV therapy or known HCV carriage. This kind of population-based investigations of a subgroup of patients with a defined entity (HCV infection in this case) requires that the control group should be representative of the general population and not be skewed. The validity of our control population is supported by the fact that the cancer incidence in the control population reproduces the figures of the registry maintained by the Spanish Association Against Cancer (AECC).[35] This splits Spain into separate areas and reports the cancer incidence along the years of age, and we selected citizens older than 15 years. In 2012, it was reported that 39,237 new cases of cancer were diagnosed in Catalonia, corresponding to an incidence of 614 cases per 100,000 person-years. Stratified by gender, the incidences were 713 cases per 100,000 person-years in men and 519 cases in women. The present results in the control population of 514.9, 710.8 and 815.3 cases per 100,000 person-years for IFN monotherapy, IFN+DAA and DAA, respectively. These incidences are higher than those described in AECC registries. This increase may be explained, at least in part, due to the selection of controls which has been conditioned by the tobacco and alcohol consumptions in the population with HCV infection. In other epidemiological evaluation in Catalonia,[36] the percentage of smokers was around 40% in 2012 and similar to Spain[37] for diary consumption in the last 30 days, between 12% to 29% less than for our selected controls. In relation to alcohol consumption, the proportion of subjects with daily consumption in the last 30 days[37] was close to 10% and binge drinking around 20% of subjects in the last year. Notably, in the selected controls, consumption of alcohol it was just under 30%.

The increased risk of cancer in HCV patients includes both hepatic and extrahepatic malignancies as occurs in alcohol-related cirrhosis,[38] non-alcoholic steatohepatitis[39] or fatty liver disease. In these cases, chronic inflammation is responsible for such increased risk that is further intensified by coexisting oncogenic factors, such as smoking, specific dietary habits or environmental conditions.[40-45] Since such factors may vary across the country, the selection of controls took into account the most relevant, as the geographical codification (DAP), confounders and concentrate in the factors related to HCV management. This stratification of patients according to treatment received allowed identifying an increased incidence of cancer in the cohorts treated with DAA, either after initial IFN-based therapies or as the only treatment approach. While environmental contaminants may be regarded as controlled by the geographical matching and smoking habit or alcohol consumption were adjusted in the propensity model, no reliable data for other toxics exposure were systematically available for adjustments, and represent a limitation of the present study. From a methodological standpoint, ideal control groups would have included untreated HCV infected patients, but these were not identified in administrative databases. Besides, HCV carriers were actively sought during the study period in order to be treated, so that likely crossing-over between cohorts would have occurred. The chosen approach of selecting healthy subjects provides a pragmatic information, in that it identifies a population with higher cancer risk, deserving active surveillance regardless of treatment causality of the observed risks.

The analysis of the influence of HIV coinfection allows discarding an adverse effect of the former, and to identify a group with high risk in the later, where the inherent high prevalence of cirrhosis in DAA exposed groups may be an alternative explanation for the higher incidence of cancer in those groups. This high prevalence was comparable to the contemporary figures observed in our country,[46] with a percentage of grade 3-4 fibrosis of 62.6% and cirrhosis of 40.8% of treated patients. The prevalence of grade 3-4 fibrosis (cirrhosis) in our treated DAA or INF+DAA groups was of 67.9% and 74.9% (45.1% and 52.7%), and the HIV coinfection prevalence was 42.5% and 26.1%, respectively. In 2013 in Catalonia, the percentage of patients with active HCV and diagnosis of HIV was 69%.[47]

The core finding of the study is that there was an increased incidence of cancer in the groups of patients with HCV infection in the DAA or INF-DAA groups of HCV treatments, as compared to their matched control subjects, which was due mainly, but not apparently exclusively, to the development of HCC. It should be noted that our findings are derived from cohorts of patients with a relatively short length of follow-up,

and the Kaplan-Meier curves suggest that the effect could be observed early at the beginning of treatment for HCV for the DAA and INF+DAA cohorts

It could be argued that the increased incidence of cancer in DAA-treated patients may reflect that they had undergone a more intense follow-up after initiation of DAA therapy, priming the clinical suspicion of cancer and its diagnosis and treatment. This would mean that all other cohorts have not been regularly followed after treatment. This hypothesis is highly unlikely, as country-based screening programs for breast and colon cancer are in place under the public healthcare system, while symptom-related diagnosis is unlikely to be affected by the recommended follow-up after HCV eradication. Indeed, it could be raised that, patients cured of HCV may assume that they have no health problems and be less prone to medical visits and to be concerned by any minor symptom. Again, the duration of DAA treatments is shorter than that of IFN-based regimens, and with less adverse complications, thus requiring less intensive clinical care, which would be opposed to an increased cancer detection due to frequent visiting and testing.

This potential heterogeneity is common to registries in any country that are maintained by different persons, including primary care physicians, institution administrations and pharmacy accounts. However, the large number and consistency of data across several assessments, as applied in our study, provide assurance of the validity of the data generated by this and other epidemiological studies in large population registries for general estimations[48] or evaluation of specific risk factors for toxic habits [38] or pharmacological side effects [49] that, in this particular case shows a similar incidence of HCC in patients treated with IFN+DAA or DAA only.

This study shows the inherent limitations of the analysis of population registries, such as missing data and paucity of variables to adjust for potential differences in risk factors between treated cohorts. In addition, the expected low counts of patients with HIV coinfection or cirrhosis in several groups did not allow a robust estimate of the stratified cancer incidence, nor it was possible to assess the incidence by cancer type.

This study describes the incidence of cancer in patients with HCV receiving different treatments, IFN or sequential IFN-DAA or DAA, where data are not contemporary. IFN-based treatments were used up to 2015, and DAA treatments were used thereafter. Since we cannot exclude that cancer risk or cancer detection has changed along the study period, incidences across treatment cohorts should be interpreted with caution. In fact, while the general incidence figure in controls is consistent with expected epidemiological data, we observed differences in the incidence of cancer between the control cohorts for the IFN and DAA groups. This may be influenced by differences in age and in unknown external factors, as well as a selection bias due to the fact that the tolerability profile of the drugs differ substantially, so that the

DAA can be given to older patients with more known risk factors for cancer, such as obesity or diabetes, and also HIV infection. In any case, despite the PS matching, considering the paucity of available data to adjust for indication bias or other confounders, we cannot exclude that residual confusion remains, limiting the comparability between exposed and control cohorts for subgroups analyses, as well as across treated cohorts.

In summary, this population-based study of cancer outcomes in HCV patients shows that the cancer incidence in Catalonia was significantly higher in the DAA or IFN-DAA treated patients as compared with their matched uninfected controls. While the absolute risk remains low and benefits of treating HCV are not questioned, increased awareness of the potential occurrence of uncommon malignant events, especially HCC, after DAA therapy is necessary.

Abbreviations

AECC: Spanish Association Against Cancer
ATC: Anatomic Therapeutic Chemical Classification System
BMI: body mass index
CatSalut: Catalan Health Service
CI: confidence interval
DAA: direct-acting antivirals
DAP: geophaphical codification of healthcare units (*Direcció d'Atenció Primària*)
eCAP: electronic health records in primary care
EU: European Union
GDPR: European Data Protection Regulation
HBV: hepatitis B virus
HCC: hepatocellular carcinoma
HCV: hepatitis C virus
HR: hazard ratio
ICD: International Classification of Diseases
ICS: Catalan Institute of Health
IDIAP: Institute of Research in Primary Care (IDIAP Jordi Gol)
IFN: interferon
IQR: interquartile range
MEDEA: deprivation index (Spanich acronym for "Mortality in Small Spanish Areas and Socioenconomic and Environmental Inequalities")
PADRIS: Public Data Analysis for Health Research and Innovation Program
PC-MBDS: primary care minimum basic data set

PS: propensity score

RPT: Registry of Patients and Treatments

RR: rate ratio

SAS: Statistical Analysis Systems

SD: standard deviation

SIDIAP: System for the Development of Research in Primary Care

STD: standardized difference

TSI: individual health card

Data availability statement

The study data are available from the authors (J.R.) upon request.

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Table 1. Main characteristics of HCV patients and their matching controls

Variables	Interferon (IFN) only			IFN + direct-acting antivirals (DAA)			Direct-acting antivirals (DAA) only		
	Matching controls (n = 19,376)	IFN (n = 4,329)	Absolute standardized differences %	Matching controls (n = 3,507)	IFN + DAA (n = 794)	Absolute standardized differences %	Matching controls (n = 26,662)	DAA (n = 6,533)	Absolute standardized differences %
Age, years, median (IQR)	36.8 (29.6-44.1)	36.5 (29.1-43.8)	3	43.9 (39.2-49.3)	43.8 (39-48.9)	2	45 (39.5-55.8)	45.5 (39.5-57)	4
Gender, n (%)			1			0			0
Men	9,092 (46.9)	2,024 (46.8)		2,339 (66.7)	531 (66.9)		16,131 (60.5)	3,948 (60.4)	
Women	10,284 (53.1)	2,305 (53.2)		1,168 (33.3)	263 (33.1)		10,531 (39.5)	2,585 (39.6)	
Height, cm, median (IQR)	165 (159-172)	166 (159.5-173)	5	167.7 (160-174)	168 (160-174)	6	166 (158-173)	166 (158-172.3)	3
Missing, n	5,648	1,072		903	159		5,744	1,194	
Weight, kg, median (IQR)	72 (62-83.5)	69.7 (60.9-80)	17	77 (66.6-87.8)	73.8 (65.3-83.3)	17	76 (66.1-86.6)	72.5 (63.2-82)	25
Missing, n	5,132	905		804	127		4,858	930	
BMI, kg/m^2, median (IQR)	26.1 (23.2-29.5)	25 (22.4-28.2)	22	27.3 (24.5-30.6)	26.2 (23.9-29.1)	22	27.5 (24.7-30.8)	26.4 (23.6-29.4)	26
Missing, n	5,411	984		849	146		5,202	1,057	
BMI, WHO categories, n (%)			21			21			24
Underweight (< 18.5)	269 (1.9)	85 (2.5)		29 (1.1)	1 (0.2)		170 (0.8)	74 (1.4)	
Normal weight (18.5-24.9)	5,401 (38.7)	1,590 (47.5)		745 (28)	240 (37)		5,742 (26.8)	1,960 (35.8)	
Pre-obesity (25.0-29.9)	5,141 (36.8)	1,147 (34.3)		1,121 (42.2)	273 (42.1)		9,092 (42.4)	2,258 (41.2)	
Obesity class I (30.0-34.9)	2,223 (15.9)	374 (11.2)		538 (20.2)	98 (15.1)		4,657 (21.7)	904 (16.5)	
Obesity class II (35.0-39.9)	666 (4.8)	113 (3.4)		161 (6.1)	30 (4.6)		1,343 (6.3)	223 (4.1)	
Obesity class III (> 40)	265 (1.9)	36 (1.1)		64 (2.4)	6 (0.9)		456 (2.1)	57 (1)	
Missing, n	5,411	984		849	146		5,202	1,057	
Smoking habit, n (%)			5			5			5
Non-Smoker	8,779 (45.3)	2,060 (47.6)		1,249 (35.6)	301 (37.9)		10,269 (38.5)	2,686 (41.1)	
Smoker or Ex-Smoker	10,597 (54.7)	2,269 (52.4)		2,258 (64.4)	493 (62.1)		16,393 (61.5)	3,847 (58.9)	
Alcohol Consumption, n (%)			4			5			8
No	13,203 (68.1)	3,038 (70.2)		2,426 (69.2)	568 (71.5)		17,177 (64.4)	4,457 (68.2)	
Yes	6,173 (31.9)	1,291 (29.8)		1,081 (30.8)	226 (28.5)		9,485 (35.6)	2,076 (31.8)	
MEDEA index, quintiles, n (%)			3			8			7
Q1	2,890 (20.5)	617 (19.5)		437 (16.8)	94 (16.2)		3,782 (18.8)	849 (17.8)	
Q2	2,783 (19.7)	632 (20)		473 (18.2)	96 (16.6)		3,894 (19.4)	867 (18.1)	
Q3	2,837 (20.1)	634 (20)		514 (19.8)	121 (20.9)		3,975 (19.8)	978 (20.5)	
Q4	2,768 (19.6)	659 (20.8)		584 (22.5)	118 (20.3)		4,150 (20.6)	964 (20.2)	
Q5	2,847 (20.2)	622 (19.7)		592 (22.8)	151 (26)		4,318 (21.5)	1,119 (23.4)	
Missing	5,251	1,165		907	214		6,543	1,756	
Diabetes mellitus, n (%)	1,213 (6.3)	285 (6.6)	1	379 (10.8)	132 (16.6)	17	3,579 (13.4)	1,251 (19.1)	16
HIV infection, n (%)	94 (0.5)	287 (6.6)	33	20 (0.6)	207 (26.1)	81	102 (0.4)	2,775 (42.5)	100
Cirrhosis, n (%)	47 (0.2)	12 (0.3)	1	17 (0.5)	367 (46.2)	100	115 (0.4)	2,824 (43.2)	100

IQR; interquartile range (25th-75th percentile); BMI: body mass index; HIV: human immunodeficiency virus.

Table 2. Incidence and rate ratios of cancer, hepatocellular carcinoma (HCC) and non-liver cancer in HCV patients and their matching controls

Malignancy and antiviral treatment	Events	Patients at risk	Follow-up (person-years)	Incidence per 100,000 person-years (95% CI)	Rate ratio (95% CI)	*P* value
Cancer (all types)						
Interferon (IFN) only						
Control	555	19,376	107,207	514.9 (472.3-561.3)	Ref.	
IFN	141	4,329	24,774	569.1 (482.5-671.4)	1.11 (0.92-1.32)	0.2771
Interferon and direct-acting antivirals (DAA)						
Control	123	3,507	17,163	710.8 (590.7-855.4)	Ref.	
IFN + DAA	49	794	3,904	1,255.3 (947.9-1,662.2)	1.77 (1.27-2.46)	0.0008
DAA only						
Control	633	26,662	77,271	815.3 (752.9-882.9)	Ref.	
DAA	283	6,533	18,170	1,552.0 (1,380.1-1,745.3)	1.90 (1.66-2.19)	< 0.0001
Hepatocellular carcinoma (HCC)						
Interferon (IFN) only						
Control	100	19,376	107,207	91.4 (75.0-111.5)	Ref.	
IFN	34	4,329	24,774	137.2 (98.1-192.1)	1.50 (1.02-2.22)	0.0409
Interferon and direct-acting antivirals (DAA)						
Control	26	3,507	17,163	151.5 (102.3-224.3)	Ref.	
IFN + DAA	23	794	3,904	589.2 (391.3-887.1)	3.89 (2.26-6.69)	< 0.0001
DAA only						
Control	88	26,662	77,271	112.6 (90.9-139.4)	Ref.	
DAA	132	6,533	18,170	726.5 (612.2-862.0)	6.45 (4.90-8.49)	< 0.0001
Non-liver cancer						
Interferon (IFN) only						
Control	455	19,376	107,207	423.5 (385.1-465.7)	Ref.	
IFN	107	4,329	24,774	431.9 (357.3-522.1)	1.02 (0.83-1.25)	0.8521
Interferon and direct-acting antivirals (DAA)						
Control	97	3,507	17,163	559.3 (453.6-689.8)	Ref.	
IFN + DAA	26	794	3,904	666.1 (453.2-978.9)	1.19 (0.77-1.84)	0.4325
DAA only						
Control	545	26,662	77,271	702.7 (645.4-765.1)	Ref.	
DAA	151	6,533	18,170	825.5 (703.1-969.2)	1.17 (0.98-1.41)	0.0793

Ref: reference group for risk calculaton.

Table 3. Incidence and rate ratios of cancer in HCV patients and their matching controls according to HIV coinfection and cirrhosis as comorbidities

Comorbidity and antiviral treatment	Events	Patients at risk	Follow-up (person-years)	Incidence per 100,000 person-years (95% CI)	Rate ratio (95% CI)	*P* value
HIV coinfection						
Interferon (IFN) only						
No HIV	130	4,042	23,336	557.1 (469.1-661.6)	Ref.	
Yes HIV	11	287	1,439	764.6 (423.4-1,380.6)	1.37 (0.74-2.54)	0.3133
Interferon and direct-acting antivirals (DAA)						
No HIV	43	587	2,855	1,506.4 (1,117.2-2,031.1)	Ref.	
Yes HIV	6	207	1,049	572.0 (257.0-1,273.1)	0.38 (0.16-0.89)	0.0263
DAA only						
No HIV	180	3,758	10,582	1,701.1 (1,469.9-1,968.2)	Ref.	
Yes HIV	103	2,775	7,589	1,344.1(1,170.0-1,632.0)	0.79 (0.62-1.01)	0.0574
Cirrhosis						
Interferon (IFN) only						
No cirrhosis	140	4,317	24,713	566.5 (480.0-668.6)	Ref.	
Yes cirrhosis	1	12	62	1,620.4 (228.3-11,503.2)	2.86 (0.4-20.4)	0.2950
Interferon and direct-acting antivirals (DAA)						
No cirrhosis	24	427	2,106	1,139.6 (763.8-1,700.1)	Ref.	
Yes cirrhosis	25	367	1,798	1,390.8 (939.8-2,058)	1.22 (0.7-2.14)	0.4856
DAA only						
No cirrhosis	112	3,709	10,078	1,101.3 (914.4-1,326.5)	Ref.	
Yes cirrhosis	171	2,824	8,092	2,113.3 (1,819.2-2,455)	1.92 (1.51-2.44)	< 0.0001

Ref: reference group for risk calculaton.

Table S1

Definition of diagnosis of cancer, date at diagnosis and data source

Pharmacy billing registry from hospitals or community pharmacies for Catalan Health System prescriptions from PADRIS and SIDIAP registries for antineoplastic agents:

ATC codes group 'L01', 'L02' (with exception 'L01BA01', 'L01XX33', 'L02AB01', 'L02AB02') and code 'L03AX91'.

SIDIAP registry from general practitioners (ICD-10), codes for malignancy:

C00, C00.0, C00.1, C00.2, C00.4, C00.8, C00.9, C01, C02, C02.0, C02.1, C02.4, C02.8, C02.9, C03, C03.1, C03.9, C04, C04.1, C04.8, C05, C05.0, C05.2, C05.8, C05.9, C06, C06.0, C06.2, C06.8, C06.9, C07, C08, C08.0, C08.1, C08.8, C08.9, C09, C09.1, C09.8, C09.9, C10, C10.0, C10.1, C10.2, C10.3, C10.4, C10.8, C10.9, C11, C11.0, C11.1, C11.2, C11.8, C11.9, C12, C13, C13.0, C13.1, C13.8, C13.9, C14, C14.0, C14.2, C14.8, C15, C15.0, C15.1, C15.2, C15.3, C15.4, C15.5, C15.8, C15.9, C16, C16.0, C16.1, C16.2, C16.3, C16.8, C16.9, C17, C17.0, C17.1, C17.2, C17.3, C17.8, C17.9, C18, C18.0, C18.1, C18.2, C18.3, C18.4, C18.6, C18.7, C18.8, C18.9, C19, C20, C21, C21.0, C21.1, C21.8, C22, C22.0, C22.1, C22.2, C22.3, C22.7, C22.9, C23, C24, C24.0, C24.1, C24.8, C24.9, C25, C25.0, C25.1, C25.2, C25.3, C25.4, C25.8, C25.9, C26, C26.0, C26.1, C26.8, C26.9, C30, C30.0, C30.1, C31, C31.0, C31.1, C31.8, C31.9, C32, C32.0, C32.1, C32.2, C32.3, C32.8, C32.9, C33, C34, C34.0, C34.1, C34.2, C34.3, C34.8, C34.9, C37, C38, C38.0, C38.1, C38.2, C38.3, C38.4, C38.8, C39, C39.0, C39.8, C39.9, C40, C40.0, C40.1, C40.2, C40.3, C40.8, C40.9, C41, C41.0, C41.1, C41.2, C41.3, C41.4, C41.8, C41.9, C43, C43.3, C43.4, C43.5, C43.6, C43.7, C43.9, C44, C44.0, C44.1, C44.2, C44.3, C44.4, C44.5, C44.6, C44.7, C44.8, C44.9, C45, C45.0, C45.2, C45.9, C46, C46.0, C46.1, C46.7, C46.8, C46.9, C47, C47.0, C47.8, C48, C48.0, C48.1, C48.2, C48.8, C49, C49.0, C49.1, C49.2, C49.4, C49.5, C49.8, C49.9, C50, C50.0, C50.1, C50.2, C50.3, C50.4, C50.5, C50.6, C50.8, C50.9, C51, C51.8, C51.9, C52, C53, C53.0, C53.1, C53.8, C53.9, C54, C54.0, C54.1, C54.2, C54.3, C54.8, C54.9, C55, C56, C57, C57.4, C57.7, C57.8, C57.9, C60, C60.1, C60.2, C60.8, C60.9, C61, C62, C62.0, C62.1, C62.9, C63, C63.1, C63.2, C63.7, C63.8, C63.9, C64, C65, C66, C67, C67.0, C67.1, C67.2, C67.3, C67.4, C67.5, C67.6, C67.7, C67.8, C67.9, C68, C68.0, C68.8, C68.9, C69, C69.0, C69.2, C69.3, C69.5, C69.6, C69.8, C69.9, C70, C70.0, C70.1, C70.9, C71, C71.0, C71.1, C71.2, C71.3, C71.4, C71.5, C71.6, C71.8, C71.9, C72, C72.0, C72.2, C72.4, C72.8, C72.9, C73, C74, C74.1, C74.9, C75, C75.0, C75.1, C75.2, C75.5, C75.9, C76, C76.0, C76.1, C76.2, C76.3, C76.4, C76.5, C76.7, C76.8, C77, C77.0, C77.1, C77.2, C77.4, C77.8, C77.9, C78, C78.0, C78.1, C78.2, C78.4, C78.5, C78.6, C78.7, C78.8, C79, C79.0, C79.1, C79.2, C79.3, C79.5, C79.6, C79.7, C79.8, C80, C81, C81.0, C81.1, C81.2, C81.3, C81.7, C81.9, C82, C82.0, C82.1, C82.2, C82.7, C82.9, C83, C83.3, C83.4, C83.5, C83.6, C83.7, C83.8, C83.9, C84, C84.0, C84.1, C84.2, C84.3, C84.4, C84.5, C85, C85.0, C85.1, C85.7, C85.9, C88, C88.0, C88.2, C88.7, C88.9, C90, C90.0, C90.1, C90.2, C91, C91.0, C91.1, C91.2, C91.3, C91.4, C91.7, C91.9, C92, C92.0, C92.1, C92.2, C92.3, C92.4, C92.7, C92.9, C93, C93.0, C93.1, C94, C94.2, C94.4, C94.5, C94.7, C95, C95.0, C95.1, C95.7, C95.9, C96, C96.1, C96.2, C96.3, C96.7, C96.9, C97

MBDR databases from SIDIAP and PADRIS registries (ICD-9), codes for malignancy:

1400, 1401, 1403, 1404, 1405, 1406, 1408, 1409, 1410, 1412, 1419, 1420, 1453, 1460, 1463, 1469, 1471, 1478, 1479, 1481, 1489, 1490, 1501, 1503, 1504, 1508, 1509, 1510, 1512, 1513, 1514, 1518, 1519, 1520, 1521, 1522, 1528, 1529, 1530, 1531, 1532, 1533, 1534, 1535, 1536, 1537, 1538, 1539, 1540, 1541, 1542, 1548, 1550, 1551, 1552, 1560, 1562, 1569, 1570, 1571, 1572, 1578, 1579, 1580, 1588, 1589, 1590, 1599, 1610, 1611, 1619, 1622, 1623, 1624, 1625, 1628, 1629, 1638, 1639, 1640, 1642, 1648, 1649, 1659, 1700, 1704, 1709, 1713, 1715, 17310, 1742, 1744, 1745, 1748, 1749, 179, 1800, 1809, 1820, 1828, 1830, 1844, 185, 1880, 1882, 1885, 1888, 1889, 1890, 1891, 1892, 1893, 1899, 1910, 1911, 1912, 1913, 1915, 1916, 1918, 1919, 193, 1940, 1950, 1953, 1960, 1961, 1962, 1963, 1965, 1966, 1968, 1969, 1970, 1971, 1972, 1974, 1975, 1976, 1977, 1978, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 19882, 19889, 1990, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2362, 2367, 23691, 2372, 2375, 2376, 2380, 2381, 2382, 2383, 2385, 2386, 2387, 23879, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 23989, 25801

Table S2 Supplementary clinical information of patients

	Interferon Only		Interferon + Direct antiviral agents		Direct antiviral agents only	
	Control	IFN	Control	IFN+DAA	Control	DAA
	n=19,376	*n=4,329*	*n=3,507*	*n=794*	*n=26,662*	*n=6,533*
Fibrosis degree, n (%)						
F0	0 (0)	14 (12)	0 (0)	13 (1.9)	0 (0)	126 (2)
F1	0 (0)	53 (45.3)	0 (0)	45 (6.5)	0 (0)	422 (6.7)
F2	0 (0)	27 (23.1)	0 (0)	123 (17.6)	0 (0)	1,460 (23.3)
F3	0 (0)	11 (9.4)	0 (0)	149 (21.4)	0 (0)	1,431 (22.8)
F4	47 (100)	12 (10.3)	17 (100)	367 (52.7)	115 (100)	2,824 (45.1)
Missing/NA	19,329	4,212	3,490	97	26,547	270
Genotype, n (%)						
1	0 (0)	41 (35)	0 (0)	504 (64.9)	0 (0)	4,804 (74.3)
2	0 (0)	5 (4.3)	0 (0)	24 (3.1)	0 (0)	157 (2.4)
3	0 (0)	40 (34.2)	0 (0)	151 (19.4)	0 (0)	717 (11.1)
4	0 (0)	31 (26.5)	0 (0)	98 (12.6)	0 (0)	784 (12.1)
5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (0.1)
Missing/NA	19,376	4212	3,507	17	27,662	66
Viral load (log, count), median (IQR)	N=0	N=117 / 13.9 (11.7-15.2)	N=0	N=777 / 14 (12.8-14.9)	N=0	N=6,454 / 13.9 (12.6-14.9)
IGG HCV, median (IQR)	N=0	N=138 / 0.1 (0.1-10.5)	N=0	N=16 / 11.6 (10.3-26.3)	N=0	N=245 / 11.4 (10-26.7)
Exposure to, n (%):						
ribavirina						
No	19,376 (100)	3,125 (72.2)	3,507 (100)	37 (4.7)	*26,662* (100)	4,039 (61.8)
Yes	0 (0)	1,204 (27.8)	0 (0)	757 (95.3)	0 (0)	2,494 (38.2)
telaprevir						
No	19,376 (100)	4,329 (100)	3,507 (100)	619 (78)	*26,662* (100)	6,533 (100)
Yes	0 (0)	0 (0)	0 (0)	175 (22)	0 (0)	0 (0)
boceprevir						
No	19,376 (100)	4,329 (100)	3,507 (100)	725 (91.3)	*26,662* (100)	6,533 (100)
Yes	0 (0)	0 (0)	0 (0)	69 (8.7)	0 (0)	0 (0)
simeprevir						
No	20,038 (100)	4,329 (100)	3,507 (100)	578 (72.8)	*26,662* (100)	5,589 (85.6)
Yes	0 (0)	0 (0)	0 (0)	216 (27.2)	0 (0)	944 (14.4)
daclatasvir						
No	19,376 (100)	4,329 (100)	3,507 (100)	692 (87.2)	*26,662* (100)	5,779 (88.5)
Yes	0 (0)	0 (0)	0 (0)	102 (12.8)	0 (0)	754 (11.5)
sofosbuvir						
No	19,376 (100)	4,329 (100)	3,507 (100)	84 (10.6)	*26,662* (100)	1,736 (26.6)
Yes	0 (0)	0 (0)	0 (0)	710 (89.4)	0 (0)	4,797 (73.4)
dasabuvir						
No	19,376 (100)	4,329 (100)	3,507 (100)	737 (92.8)	*26,662* (100)	5,056 (77.4)
Yes	0 (0)	0 (0)	0 (0)	57 (7.2)	0 (0)	1,477 (22.6)
ledipasvir						
No	19,376 (100)	4,329 (100)	3,507 (100)	490 (61.7)	*26,662* (100)	3,598 (55.1)
Yes	0 (0)	0 (0)	0 (0)	304 (38.3)	0 (0)	2,935 (44.9)
ombitasvir						
No	19,376 (100)	4,329 (100)	3,507 (100)	711 (89.5)	*26,662* (100)	4,812 (73.7)
Yes	0 (0)	0 (0)	0 (0)	83 (10.5)	0 (0)	1,721 (26.3)
pertaprevir						
No	19,376 (100)	4,329 (100)	3,507 (100)	711 (89.5)	*26,662* (100)	4,812 (73.7)
Yes	0 (0)	0 (0)	0 (0)	83 (10.5)	0 (0)	1,721 (26.3)
ritonavir						
No	19,376 (100)	4,329 (100)	3,507 (100)	711 (89.5)	*26,662* (100)	4,812 (73.7)
Yes	0 (0)	0 (0)	0 (0)	83 (10.5)	0 (0)	1,721 (26.3)
CKD-EPI ($mL/min^{-1}/1.73m^3$), median (IQR)	N=14,903 / 90.1 (87.3-90.1)	N=3,839 / 90.1 (89.8-90.1)	N=2,856 / 90.1 (84.2-90.1)	N=717 / 90.1 (86.9-90.1)	N=22,434 / 90.1 (80.4-90.1)	N=5,891 / 90.1 (81.4-90.1)
GGT (IU/L), median (IQR)	N=10,760 / 21 (14-34)	N=3,331 / 24 (16-44)	N=2,154 / 26 (17-44)	N=648 / 79 (38.5-152.5)	N=17,643 / 25 (17-42)	N=5,402 / 63 (33-126)

ALT (IU/L), median (IQR)	N=12,683 / 19 (14-29)	N=3,331 / 24 (16-45)	N=2,472 / 22.5 (16-33)	N=632 / 64 (39-109)	N=20,029 / 21 (16-31)	N=5,368 / 61 (38-98)
Platelets (10^9 count), median (IQR)	N=13,111 / 245 (209-287)	N=3,569 / 225 (187-267)	N=2,520 / 239 (204-281)	N=665 / 170 (128-216)	N=20,414 / 237 (202-278)	N=5,539 / 173 (124-222)
Total bilirrubin (mg/dL), median (IQR)	N=8,504 / 0.5 (0.4-0.7)	N=3,175 / 0.5 (0.4-0.7)	N=1,792 / 0.5 (0.4-0.7)	N=610 / 0.6 (0.5-0.9)	N=14,339 / 0.5 (0.4-0.7)	N=5,192 / 0.7 (0.5-0.9)
AST (IU/L), median (IQR)	N=6,862 / 21 (17-28)	N=3,018 / 24 (18-37)	N=1,437 / 23 (18-30)	N=608 / 58 (36-95)	N=11,545 / 22 (18-29)	N=5,212 / 54 (36-85)
Prothombine time (%), median (IQR)	N=2,535 / 100 (95-107)	N=1,196 / 100 (92-103)	N=475 / 100 (93.1-106)	N=320 / 96 (87-100)	N=4,173 / 100 (93.5-104)	N=2,840 / 96 (85-100)
Albumin (mg/dL), median (IQR)	N=2,362 / 4.4 (4.1-4.6)	N=1,842 / 4.4 (4.1-4.6)	N=550 / 4.4 (4.1-4.6)	N=476 / 4.3 (4-4.5)	N=4,295 / 4.3 (4.1-4.6)	N=3,800 / 4.2 (3.9-4.5)
INR, median (IQR)	N=1,824 / 1 (0.9-1.1)	N=927 / 1 (1-1.1)	N=376 / 1 (0.9-1)	N=278 / 1 (1-1.1)	N=3,053 / 1 (0.9-1.1)	N=2,436 / 1 (1-1.1)
IGG HBV (mg/dL), median (IQR)	N=110 / 0.2 (0.2-0.3)	N=178 / 0.2 (0.1-0.4)	N=29 / 0.2 (0.1-2)	N=37 / 0.2 (0.1-2.6)	N=182 / 0.2 (0.1-0.3)	N=402 / 0.2 (0.2-1.5)

IFN: Interferon, DAA: Direct antiviral agents, Ref.: Reference group for risk calculation, NE: Not Estimable, 100k/PY: 100.000 patients-years, NA. Not Applicable
*Control groups are control patients matched for each hepatitis C virus therapy regimen

Figure 1 - Flow Chart

Click here to access/download;Figure;Figure 1 Flow Chart.png

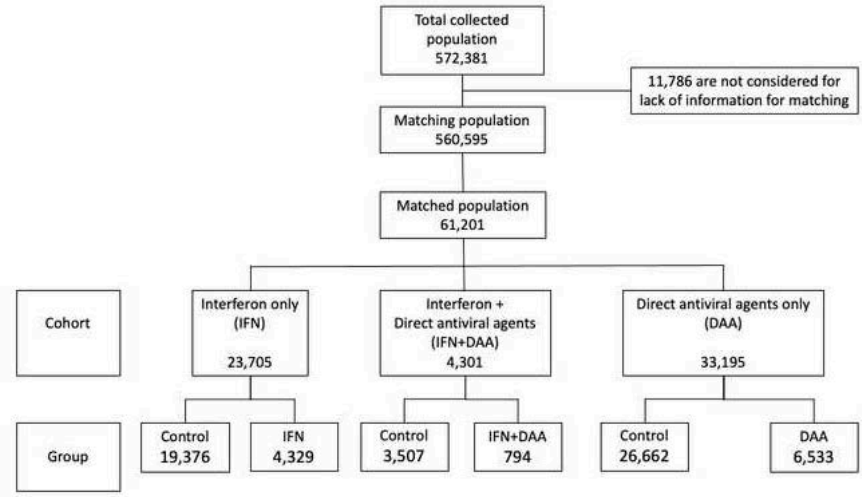


Figure 2a

Click here to access/download;Figure;Figure2a.png

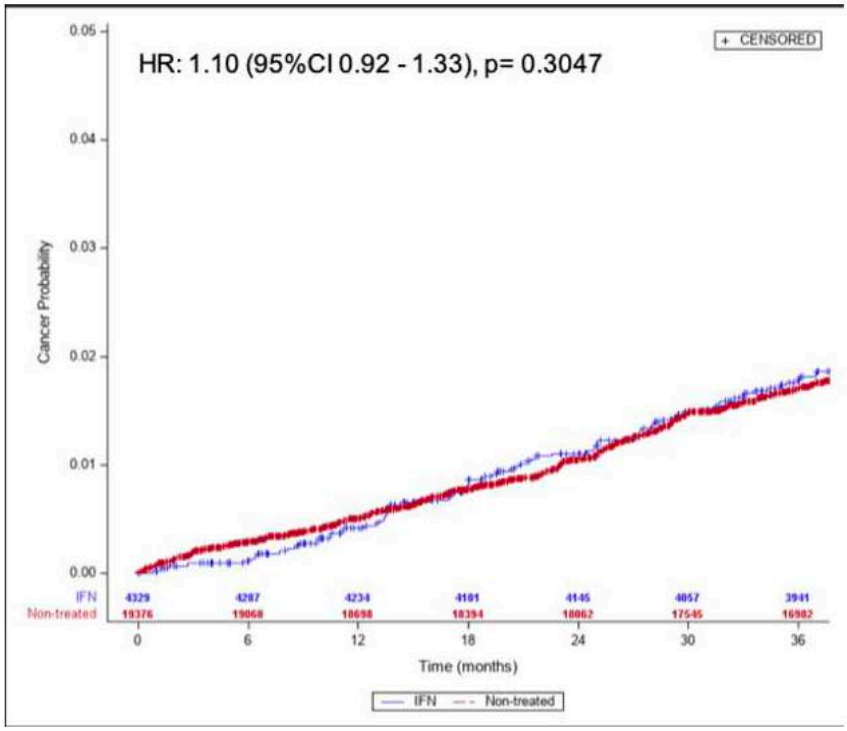


Figure 2b

Click here to access/download;Figure;Figure 2b.png

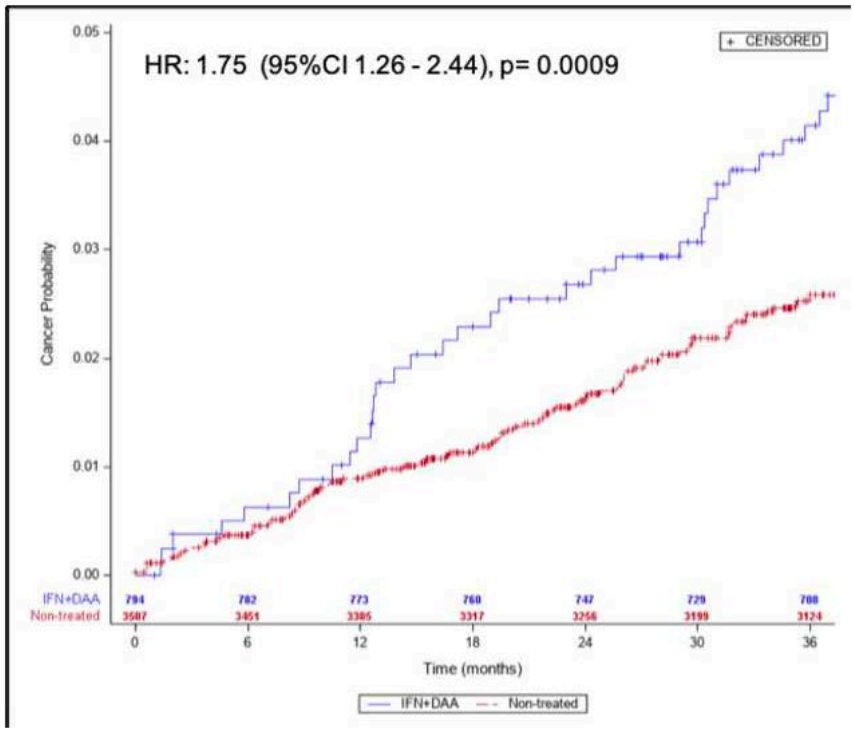
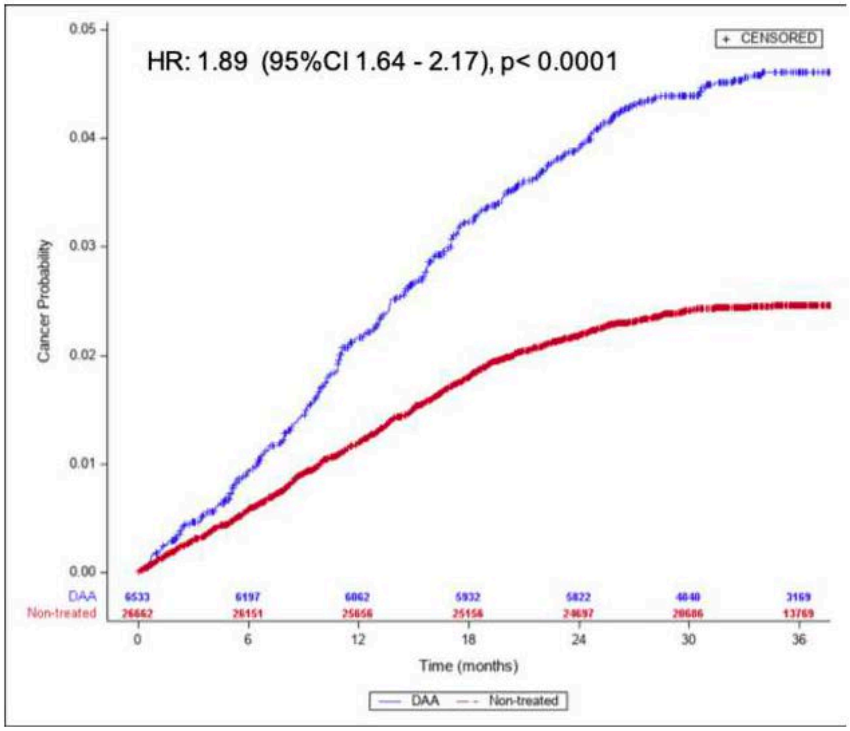


Figure 2c

Click here to access/download;Figure;Figure 2c.png



14.4 Annex 4: Study Protocol

Protocol d'Estudi

Incidencia de càncer en relació amb el tractament farmacològic de la infecció pel virus de l'hepatitis C

Investigador principal: Maria Reig

e-mail: mreig1@clinic.cat

Professió: Metge

Institució: Unidad funcional de oncologia hepática. Servei Hospital Clínic de Barcelona

Col·laboradors **Institut Universitari d'Investigació en Atenció Primària (IDIAP) Jordi Gol:**

- Rosa Morros

CatSalut: Àrea del Medicament

- Gerència d'Harmonització Farmacoterapèutica:
  - Caridad Pontes, Marta Roig

Hospital Clínic:

- Institut Clínic de Malalties Digestives i Metabòliques (ICMDiM)
  - Unidad funcional de hepatitis víricas:
    - Zoe Mariño, Sabela Lens, Xavier Forns
  - Unidad funcional de oncologia hepática:
    - Maria Reig, Victor Sapena, Jordi Bruix
  - IDIBAPS - Medical Statistics Core Facility:
    - Ferran Torres, Jose Rios

Promotor **Servei Català de la Salut**

Versió 1: 30 de gener 2017

Títol i resum

Incidència de càncer en relació amb el tractament farmacològic de la infecció pel virus de l'hepatitis C

L'any 2014 es van iniciar a Catalunya els primers tractaments dels pacients infectats pel virus de l'hepatitis C (VHC) amb agents antivirals directes (AAD). Aquests fàrmacs tenen eficàcies superiors i un millor perfil de seguretat que els tractaments previs basats en interferó (IFN). L'efectivitat i la seguretat a curt termini dels tractaments en condicions de pràctica clínica ha mostrat ser similar a la descrita en els assaigs clínics, però la seguretat a llarg termini o en poblacions especials està poc establerta. Recentment s'han descrit alguns senyals de seguretat que inclouen riscos de descompensació hepàtica en pacients amb fibrosi avançada, reactivació de la infecció per hepatitis B i un possible augment del risc de recidiva d'hepatocarcinoma després del tractament amb AAD. Fisiopatològicament és plausible que augmenti el risc de qualsevol càncer, no només de l'hepatocarcinoma. Aquestes comunicacions han generat recentment una alerta de seguretat regulatòria a nivell europeu.

El present estudi de cohorts retrospectiu pretén valorar a nivell poblacional la incidència de qualsevol càncer en pacients tractats amb AAD per a la infecció per VHC, i comparar-la amb la incidència en controls infectats per VHC tractats amb pautes basades en interferó abans del 2014, i amb pacients no infectats per VHC, per tal de valorar possibles diferències i factors que puguin influir en les mateixes. L'establiment d'inicidències i de risc relatius per als ADD mitjançant models de Poisson ajustats permetrà descriure si existeixen aquests riscs, i en cas afirmatiu, establir-ne el risc atribuïble i dissenyar un pla de gestió dels mateixos.

Títol i resum en anglès

Incidence of cancer related with pharmacological treatment of the infection by hepatitis C virus.

In 2014 the first treatments with direct antiviral agents (AAD) for the treatment of patients infected with hepatitis C virus (HCV) were started in Catalunya. These drugs had higher efficiencies and better safety profile than previous treatments based on interferon (IFN). The effectiveness and safety of treatment in clinical practice conditions are shown to be similar in the short-term to those described in clinical trials, but their long-term safety or in special populations is yet uncertain. Some potential risks including liver decompensation in patients with advanced fibrosis, reactivation of hepatitis B infection and a possible increased risk of recurrence of hepatocellular carcinoma after treatment with AAD have been described. It seems physio-pathologically plausible that risk of any cancer could be augmented, not only that of hepatocellular carcinoma. The communication of these safety signals have generated a recent alert issued by the European Medicines Agency.

This retrospective cohort study aims to assess the incidence of any cancer in patients treated with AAD for HCV infection at the population-level, and compare it with the incidence in controls infected with HCV who received treatments based on interferon before 2014, and with patients not infected by HCV, in order to assess potential differences and factors that can influence them. Describing incidences and relative risks for cancer for each type of treatment using adjusted Poisson models will allow to detect such risks, and to establish the attributable risk if appropriate, as well as to design risk management strategies.

Contingut

Títol i resum ... 2
Títol i resum en anglès ... 2
Antecedents, Justificació i Bibliografia ... 4
Hipòtesi i Objectius ... 6
Hipòtesi ... 6
Objectius Generals ... 6
Objectius Específics ... 6
Metodologia ... 6
Disseny ... 6
Àmbit i període d'estudi ... 6
Població de referència ... 7
Població d'estudi amb els criteris d'inclusió i exclusió ... 7
Mida de la mostra i procediment de mostratge ... 7
Variables (diferenciar entre dependents i independents) ... 7
Recollida de dades i fonts d'informació ... 9
Anàlisi de dades ... 10
Dificultats i limitacions de l'estudi ... 10
Consideracions ètiques i Confidencialitat de les dades ... 11
Procediments addicionals derivats de l'estudi (si procedeix) ... 11
Pla de treball (tasques, fites i cronologia) ... 11
Experiència de l'equip investigador sobre el tema ... 11
Historial científic-tècnic ... 11
Aplicabilitat i utilitat pràctica dels resultats de l'estudi ... 12
Mitjans disponibles per a la realització del projecte ... 12
Justificació del Finançament i Pressupost Sol·licitat ... 12
Possibles conflictes d'interès ... 13
Annexes (si procedeix) (Full d'informació al pacient o participant, Full de consentiment informat) ... 13

Antecedents, Justificació i Bibliografia

L'hepatitis C és una infecció vírica que afecta principalment el fetge, de transmissió parenteral i sexual, que es cronifica en un 80% dels infectats, els qui entren en període de dècades de malaltia silent, amb discretes alteracions dels enzims hepàtics. En alguns pacients la infecció crònica evoluciona a fibrosi hepàtica, i en menys d'un 5% a hepatocarcinoma (1). Es considera que un 3% de la població mundial està infectada pel virus de l'hepatitis C (VHC), i que aquesta és la principal causa de cirrosi, trasplantament hepàtic i hepatocarcinoma (HCC) (2).

El tractament dels pacients infectats pel virus de l'hepatitis C (VHC) fins a l'any 2014 consistia en pautes de 24 a 52 setmanes que combinaven interferó (IFN) i ribavirina, amb una eficàcia en termes de resposta virològica sostinguda (RVS) entre el 50 i el 80% segons el genotip viral, i mala tolerabilitat i seguretat. L'any 2011 van aparèixer els primers agents antivirals directes (AAD), amb eficàcies superiors al 90% i un millor perfil de seguretat. Al 2014 es van iniciar a Espanya els primers tractaments amb aquests fàrmacs i lliures de interferó (IFN).

La millora de les opcions terapèutiques ha motivat que es recomani el tractament del VHC en pacients que per la seva gravetat abans no es tractaven amb IFN, com ara aquells amb antecedents d'hepatocarcinoma cel·lular (HCC) o en espera de trasplantament de fetge (3) . L'avanç substancial que representen els AAD s'ha acompanyat d'una ràpida introducció d'aquests en la clínica, inicialment en pacients amb un nivell de gravetat elevat, amb fibrosi avançada, i posteriorment en pacients de menys gravetat o en subpoblacions escassament estudiades en assajos clínics. L'efectivitat i la seguretat a curt termini dels tractaments en condicions de pràctica clínica ha mostrat ser similar a la descrita en els assaigs clínics (4).

No obstant això, la seguretat a llarg termini o en poblacions especials, per la limitació de la població inclosa, no està ben qualificada en els assajos clínics. Així, després de la comercialització s'han descrit alguns senyals de seguretat que inclouen riscos de descompensació hepàtica en pacients amb fibrosi avançada (4), reactivació de la infecció per hepatitis B i un possible augment del risc de recidiva d'hepatocarcinoma després del tractament amb AAD (5,6). També s'han comunicat dades d'aparició de HCC en subjectes sense antecedents de tumor, i les dades preliminars comunicats suggereixen que el patró d'agressivitat tumoral d'aquests casos és de pitjor pronòstic que l'esperable (7,8). Aquestes comunicacions han generat recentment un avís de seguretat per part de l'agència europea de medicaments (EMA) (9), si bé aquestes dades estan pendents de confirmació mitjançant estudis específics.

S'ha proposat que la plausibilitat biològica del risc de recidiva de HCC es basa en que la ràpida desaparició de la infecció crònica per VHC tindria un efecte disruptor de la vigilància immunològica comuna antivírica i antitumoral, facilitant l'emergència de tumors preexistents. Per tant, és teòricament possible que augmenti el risc de qualsevol càncer, no només del HCC.

L'absència de seguiments a llarg termini d'aquests pacients un cop curats de la seva infecció per VHC dificulta la detecció i l'establiment de sospites de causalitat amb tumors posteriors a nivell individual, de manera que la metodologia més apropiada per al seu estudi en aquest moment és la aproximació poblacional mitjançant registres de dades sanitàries.

Considerant la prevalença de la infecció a la nostra població, el fet que es considera ampliar progressivament la utilització dels tractaments a poblacions amb un grau menor d'afectació

hepàtica, i el potencial l'impacte sanitari i sobre la salut pública d'un risc associat a aquests tractaments que es podria produir a llarg termini i que és difícilment detectable en assaigs clínics, esdevè prioritari estudiar amb metodologia farmacoepidemiològica si existeix una possible associació causal entre els nous tractaments AAD i l'aparició de neoplàsies.

Això motiva el present estudi, que pretén valorar a nivell poblacional la incidència de càncer en pacients tractats amb AAD per a la HCV, i comparar-la amb la incidència en controls infectats per VHC tractats amb pautes basades en interferó abans del 2014, i amb pacients no infectats per VHC, per tal de valorar possibles diferències i factors que puguin influir en les mateixes.

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Hipòtesi i Objectius

HIPÒTESI

La disminució ràpida de la càrrega viral del VHC observada amb els tractaments amb AAD pot canviar l'entorn immunitari a nivell hepàtic. La reducció de l'estímul crònic de la infecció viral pot alterar el control de les cèl·lules canceroses latents en el fetge, promovent la recurrència de tumors pre-existents i/o el desenvolupament de neoplàsies *de novo*. Atès que la infecció per VHC es considera una malaltia sistèmica, la disminució extremadament ràpida de la càrrega viral també podria canviar la homeòstasi immunològica sistèmica, i associar-se a un increment inesperat de la incidència i/o la recurrència dels tumors malignes extrahepàtics en aquests pacients.

OBJECTIUS GENERALS

- Descriure la incidència de tumors malignes en els pacients amb VHC tractats (independentment del tractament anti-VHC) i en pacients sense infecció pel VHC a Catalunya.

OBJECTIUS ESPECÍFICS

- Comparar el risc de neoplàsies en pacients amb VHC que han rebut tractament amb ADD o amb combinacions basades en IFN a Catalunya, en el període entre 2012 i 2016.
- Comparar la incidència de càncer en pacients tractats amb AAD per al VHC respecte de la incidència en controls similars però sense infecció crònica pel VHC.
- Comparar la incidència de càncer en pacients tractats amb pautes antivirals per al VHC basades en interferó respecte de la incidència en controls similars però sense infecció crònica pel VHC.
- Avaluar l'associació temporal entre el diagnòstic de tumors malignes i el tractament de la infecció per VHC.
- Avaluar el tipus de neoplàsies extrahepàtiques que pateixen els pacients amb VHC que han rebut tractament amb ADD o amb combinacions basades en IFN, i respecte de la població que no està infectada pel VHC.
- Descriure i analitzar comparativament la incidència de HCC i de recurrència d'HCC en pacients tractats amb ADD respecte dels controls.

Metodologia

Es proposa fer un estudi observacional retrospectiu de cohorts, per avaluar la seguretat relativa a la inducció de neoplàsies de dos tipus de tractaments antivirals per a la infecció per VHC en condicions de pràctica clínica habitual a Catalunya.

Disseny

ÀMBIT I PERÍODE D'ESTUDI

Es proposa estudiar el període entre 2012 i 2016, considerant que en el període entre 2012 i 2014 es van emprar només tractaments basats en IFN i en el període entre 2014 i 2016 predominantment tractaments basats en ADD. Els dos tipus de tractament es van solapar

durant uns mesos de l'any 2014. Els pacients que hagin rebut tos dos s'analizaran addicionalment com a subgrup.

POBLACIÓ DE REFERÈNCIA

La població de referència serà la població que rep assistència primària en dispositius d'atenció primària de l'Institut Català de la Salut.

POBLACIÓ D'ESTUDI AMB ELS CRITERIS D'INCLUSIÓ I EXCLUSIÓ

La identificació de les cohorts es farà a partir dels pacients que han rebut tractament per a la infecció per VHC registrats en el Registre de Pacients i Tractaments del CatSalut, un registre obligatori vinculat a la facturació dels fàrmacs, que conté un identificador personal que permetrà vincular les dades sanitàries.

S'identificaran les següents cohorts:

- Cohort A: Pacients que han rebut un tractament amb AAD a càrrec de la Seguretat Social.
- Cohort B: Pacients que han rebut un tractament antiVHC no AAD a càrrec de la Seguretat Social.
- Cohort C: Subjectes amb registres sanitaris actius en un centre d'atenció primària usuari de eCAP, sense infecció documentada per VHC, i que no han rebut cap tractament per a la infecció per VHC a càrrec de la seguretat social; la cohort es seleccionarà per incloure subjectes semblants a la cohort A en edat, sexe, zona geogràfica, consum de tabac, consum de risc d'etanol i diabetis mellitus.
- Cohort D: Subjectes registrades en un centre d'atenció primària usuari de eCAP amb registres actius, sense infecció documentada per VHC, i que no han rebut cap tractament per a la infecció per VHC a càrrec de la seguretat social; la cohort es seleccionarà per incloure subjectes semblants a la cohort B en edat, sexe, zona geogràfica, consum de tabac, consum de risc d'etanol i diabetis mellitus.

Per identificar les cohorts A i B s'utilitzarà el Registre de Pacients i Tractaments del CatSalut (RLT), que inclou dades clíniques i de facturació de tractaments de dispensació hospitalària. Per identificar les cohorts C i D s'utilitzaran el Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària (SIDIAP) i el Conjunt Mínim Bàsic de Dades (CMBD).

MIDA DE LA MOSTRA I PROCEDIMENT DE MOSTRATGE

La mida de la mostra vindrà de la cohort A vindrà determinada pel número de pacients que hagin rebut tractament amb AAD a Catalunya a càrrec de la seguretat social i estiguin registrats en el Registre de Pacients i Tractaments fins al 31 de desembre del 2016; aquests representen aproximadament uns 12.000 pacients.

La cohort B vindrà determinada pel número de pacients amb infecció per VHC tractats amb pautes basades en INF en el període equivalent previ al primer tractament registrat amb AAD.

Les cohorts C i D seran aparellades 2:1 pels factors descrits a les cohorts A i B.

VARIABLES (DIFERENCIAR ENTRE DEPENDENTS I INDEPENDENTS)

En funció de la disponibilitat de registres, s'extrauran dades retrospectives de tipus clínic de cada cohort, incloent informació sobre característiques demogràfiques, factors de risc de

tumors, indicadors de morbiditat, tractaments concomitants potencialment modificadors del risc de càncer, característiques clíniques dels tumors identificats i els tractaments aplicats.

Es necessitaran les següents variables:

Variable principal:

- Codi ICD-10, descripció i data d'inici del diagnòstic per a les següents malalties:
  - C00-C96 (neoplasies malignes) i D37-D48 (neoplasies de comportament inciert, policitèmia vera i síndromes mielodisplàsics) (identificació de casos)

Per a aquesta variable es desglossaran els codis ICD-10 per descriure els tipus de tumors observats més freqüents.

- Codi ICD-9, descripció i data d'inici del diagnòstic per a les següents malalties:
  - Neoplasies (140-209) (codis de neoplasies excloent 210-229 neoplasies benignes, 230-234 Carcinoma in situ, 235-238 Neoplasies de comportament incert i 239 neoplasies de naturalesa no especificada)
- Codi ATC:
  - Grup L04 + L01AA01 + L01BA01 (inmunosupresors + ciclofosfamida + metotrexat, com a com a factor d'augment de risc oncològic), L02B (antagonistes d'hormones sexuals, com a tractament oncològic de càncers de mama o pròstata amb dispensació en oficina de farmàcia) (identificació de casos)

Altres variables destinades a descriure les poblacions i com a potencials ajustaments de les dades:

- Edat, Data de Naixement, Sexe, Nacionalitat, Equip d'Atenció Primària que presta serveis al pacient, Data d'assignació del pacient a l'EAP. Freqüentació.
- Codis ATC:
  - Grup A10 (hipoglucemiants, per tal de completar un algoritme diagnòstic de diabetes, com a factor d'augment de risc oncològic),
  - Número de tractaments actius per cada subjecte (com a indicador indirecte de morbiditat).
- Codi ICD-10, descripció i data d'inici del diagnòstic per a les següents malalties:
  - B15-B19 Hepatitis viral (identificació de cohorts)
  - K70-K78: Malalties del fetge (control de factors de risc d'hepatocarcinoma i comparabilitat de les cohorts estudiades)
  - D12.6 Neoplasia benigna de colon, no especificada (factor de risc oncològic per a ajustament)
  - E08-E13 Diabetes mellitus (factor de risc oncològic per a ajustament)
  - Z80 Història familiar de neoplasia maligna primària (factor de risc oncològic per a ajustament)
  - Z85 Història personal de neoplasia maligna (factor de risc oncològic per a ajustament)
  - Z94.0 estat de transplantament renal, Z94.1 estat de transplantament de cor, Z94.2 estat de transplantament de pulmó, Z94.3 estat de transplantament de cor i pulmó, Z94.4 estat de transplantament de fetge, Z94.81 estat de transplantament de medul·la òssia, Z94.82 estat de transplantament d'intestí, Z94.83 estat de transplantament de pàncrees (factors de risc oncològic per a ajustament)
  - M30-M36 trastorns sistèmics del teixit connectiu, M05-M14 poliartropaties inflamatòries, K50 malaltia de Crohn, K51 colitis ulcerosa, L40 psoriasi (inclou

artritis psoriasica), G35 esclerosi múltiple, G70.0 Miastenia gravis, I27.0 hipertensió pulmonar primària (tractats sovint amb inmunosupressoors, factors de risc oncològic per a ajustament)
    - B97.7 Papilomavirus com a causa de malalties classificades sota altre concepte (factors de risc oncològic per a ajustament)
    - B20 Malaltia per virus de la inmunodeficiència humana [VIH], Z21 Estat d'infecció asimptomàtica pel virus de inmunodeficiència humana [VIH], B27.0 Mononucleosi deguda a herpes virus gamma (factors de risc oncològic per a ajustament)
- Pes, Talla, IMC, Consum d'alcohol, Consum de tabac (factors de risc oncològic per a ajustament)
- Bioquímica en sang: transaminases, bilirubina, albúmina, temps de protrombina/ INR, funció renal (MDRD) (factors de risc oncològic per a ajustament)
- Hematologia: recompte plaquetar, CD4 (factors de risc oncològic per a ajustament)
- Immunologia humoral: anticossos anti VHC, antiHBs, antiHBc y HBSAg, Epstein-Barr virus, VIH (factors de risc oncològic per a ajustament)
- Microbiologia sèrica: rnaVHC, dnaVHB (factors de risc oncològic per a ajustament)
- Nivell socioeconòmic censal: índex MEDEA

Tanmateix per a disposar d'un control negatiu intern a l'estudi, es considerarà una patologia aguda independent de les exposicions estudiades i ben validada a SIDIAP, en concret la cardiopatia isquèmica aguda definida pels següents codis ICD:

- Registre d'ingrés hospitalari a CMBD-AA per algun dels següents codis diagnòstics ICD-9:
    - 411.1, 411.8 Angina inestable i altres formes agudes de cardiopatia isquèmica;
    - 410.0 - 410.9 Infart agut de miocardi;
    - 36.09 Angioplàstia coronaria (transluminal percutània (baló) 00.66); 36.03 Angioplàstia via torax obert;
    - 39.50 Angioplàstia baló (transluminal percutània) d'artèria coronària (un sol vas) 00.66.
- Registre en e-CAP d'algun dels següents codis ICD-10:
    - I20.0 Angina inestable;
    - I21.0 - I21.9 Infart agut de miocardi;
    - I22.0 - I22.9 Re-infart agut de miocardi
- Mort referida en e-CAP en els 30 dies posteriors a un diagnòstic hospitalari d'algun dels següents codis ICD-10:
    - I20.0 Angina inestable;
    - I21.0 - I21.9 Infart agut de miocardi;
    - I22.0 - I22.9 Re-infart agut de miocardi;
    - I46 Mort sobtada

RECOLLIDA DE DADES I FONTS D'INFORMACIÓ

- Es sol·liciten a SIDIAP:
    - Variables Sociodemogràfiques (inclou índex de privació MEDEA)
    - Problemes de Salut i/o Variables Clíniques
    - Farmàcia (dispensació farmacèutica generada per l'ICS)
    - Laboratori
    - CMBD

- Fonts Externes (s'entén per fonts externes, altres registres existents o bases de dades pròpies de l'equip investigador)
    - Registre de Pacients i Tractaments del CatSalut

Període de creuament. De 2012 a 2016

ANÀLISI DE DADES

Les dades es descriuran conforme a la seva naturalesa, així les variables quantitatives es descriuran com a mitjana o mediana i desviació estàndard o rang interquartílic i les qualitatives com a freqüència absoluta i relativa.

Les taxes d'incidència seran calculades com a esdeveniments / pacients-mes donat que s'espera que el principal esdeveniment d'interès, l'aparició o recurrència de càncer es presenti a curt-mig termini en el temps segons la nostra hipòtesi. En cas de requerir models ajustats de taxes d'incidència es realitzaran models de regressió de Poisson, ajustats per l'índex de propensió (*propensity score*). Els riscs relatius (*rate ratios*) s'estimaran a partir del mateix model de Poisson. S'intentarà valorar les diferents taxes d'incidència anual o semestral mitjançant models de cadena de Markov per tal d'explorar la seva utilitat en l'establiment de taxes d'incidència de recurrència de càncer esperades en funció de diversos escenaris estadístics.

Dificultats i limitacions de l'estudi

La principal limitació de l'estudi és que, en ser observacional retrospectiu i considerant la gran quantitat de factors de risc del càncer, possiblement no es poden recollir les dades de totes les variables relacionades amb l'esdeveniment estudiat. Una altra important limitació de l'estudi és que les cohorts venen determinades pel nombre de tractaments realitzats en els últims tres anys, i considerant el període d'inducció de les alteracions neoplàsiques, el període d'observació pot esdevenir massa breu. No obstant, la fisiopatologia proposada i les observacions preliminars referides a hepatocarcinoma suggereixen que, de ser certa la hipòtesi, el risc podria augmentar ràpidament en les setmanes posteriors a l'exposició al tractament amb AAD.

Pel que fa a la identificació dels càncers, no podem garantir que sigui viable identificar tots els casos incidents de neoplasia, doncs pot ser que aquests no s'enregistrin com a diagnòstic principal d'alta en un centre hospitalari o es transcriguin a la història clínica d'atenció primària; tanmateix, es possible que la data d'inici de les neoplàsies no es correspongui amb la data d'inici de la patologia, bé perquè hi hagi un retard diagnòstic, o bé perquè el registre de la patologia es faci amb posterioritat i emprant la data de la visita en la qual es registra la informació.

Respecte de les exposicions que determinen la inclusió en cada una de les cohorts proposades, mentre es considera un indicador d'exposició força fiable que el pacient consti al Registre de Pacients i Tractaments, també es pot donar que la data de registre no es correspongui fidelment amb la data de tractament, especialment en els dos o tres primers mesos d'introducció dels AAD durant 2014, ja que alguns pacients varen iniciar el tractament amb AAD de manera prèvia i la data que consta al registre pot indicar la data de transcripció de la

informació, essent el tractament anterior a aquesta data. Pel que fa al registre de la informació clínica, aquesta pot ser incompleta o inexacta, ja que s'empren registres assistencials la finalitat no és l'exhaustivitat de la informació sinó la seva utilitat pràctica. D'altra banda, les bases de dades no solen proporcionar informació sobre l'estadi o grau de les malalties en el moment del diagnòstic, el tipus de dieta, els exàmens físics i els resultats de les proves diagnòstiques. Els hàbits de salut (per exemple, el tabaquisme, consum d'alcohol, l'activitat física) es registren parcialment en aquestes bases de dades, i de manera heterogènia en funció de diversos factors. Respecte de la infecció per VHC, és possible que una proporció de pacients que es considerin no infectats pel VHC realment ho estiguin però no hagin estat diagnosticats, o bé pot passar que aquest diagnòstic no consti a les seves dades clíniques. No obstant, ja que es tracta d'informació recollida de forma independent a la realització de l'estudi, aquestes limitacions suposadament afecten per igual totes les cohorts, de manera que la probabilitat de biaixos es pot considerar menor que en les metodologies de recollida d'informació primària.

Consideracions ètiques i Confidencialitat de les dades

S'elaborarà un protocol d'estudi, se sol·licitarà autorització del Comitè Ètic d'Investigació de l'Institut D'Investigació en Atenció Primària Jordi Gol i es notificarà l'estudi com EPA-OD a l'Agència Espanyola de Medicaments i Productes Sanitaris, prèviament al seu inici.

Les dades s'extrauran de manera anonimitzada i per part de personal independent de l'equip investigador, emprant un identificador que permeti aparellar cada grup de controls amb el seu cas corresponent.

Procediments addicionals derivats de l'estudi (si procedeix)

No aplica

Pla de treball (tasques, fites i cronologia)

Es proposa iniciar l'extracció de dades al mes d'abril del 2017, i analitzar-les durant els mesos de maig i juny. Es preveu obtenir els resultat preliminars a finals del mes de juny del 2017, i disposar d'un informe final de l'estudi al mes d'octubre.

Experiència de l'equip investigador sobre el tema

HISTORIAL CIENTÍFIC-TÈCNIC

Els Drs Maria Reig i Jordi Bruix són experts clínics en hepatocarcinoma, i han descrit per primer cop el senyal de seguretat relatiu a un possible increment del risc de càncer associat al tractament amb AAD.

La Dra Rosa Morros té una àmplia experiència en estudis de Farmacoepidemiologia semblants a l'estudi que ens ocupa en aquest protocol, i forma part de l'Institut de Recerca i d'Atenció Primària Jordi Gol, i la seva participació garantirà la correcció de la metodologia aplicada, i la

pertinença de les variables triades. La Dra Caridad Pontes compta amb una àmplia experiència en estudis de casos i controls, quatre dels quals emprant la base de dades del SIDIAP, i també en el Programa d'Harmonització Farmacoterapèutica del CatSalut; la seva contribució, de manera semblant a la Dra Morros, garantirà la correcció de la metodologia aplicada, i la pertinença de les variables triades . La Marta Roig és tècnica del Programa d'Harmonització Farmacoterapèutica del CatSalut i té una llarga experiència i bon coneixement respecte del Registre de Pacients i Tractaments, proporcionant el coneixement necessari per a avaluar les particularitats de les dades del registre.

Ferran Torres, Jose Rios i Victor Sapena són experts en estadística, i reuneixen els coneixements necessaris per a fer les anàlisis de dades requerides.

Els Drs Zoe Mariño, Sabela Lens i Xavier Forns són especialistes en el tractament de les hepatitis de causa vírica i ajudaran a interpretar els resultats de l'estudi.

APLICABILITAT I UTILITAT PRÀCTICA DELS RESULTATS DE L'ESTUDI

Considerant la prevalença de la infecció a la nostra població, el fet que es considera ampliar progressivament la utilització dels tractaments a poblacions amb un grau menor d'afectació hepàtica, i el potencial l'impacte sanitari i sobre la salut pública d'un risc associat a aquests tractaments que es podria produir a llarg termini i que és difícilment detectable en assaigs clínics, esdevè prioritari estudiar amb metodologia farmacoepidemiològica si existeix una possible associació causal entre els nous tractaments AAD i l'aparició de neoplàsies.

Això motiva el present estudi, que pretén valorar a nivell poblacional la incidència de càncer en pacients tractats amb AAD per a la HCV, i comparar-la amb la incidència en controls infectats per VHC tractats amb pautes basades en interferó abans del 2014, i amb pacients no infectats per VHC, per tal de valorar possibles diferències i factors que puguin influir en les mateixes.

La identificació de potencials riscs permetrà establir les mesures necessàries per a la seva gestió i prevenció.

MITJANS DISPONIBLES PER A LA REALITZACIÓ DEL PROJECTE

No es disposa de cap ajut específic per a la realització del present estudi; les hores de dedicació requerides per a la seva realització seran aportades per cadascun dels investigadors implicats.

Les despeses derivades de l'extracció de les dades i dels possibles honoraris de personal no investigador seran cobertes pel Servei Català de la Salut prèvia conformitat amb un pressupost específic presentat amb anterioritat a l'execució de la tasca, i en el marc de la signatura d'un conveni específic amb la part implicada.

JUSTIFICACIÓ DEL FINANÇAMENT I PRESSUPOST SOL·LICITAT

No aplica

POSSIBLES CONFLICTES D'INTERÈS

Els següents investigadors declaren tenir com a conflictes d'interès els següents:

- Jordi Bruix: és consultor, i rep ajuts de Bayer, Daiichi i ArQule, i és consultor de BioCompatibles, Novartis, Abbott, Bristol-Myers Squibb, GlaxoSmithKline, Kowa, Lilly i Roche.
- Xavier Forns: rep ajusts sense restriccions de Roche i MSD; és consultor per a Jansen, Abbvie, Gilead i MSD
- Ferran Torres ha format part de Data Safety Monitoring Boards d'assaigs clínics per a ImClone, Daiichi-Sankyo Pharma Development and ArQule
- Zoe Mariño, ha rebut honoraris per consultoria de BMS.
- Sabela Lens ha rebut honoraris per consultoria de Janssen, Gilead i Abbvie. Maria Reig, Rosa Morros, Marta Roig, Caridad Pontes, Jose Rios i Victor Sapena declaren no tenir cap conflicte d'interès.

Annexes (si procedeix) (Full d'informació al pacient o participant, Full de consentiment informat)

No aplica