Universitat Autònoma de Barcelona

Impacto de la enfermedad hepática grasa no alcohólica a nivel poblacional y en grupos de riesgo. Papel de la elastografía de transición en el diagnóstico, estadiaje y predicción de eventos clínicos

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Tesis doctoral

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A mi madre

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LISTADO DE ABREVIATURAS

ALT, Alanina aminotransferasa

AST, Aspartato aminotransferasa

BCLC, Barcelona Clinic Liver Cancer

CHC, Carcinoma hepatocelular

CRN, Clinical Research Network

CV, Cardiovascular

DM2, Diabetes mellitus tipo 2

ECG, Eventos clínicos globales

ECV, Evento cardiovascular

EHCAC, Enfermedad hepática crónica avanzada compensada

EHGNA, Enfermedad hepática grasa no alcohólica

EHNA, Esteatohepatitis no alcohólica

ERC, Enfermedad renal crónica

ET, Elastografía de transición

ETHON, Estudio poblacional de enfermedades hepáticas nacional

FIB-4 index, Fibrosis 4 index

FLI, Fatty liver index

GPVH, Gradiente de presión venosa hepática

HbA1c, Hemoglobina glicosilada

HFS, Hepamet fibrosis score

HP, Hipertensión portal

HPCS, Hipertensión portal clínicamente significativa

HR, Hazard ratio

HSI, Hepatic Steatosis Index

IC, Intervalo de confianza

IMC, Índice de masa corporal

MELD, Model for end-stage liver disease

NAFLD, Non-alcoholic fatty liver disease

NAS, NASH activity score

NASH, Non-alcoholic steatohepatitis

ND, Nefropatía diabética

NFS, NAFLD Fibrosis score

OR, Odds Ratio

PAC, Parámetro de Atenuación Controlada

PRECISED, Preventing Cardiovascular Ischemic Events and Arresting Their Consequences in Type 2 Diabetic Population

RIC, Rango intercuartílico

RM, Resonancia magnética

SM, Síndrome metabólico

TEFG, Tasa estimada de filtrado glomerular

TH, Trasplante hepático

TNI, Test no invasivos

VHB, Virus hepatitis B

VHC, Virus hepatitis C

VIH, Virus de la inmunodeficiencia humana

VPN, Valor predictivo negativo

VPP, Valor predictivo positivo

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RESUMEN

La enfermedad hepática grasa no alcohólica (EHGNA) es considerada la enfermedad hepática crónica más frecuente a nivel global y se ha convertido en un problema de salud pública de primer orden. Afecta alrededor del 25-35% de la población general en países occidentales y su prevalencia aumenta hasta el 60-80% en pacientes con diabetes mellitus tipo 2 (DM2) u obesidad. La EHGNA presenta una fisiopatología multifactorial y un curso clínico variable, en el que una proporción de pacientes desarrollará una enfermedad progresiva con inflamación (esteatohepatitis) y fibrosis hepática en diferentes grados, lo que implica un mayor riesgo de complicaciones y un peor pronóstico.

La creciente incidencia de la EHGNA en los últimos años ha motivado la implementación de herramientas diagnósticas no invasivas, entre las que destaca la elastografía de transición (ET). La ET permite una estimación indirecta de la fibrosis y esteatosis hepática, está ampliamente validada en hepatopatía por virus o alcohol y se usa de forma rutinaria en la evaluación de pacientes con sospecha de enfermedad hepática avanzada.

La presente tesis pretende estudiar el impacto de la EHGNA sobre diferentes poblaciones y el papel de la ET en el abordaje diagnóstico y pronóstico de la enfermedad. Los objetivos de la tesis son evaluar la utilidad de la ET en la identificación de EHGNA y la estratificación de fibrosis hepática para estimar la prevalencia de la enfermedad; y, por otra parte, investigar la asociación de la fibrosis hepática con el desarrollo de complicaciones y la capacidad de la ET de predecir eventos clínicos.

Para alcanzar dichos objetivos se han llevado a cabo tres estudios independientes donde se estudia: 1) la prevalencia de los diferentes estadios de fibrosis por EHGNA en población general española; 2) la incidencia de carcinoma hepatocelular (CHC) y los factores asociados en pacientes con EHGNA, así como el rol de la ET en la predicción de CHC y; 3) la asociación de la EHGNA y la fibrosis hepática evaluada por ET con el desarrollo de eventos clínicos en pacientes con DM2.

Tras la realización de estos estudios se puede concluir que: 1) la ET es una herramienta útil en el abordaje global de la EHGNA, ya que permite estimar de

identificar la presencia de esteatosis, estadiar la severidad de la enfermedad y predecir la aparición de eventos clínicos; 2) la prevalencia de fibrosis por EHGNA en población general de España se estima en 2,03% y 0,7% para fibrosis significativa (F ≥ 2) y cirrosis, respectivamente; 3) la presencia de fibrosis avanzada medida por histología o por ET se asocia al desarrollo de CHC en pacientes con EHGNA y; 4) la presencia de EHGNA y la elasticidad hepática medida por ET se relacionan de forma independiente con la enfermedad renal crónica y con el riesgo incidente de presentar eventos clínicos en pacientes con DM2.

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and a global public health issue. It affects 25-35% of the general population in Western countries and the prevalence rises to 60-80% in patients with type 2 diabetes or obesity. NAFLD is a heterogenous disease and has a variable natural history. A proportion of patients may progress to steatohepatitis with different fibrosis stages, which implies a higher risk of developing complications and a worse prognosis.

The rapid increase in NAFLD incidence in recent years has led to the implementation of non-invasive diagnostic techniques, amongst which transient elastography (TE) stands out. TE allows an indirect point-of-care estimation of liver steatosis and fibrosis; it is widely used in patients with suspected compensated chronic advanced liver disease and has been validated for viral or alcohol-related liver disease.

The present doctoral thesis aims to study the impact of NAFLD in different populations and to assess the usefulness of TE in the diagnostic and prognostic approach to the disease. Thus, the objectives are to evaluate the role of TE in NAFLD detection and fibrosis stratification, allowing us to estimate the prevalence of the disease and, besides, to investigate the association between liver stiffness and the development of complications, as well as the TE's ability to predict clinical events.

In order to achieve these goals, three independent studies have been carried out to investigate: 1) the prevalence of the different stages of fibrosis due to NAFLD in the general population from Spain; 2) the incidence of hepatocellular carcinoma (HCC) amongst NAFLD patients and the associated risk factors, as well as the predictive role of TE for HCC within this population and; 3) the association of NAFLD and liver stiffness by TE with the development of clinical events in patients with type 2 diabetes.

After conducting these studies, it can be concluded that: 1) TE is a useful tool for the NAFLD approach. TE is capable of identifying steatosis, stratifying disease severity, and predicting clinical events in NAFLD subjects; 2) the prevalence of significant fibrosis and cirrhosis due to NAFLD in the general population has been estimated in 2,03% and 0,7%, respectively; 3) the presence of liver fibrosis measured by histology or TE is associated to HCC development in NAFLD subjects, 3) NAFLD and liver fibrosis by TE are associated with chronic kidney disease and the risk of clinical events in people with type 2 diabetes.

1. INTRODUCCIÓN

1. INTRODUCCIÓN

1.1. Enfermedad hepática grasa no alcohólica (EHGNA)

1.1.1. Definición y fisiopatología

La enfermedad hepática grasa no alcohólica (EHGNA) se caracteriza por la presencia de lípidos en más de un 5% de los hepatocitos.[1] La hipótesis de múltiples impactos es la teoría fisiopatológica más aceptada en la actualidad.[2] Según esta teoría, la interacción de diversos agentes (metabólicos, genéticos, epigenéticos e inmunomediados) facilitaría la acumulación de lípidos tóxicos en el hepatocito, provocando estrés oxidativo y apoptosis celular, lo que activaría una cascada inflamatoria y a las células estrelladas, provocando finalmente la formación de fibrosis.

Desde el punto de vista clínico, el diagnóstico clásico de la EHGNA se realizaba por exclusión, es decir, quedaba establecido una vez descartadas el resto de etiologías de enfermedad hepática y las causas secundarias de esteatosis (p.ej. alcohol o fármacos).[3] Sin embargo, en 2020 se propusieron nuevos criterios diagnósticos[4] y una nueva nomenclatura (esteatosis hepática metabólica), que pretendía reflejar la elevada carga metabólica asociada a la enfermedad y reducir el estigma asociado a términos como “graso” o “alcohólico”.[5] No obstante, aún no se ha alcanzado un claro consenso internacional, por lo que la terminología sigue siendo objeto de debate.

El abordaje de la enfermedad desde la perspectiva clásica (enfermedad hepática grasa no alcohólica) o desde un enfoque inclusivo (esteatosis hepática metabólica) tiene implicaciones epidemiológicas y condiciona el diseño de estudios de investigación.[6,7] Si bien es cierto que el concepto de esteatosis hepática metabólica reproduce de forma más fidedigna la práctica clínica real, donde los pacientes con EHGNA consumen cantidades variables de alcohol o pueden presentar otras enfermedades hepáticas concomitantes, en la presente tesis doctoral el diagnóstico de EHGNA se ha establecido por exclusión, permitiendo una identificación etiológica de la EHGNA más granular y obtener unos resultados más robustos, fácilmente interpretables y extrapolables.

1.1.2. Epidemiología e impacto en diferentes poblaciones

La EHGNA es considerada actualmente la enfermedad hepática crónica más frecuente en los países occidentales, afectando alrededor del 25-35% de los adultos de la población general en nuestra área geográfica.[8,9] La incidencia de EHGNA ha aumentado de forma exponencial en los últimos años, de forma paralela a las epidemias de obesidad, diabetes mellitus 2 (DM2) y síndrome metabólico (SM), lo que la ha convertido en una importante amenaza para la salud pública a escala mundial.[10] De hecho, se ha demostrado en los últimos años que el 3-4% y el 0,5% de la población adulta podría tener una EHGNA con fibrosis avanzada o cirrosis, respectivamente.[11-13] Además, se estima que el número de pacientes con EHGNA en estadios avanzados de enfermedad podría aumentar entre un 60 y un 150% en 2030.[14]

El perfil fenotípico más frecuente de la EHGNA (80-90%) es el de un paciente con un estilo de vida sedentario y comorbilidad metabólica concomitante (obesidad, hipertensión arterial, DM2 y/o dislipemia), lo que ha llevado a considerar a la EHGNA como la expresión hepática del SM.[15] Cabe destacar que los sujetos con obesidad o DM2 presentan una prevalencia de EHGNA de un 60-80% (pudiendo alcanzar un 90-100% si ambas condiciones están presentes) y mayores tasas de inflamación hepática (esteatohepatitis) y fibrosis.[11-14,16] Por otra parte, la presencia de EHGNA tiene un impacto negativo sobre la comorbilidad metabólica, confirmando así la relación bidireccional deletérea entre la EHGNA y el SM.[17,18]

Por otra parte, alrededor de un 10-20% de los pacientes no presentan un evidente SM y son conocidos como “EHGNA delgados”.[19,20] En este subgrupo de pacientes es habitual la coexistencia de patología endocrina (p.ej. ovario poliquístico o hipotiroidismo), inmunomediada (p.ej. enfermedad inflamatoria intestinal o patología sistémica), enfermedades infecciosas (p.ej. virus de la inmunodeficiencia humana -VIH-) o defectos congénitos del metabolismo lipídico (p.ej. déficit lipasa ácida lisosomal o abetalipoproteinemia).[21-23] Además, se han descrito polimorfismos genéticos más frecuentes en ciertas razas o etnias, así como factores epigenéticos (p.ej. microbiota intestinal) que predisponen al desarrollo de la EHGNA y a presentar un curso clínico más agresivo.[24]

En resumen, la EHGNA es una patología altamente prevalente con una incidencia al alza que se espera siga aumentado a medio y largo plazo y que tiene importantes implicaciones sanitarias y económicas a nivel tanto individual como colectivo. Desde una perspectiva de salud pública, es esencial contar con estimaciones precisas y actualizadas de la prevalencia de la EHGNA ajustadas a cada área geográfica, tanto a nivel poblacional como en subgrupos específicos de riesgo (p.ej. pacientes con DM2, obesidad o en fases avanzadas de la enfermedad). Estos datos nos proporcionarían una idea aproximada de los esfuerzos necesarios para contener el auge de la enfermedad y sentaría las bases para el diseño de estrategias integradas multidisciplinares.

1.1.3. Historia natural

La EHGNA tiene un origen multifactorial, una expresión fenotípica heterogénea y un curso clínico ampliamente variable. El término enfermedad hepática grasa no alcohólica engloba a todo el espectro de la enfermedad, aunque se diferencian varias fases dentro de la misma.[13,25] (Figura 1).

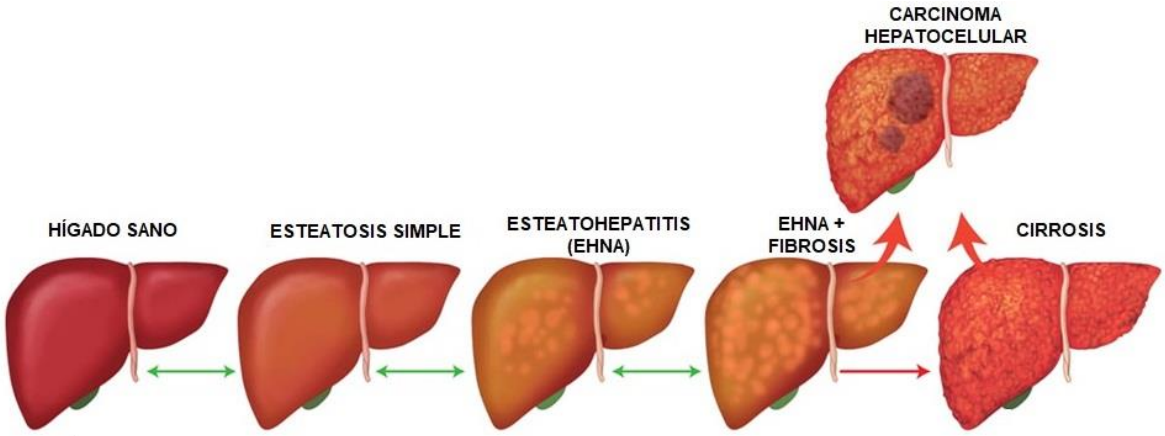

Figura 1. Historia natural de la EHGNA**.** EHNA, esteatohepatitis no alcohólica; CHC, carcinoma hepatocelular.

El grueso de pacientes afectos de EHGNA cursarán una enfermedad leve y no progresiva (esteatosis simple). En cambio, aproximadamente un 20% presentará inflamación hepática o esteatohepatitis no alcohólica (EHNA) y de éstos, hasta un 10-20% desarrollará fibrosis en diferentes grados, la cual puede evolucionar en última instancia a cirrosis y favorecer la aparición de carcinoma hepatocelular (CHC).

En la mayoría de los pacientes, la EHGNA se comporta como una enfermedad lentamente progresiva, estimándose la progresión de un estadio de fibrosis cada 7-10 años, si bien hay un 20% de pacientes que pueden progresar rápidamente.[26-29]

Por lo tanto, el curso clínico de la EHGNA varía de forma significativa según las características de cada individuo, siendo el riesgo de presentar una enfermedad agresiva y rápidamente progresiva sustancialmente más elevado en sujetos con comorbilidad metabólica y predisposición genética.[28] Identificar precozmente a estos sujetos es primordial debido a que la presencia y grado de fibrosis es el principal determinante de morbilidad y mortalidad entre los pacientes con EHGNA.[30-32] De hecho, a medida que la fibrosis progresa, el riesgo de complicaciones de origen hepático (descompensaciones de la cirrosis y CHC), así como la mortalidad de causa hepática y global aumenta de forma dramática.[30-35] Por el contrario, en estadios precoces de fibrosis las principales causas de mortalidad son extrahepáticas, como la enfermedad cardiovascular o neoplásica.[36,37]

La cirrosis representa el estadio final de cualquier enfermedad hepática crónica. Se divide en dos fases bien diferenciadas, una fase asintomática denominada cirrosis compensada, seguida de un estadio en el que aparecen complicaciones hepáticas (ascitis, hemorragia o encefalopatía hepática) o cirrosis descompensada, la cual implica un dramático ascenso de la mortalidad y de la necesidad de trasplante hepático (TH).[38]

Sin embargo, la historia natural de los pacientes con cirrosis por EHGNA no está completamente caracterizada.[39,40] Estudios recientes sitúan el riesgo anual de presentar una primera descompensación clínica en un 4-8%[29,33,41] y se ha sugerido que los pacientes con EHGNA en fases precirróticas presentan un riesgo aumentado de presentar eventos hepáticos, particularmente CHC, en comparación con el resto de las causas de enfermedad hepática crónica.[33]

Una de las consecuencias más relevantes de la cirrosis desde el punto de vista pronóstico es el desarrollo de hipertensión portal (HP). La cirrosis provoca cambios estructurales en el parénquima hepático que generan un aumento de la resistencia vascular intrahepática, induciendo un aumento de presión en el sistema porta que terminará por alterar el equilibrio hemodinámico sistémico. Así,

una presión portal > 5 mmHg medida mediante el gradiente de presión venosa hepática (GPVH) es diagnóstica de HP. El riesgo de aparición de complicaciones hepáticas se correlaciona directamente con los valores del GVPH, de modo que gradientes ≥ 10 mmHg aumentan de forma notable la probabilidad de desarrollar complicaciones clínicas hepáticas, denominándose a esta condición hipertensión portal clínicamente significativa (HPCS).[38,42] No obstante, en pacientes con EHGNA se ha descrito una menor precisión en la valoración de la presión portal tanto mediante métodos diagnósticos directos como el GPVH,[43] como indirectos, como la ET,[44] en comparación con el resto de las causas de enfermedad hepática (p.ej. hepatopatía por virus o alcohol).

En definitiva, la EHGNA es una enfermedad poliédrica, potencialmente progresiva y que asocia una importante morbimortalidad hepática y extrahepática, especialmente en estadios avanzados. Ante este escenario resulta de vital importancia la implementación y validación de técnicas no invasivas que permitan el diagnóstico de EHGNA en fases precoces de la enfermedad y estimar el riesgo de presentar eventos clínicos durante el seguimiento.

1.2. Abordaje diagnóstico y estrategias de derivación

El dramático aumento en la prevalencia de la EHGNA, sumado a un curso clínico habitualmente silente, condiciona un diagnóstico tardío en un considerable número de pacientes, una vez la cirrosis se había establecido o a raíz de una primera descompensación clínica. Además, la biopsia hepática es una técnica invasiva no exenta de complicaciones que se ha de realizar por personal entrenado en centros de referencia, lo que limita su aplicabilidad en el grueso de pacientes con EHGNA. Esta situación ha llevado a un rápido desarrollo de tests no invasivos (TNI) en los últimos años, con el objetivo de identificar pacientes asintomáticos que se encuentran en estadios iniciales de la enfermedad.

Las principales técnicas no invasivas empleadas en el abordaje diagnóstico de la EHGNA son:[45,46]

- Test serológicos: Los TNI de fibrosis se dividen en patentados (Fibrotest, Hepascore, *Enhanced Liver Fibrosis test*, etc) y no patentados, entre los que destacan el Fibrosis-4 *index* (FIB-4), el NAFLD Fibrosis *score* (NFS) y Hepamet Fibrosis *score* (HFS). Éstos últimos son los más utilizados, emplean parámetros clínicos y analíticos habituales (p.ej. edad, peso, cifras de transaminasas o plaquetas), son baratos y sencillos de calcular. También existen test serológicos de esteatosis, como el *Fatty Liver Index* (FLI) o el *Hepatic Steatosis Index* (HSI), menos utilizados en práctica habitual.
- Pruebas de imagen: La ecografía abdominal permite el diagnóstico de esteatosis y aporta datos indirectos sobre el estadio de la hepatopatía, como alteraciones en la arquitectura hepática o signos indirectos de HP. La resonancia magnética (RM), gracias al software *proton density fat fraction,* es capaz de proporcionar estimaciones precisas sobre esteatosis, aunque su disponibilidad es limitada en práctica clínica.
- Técnicas elastográficas:
    - Elastografía de transición (ET): Es el método elastográfico más utilizado. Permite descartar fibrosis significativa y confirmar cirrosis, pero es menos fiable para definir estadios intermedios de fibrosis. Además, aporta información cuantitativa sobre esteatosis a través del Parámetro de Atenuación Controlada (PAC).

- Elastografía por ecografía: La *acoustic radiation forced imaging* y el *shear-wave elastography* permiten evaluar la elasticidad en un área de interés previamente seleccionada bajo visión ecográfica directa. Están menos validadas por lo que no se emplean rutinariamente.
- Elastografía por RM: Método no invasivo más preciso para el estadiaje de fibrosis. Acceso limitado por coste y baja disponibilidad.

Por otro lado, la biopsia hepática continúa siendo la técnica de elección para el diagnóstico definitivo de la EHGNA y sus diferentes estadios. El *non-alcoholic steatohepatitis* (NASH) C*linical Research Network* (CRN) *score*[47] es la clasificación histológica más utilizada en práctica clínica y en estudios de investigación (Tabla 1). Se sirve del NASH *Activity score* (NAS) para el diagnóstico etiológico de la enfermedad (siendo necesario obtener al menos un punto en cada uno de los tres *ítems*). Por otra parte, los estadios de fibrosis abarcan desde 0 (ausencia de fibrosis) a 4 (cirrosis), empleándose los términos fibrosis significativa (F ≥ 2) o avanzada (F 3-4) para diferenciar estadios con diferente pronóstico.

Actividad (NASH Activity score, NAS)			**Estadificación de fibrosis (CRN Fibrosis Staging)**
Esteatosis	Inflamación lobulillar	Balonización	
0: <5%	0: Ausente	0: Ausente	0: Ausente
1: 5-33%	1: < 2 focos x20 campos	1: Escasa	1a: Fibrosis perisinusoidal leve 1b: Fibrosis perisinusoidal moderada 1c: Fibrosis periportal/portal exclusivamente
2: 34-66%	2: 2-4 focos x20 campos	2:Abundante	2: Fibrosis zona 3 + periportal/portal
3: > 66%	3: > 4 focos x20 campos		3: Puentes de fibrosis
			4: Cirrosis, probable o definitiva

Tabla 1. Clasificación histológica de la EHGNA según NASH Clinical Research Network (CRN) *score.*[47]

Existe un consenso generalizado respecto al abordaje diagnóstico de pacientes con EHGNA,[48,49] que se realiza de forma secuencial y se basa en (Figura 2):

1) Diagnóstico de EHGNA: Se establece en pacientes con un contexto clínico compatible, ausencia de otras enfermedades hepáticas y presencia de esteatosis (habitualmente diagnosticada mediante ecografía abdominal o el PAC asociado a la ET)

2) Estadiaje de fibrosis y estratificación de la severidad:

   a) En primer lugar, se recomienda realizar una prueba serológica no patentada (FIB4, NFS o HFS). Estos test permiten descartar la presencia de fibrosis avanzada gracias a un valor predictivo negativo (VPN) > 90%. Los sujetos con resultados negativos (EHGNA de bajo riesgo) pueden ser manejados en atención primaria con reevaluaciones periódicas de la EHGNA y una optimización del control metabólico.

   b) Si los test serológicos no pueden descartar fibrosis avanzada el siguiente paso es practicar un segundo test de fibrosis más sensible, como la ET. Valores de ET < 8 kPa descartan fibrosis avanzada por lo que, nuevamente, estos casos podrían ser remitidos a primaria, mientras que aquellos con una ET ≥ 8 kPa son clasificados como de riesgo y deben ser derivados a atención especializada.

   c) Finalmente, la biopsia hepática se indica cuando persisten dudas respecto a la etiología y para el estadiaje definitivo de fibrosis en sujetos con sospecha de hepatopatía avanzada tras la realización de TNI. Este subgrupo de pacientes ha de ser manejado en centros terciarios, donde se valorará su inclusión en estudios terapéuticos y el inicio de cribado de complicaciones hepáticas (varices esofágicas, CHC) y sistémicas (p.ej. enfermedad cardiovascular o renal)

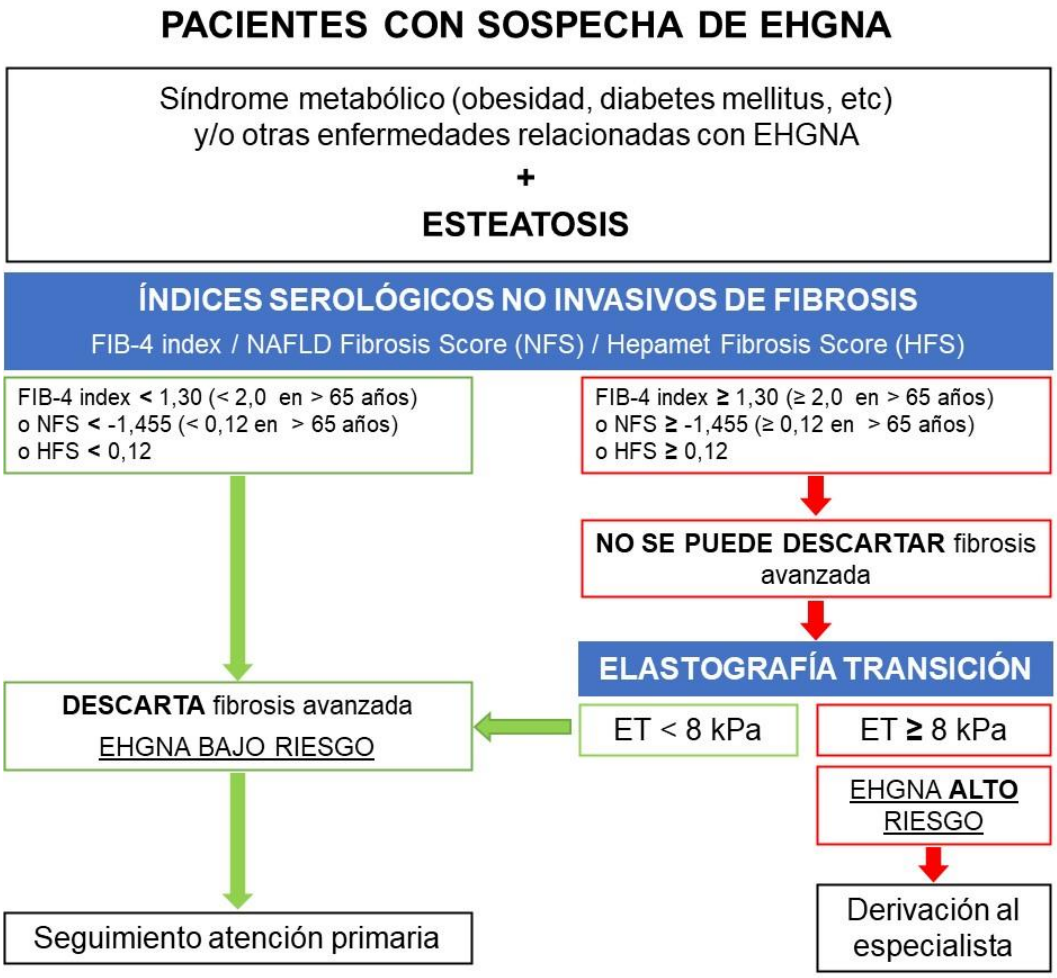

Figura 2. Propuesta de algoritmo diagnóstico de EHGNA y circuitos de derivación**.** EHGNA, Enfermedad hepática grasa no alcohólica; ET, Elastografía de transición; NFS, NAFLD Fibrosis *score*; HFS, Hepamet Fibrosis *score*.

Así pues, la aproximación diagnóstica de la EHGNA y los circuitos clínicos existentes se centran en identificar de forma no invasiva a pacientes con un mayor riesgo de presentar complicaciones, de forma que puedan ser derivados precozmente al especialista, donde se completará el estudio de la enfermedad hepática y se decidirá el seguimiento y el manejo de forma individualizada.

Diferentes estudios han evaluado estrategias de cribado tanto de fibrosis hepática en global[50] como secundaria a EHGNA,[51] así como la coste-efectividad de las mismas.[52] En nuestro medio, considerando el volumen de potenciales pacientes a estudio, la estructura sanitaria existente y los recursos disponibles, parece razonable orientar la detección de la EHGNA a poblaciones de riesgo de fibrosis avanzada siguiendo un abordaje secuencial, como el propuesto en la Figura 2.

1.3. Papel de la elastografía de transición en la evaluación de la EGHNA

1.3.1. La elastografía de transición

La ET es una herramienta basada en el ultrasonido que permite una evaluación no invasiva, precisa y rápida de pacientes con sospecha de enfermedad hepática. La ET mide la velocidad de propagación de una onda elástica a través de un tejido, la cual se relaciona directamente con la rigidez, de modo que, a mayor rigidez, mayor velocidad y, por tanto, mayor será el grado de fibrosis hepática.[53] La ET es una exploración técnicamente sencilla, dispone de diferentes sondas (S en niños, M y XL en adultos) que se emplean en función de las características del paciente, lo que permite disminuir el número de mediciones inválidas o imprecisas.[54] Se consideran fiables aquellas exploraciones con un rango intercuartílico/mediana < 0,30 y al menos 10 mediciones válidas.[55]

La ET es la técnica elastográfica más utilizada en la actualidad. Su amplia implementación en la práctica habitual de las unidades de hepatología ha modificado radicalmente la forma de diagnosticar a pacientes con enfermedad hepática crónica. Gracias a la ET se consiguen identificar precozmente sujetos asintomáticos con una cirrosis incipiente o en fases precirróticas que de otra forma no serían diagnosticados hasta fases clínicamente evidentes.

El concepto de enfermedad hepática crónica avanzada compensada (EHCAC) se acuñó para catalogar a aquellos pacientes asintomáticos diagnosticados mediante ET con fibrosis avanzada o cirrosis compensada.[56] La aplicación de los diferentes puntos de corte de ET permite clasificar a los pacientes según el riesgo de presentar EHCAC, de modo que una ET > 15 kPa confirma la presencia de EHCAC mientras que una ET < 10 kPa descarta el diagnóstico (Figura 3). Del mismo modo, la ET es capaz de estimar de forma no invasiva la presencia de HP e HPCS e inferir indirectamente el riesgo de presentar eventos hepáticos.

Además, la ET es una herramienta costo-efectiva, dado que evita la realización de exploraciones invasivas innecesarias, como estudios endoscópicos en pacientes con baja probabilidad de presentar varices esofágicas o mediciones

del GPVH para confirmar la existencia de HPCS en sujetos de alto riesgo en los que se podría asumir su presencia de forma no invasiva gracias a las mediciones de ET.[56,57]

Los puntos de corte de ET para EHCAC y HPCS en enfermedad hepática por virus y alcohol están ampliamente validados. No obstante, en el caso de la EHGNA son necesarios más estudios que ahonden en la fisiopatología de la enfermedad hepática y en el comportamiento hemodinámico de la misma, de modo que seamos capaces de definir puntos de corte óptimos de ET que nos permitan identificar sujetos con EHCAC por EHGNA y a aquellos que hayan desarrollado HPCS, especialmente el subgrupo de sujetos con obesidad, que son los que *a priori* suponen un mayor desafío.[44,56]

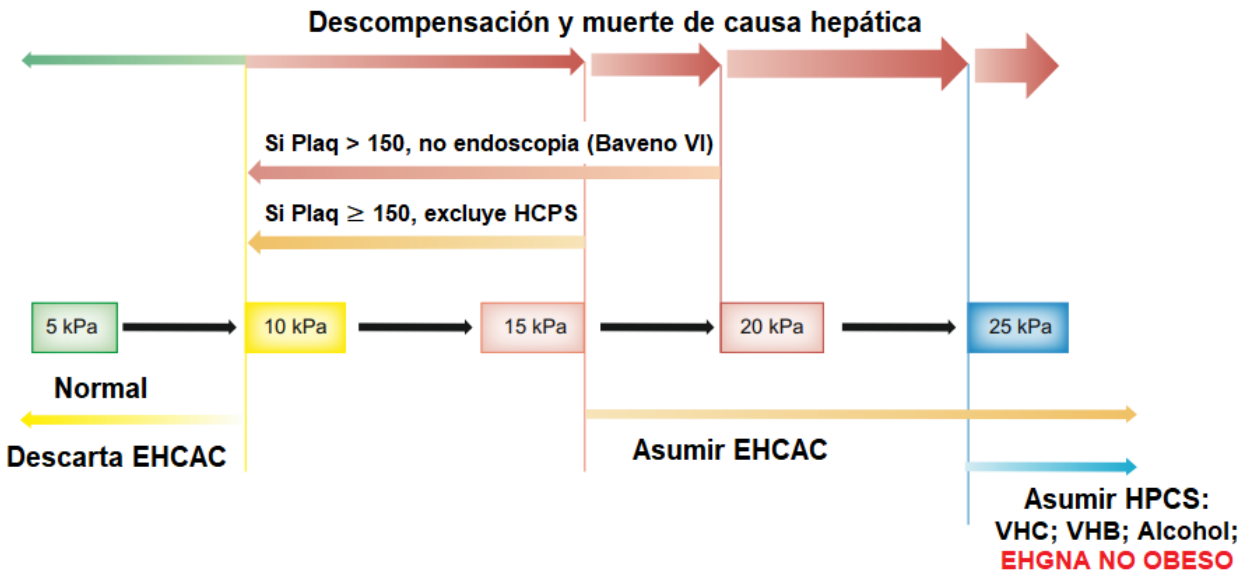

Figura 3. Algoritmo para evaluación no invasiva de EHCAC y HPCS. Figura modificada.[56] EHCAC, enfermedad hepática crónica avanzada compensada, HPCS; hipertensión portal clínicamente significativa; EHGNA, enfermedad hepática grasa no alcohólica; VHC, virus hepatitis C; VHB, virus hepatitis B.

1.3.2. Diagnóstico de esteatosis

La esteatosis hepática es el hallazgo característico de la EHGNA y condición necesaria para establecer el diagnóstico de la enfermedad.

La detección de esteatosis mediante ecografía abdominal o test serológicos ha demostrado ser subóptima (p.ej. la ecografía sólo detecta esteatosis si hay > 20-30% de los hepatocitos afectados) en comparación con el PAC de la ET.[46,47] El PAC es un *software* instalado en el aparato de ET que mide la atenuación de la onda de ultrasonido a través del tejido hepático, proporcionando datos cuantitativos que permiten estimar la presencia y severidad de la esteatosis.[58]

Estudios de ET comparados con histología han propuesto diversos puntos de corte del PAC para el diagnóstico y estadiaje de la esteatosis, sobre los que no existe un claro consenso. Un metaanálisis de Karlas et al.[59] sugirió 250 dB/m como el punto de corte más sensible para la identificación de esteatosis, siendo necesario corregir el valor de CAP en función de la etiología hepática subyacente, la presencia de DM2 o el índice de masa corporal (IMC). Sin embargo, la guía de práctica clínica europea[60] ha actualizado recientemente el punto de corte de PAC para el diagnóstico de esteatosis a 275 dB/m, con unos valores de sensibilidad y valor predictivo positivo (VPP) > 90%.

Así mismo, cabe destacar que, pese a la alta sensibilidad del PAC y la accesibilidad de la técnica, el número de estudios poblacionales que han evaluado la presencia de esteatosis mediante PAC hasta la fecha es escaso,[61] por lo que probablemente las estimaciones disponibles sobre la prevalencia en población general de la EHGNA y de sus diferentes estadios son inexactas.

En global, estos datos reflejan la necesidad de poner en marcha estudios de base poblacional que implementen el PAC de la ET como herramienta de cribado de esteatosis, lo que permitiría estimar el peso atribuible a EHGNA dentro del conjunto de enfermedades hepáticas crónicas en cada área geográfica y dar una idea aproximada de la magnitud del problema de salud pública que supone la EHGNA.

1.3.3. Estadiaje de fibrosis hepática

La fibrosis hepática es el principal condicionante de la historia natural de la EHGNA. Los pacientes con fibrosis significativa (F ≥ 2) y avanzada (F 3-4) tienen un peor pronóstico que aquellos con estadios precoces de fibrosis, por lo que resulta prioritario identificar adecuadamente a estos pacientes de riesgo.

La biopsia hepática y la elastografía por RM son las pruebas más precisas para el estadiaje de fibrosis hepática en EHGNA.[46,47,62] Sin embargo, su uso está limitado a subgrupos seleccionados de pacientes por su naturaleza invasiva o su escasa disponibilidad, respectivamente. Dado el enorme volumen de potenciales pacientes con sospecha de EHGNA, los TNI de fibrosis juegan un papel fundamental, permitiendo descartar la presencia de enfermedad hepática avanzada con un grado moderado-alto de seguridad en un importante porcentaje de pacientes evaluados en atención primaria.

En el caso específico de la ET, valores < 8 kPa descartan la presencia de fibrosis avanzada, como se ha demostrado en múltiples estudios.[45,46] No obstante, la capacidad de la ET para definir estadios intermedios de fibrosis por EHGNA es limitada, especialmente cuando se aplica en poblaciones con baja prevalencia de la enfermedad, por lo que no existe consenso sobre los valores de ET para clasificar a pacientes con F2-3. Para el diagnóstico de cirrosis, los puntos de corte empleados varían ampliamente, siendo 20 kPa el más aceptado, con una especificidad en torno al 90-95% y un VPP del 60-65%.[58,62]

Por lo tanto, en pacientes con EHGNA y una ET ≥ 8 kPa se ha de considerar la realización de una biopsia hepática para definir el estadio exacto de fibrosis, con especial interés en aquellos sujetos con sospecha de EHCAC que no presenten otros hallazgos evidentes de cirrosis como alteraciones volumétricas o signos indirectos de HP en pruebas de imagen.

En conclusión, la estimación del grado de fibrosis mediante ET permite guiar las estrategias de derivación y estratificar correctamente a los pacientes según el riesgo de presentar complicaciones (como el CHC o varices esofágicas),[56,57] lo que facilita la toma de decisiones clínicas y condiciona el manejo y seguimiento posterior (Figuras 2 y 3)

1.3.4. Predicción de eventos clínicos

Los pacientes con EHGNA tienen un riesgo aumentado de desarrollar eventos clínicos en estadios precoces de la hepatopatía en comparación con el resto de las causas de enfermedad hepática. Tanto es así que las principales causas de mortalidad global entre los pacientes con EHGNA (especialmente si tienen obesidad y/o diabetes) son de origen extrahepático (enfermedad cardiovascular y cáncer no hepático).[36,37]

En cambio, la morbimortalidad de causa hepática aumenta significativamente según progresa la fibrosis,[28-34] siendo los pacientes cirróticos los que presentan un mayor riesgo de complicaciones. Cabe destacar que la EHGNA se ha situado como la etiología subyacente de CHC de más rápido crecimiento en los últimos años y es una de las principales causas de TH a nivel global.[11,12,63,64]

En este contexto, diversos estudios han evaluado la capacidad de los TNI para predecir la aparición de eventos clínicos en pacientes con EHGNA, como el FIB-4 o la elastografía por RM.[65-67] La ET ha demostrado ser un método fiable en la predicción de eventos hepáticos y extrahepáticos (fundamentalmente cardiovasculares) y se ha asociado con la mortalidad global y de causa hepática.[68,69] Dicha asociación se ha establecido tanto con valores basales de elasticidad como con variaciones de la ET a lo largo del tiempo (un aumento > 20% de la ET respecto al valor basal aumentaría el riesgo de sufrir eventos).[70]

Así mismo, se ha descrito un aumento del riesgo de presentar complicaciones hepáticas, particularmente CHC, en fases precirróticas de la EHGNA,[33,63,64,71] a diferencia de lo que ocurre en otras enfermedades hepáticas crónicas. Sin embargo, estos datos no se han confirmado en poblaciones del sur de Europa, como España.

En definitiva, queda subrayada la importancia de disponer de métodos no invasivos que permitan predecir la probabilidad de desarrollar eventos, de cara a identificar sujetos de riesgo antes de que aparezcan las complicaciones o en fases iniciales de las mismas, de modo que un mayor porcentaje de pacientes pueda beneficiarse de tratamientos curativos y de un manejo personalizado.

1.3.5. Tratamiento y monitorización

Actualmente no existe ningún tratamiento farmacológico aprobado por las agencias reguladoras para la EHGNA. La única medida con eficacia histológica demostrada es la pérdida de al menos un 7-10% del peso.[72] Lamentablemente, el porcentaje de pacientes que alcanzan dicho objetivo y logran mantener el peso de forma sostenida mediante cambios en el estilo de vida, o que son candidatos a cirugía metabólica, es escaso, por lo que la mayoría de los pacientes permanece sin tratamiento. Esta situación ha motivado la puesta en marcha de múltiples ensayos clínicos farmacológicos en los últimos años, algunos de los cuales ya han mostrado resultados positivos y se encuentran en fases avanzadas de desarrollo.[73-75]

Ante este escenario, la correcta identificación de sujetos de riesgo resulta fundamental para realizar una intervención precoz sobre el estilo de vida, así como para valorar la indicación de cirugía metabólica o considerar su inclusión en ensayos terapéuticos con compuestos potencialmente eficaces.

Los criterios de selección y los objetivos del grueso de ensayos clínicos giran en torno a la mejoría histológica de la inflamación y/o fibrosis hepática, fundamentalmente los dirigidos a pacientes en estadios precirróticos.[76] Los TNI desempeñan un papel clave en la selección de candidatos, minimizando el número de biopsias hepáticas innecesarias y los fallos de selección.[77,78] En el caso de la ET, se han desarrollado diferentes índices compuestos con parámetros analíticos básicos o con test serológicos para optimizar la identificación de candidatos, como el Fibroscan-AST *score*.[79]

Por otra parte, disponer de TNI validados nos permitiría evaluar la respuesta a eventuales tratamientos y monitorizar de forma estrecha la evolución de la enfermedad en subgrupos de mayor riesgo. De hecho, estudios basados en ET han demostrado una correlación entre los valores dinámicos de elasticidad hepática y cambios en los estadios de fibrosis histológica.[70,73]

Así, la ET se posiciona como una herramienta potencialmente útil en la identificación de pacientes de riesgo con EHGNA, permitiendo guiar la toma de decisiones, monitorizar la progresión o regresión de la enfermedad y ajustar el seguimiento en consecuencia.

2. HIPÓTESIS

2. HIPÓTESIS

La EHGNA es una enfermedad altamente prevalente con un curso clínico habitualmente silente que puede pasar inadvertida hasta fases avanzadas con un peor pronóstico. Son necesarios, por tanto, métodos diagnósticos no invasivos, sencillos y aplicables a diferentes grupos poblacionales que permitan identificar sujetos con EHGNA de forma precoz, antes de que evolucionen a estadios finales de la enfermedad y desarrollen eventos clínicos.

La hipótesis principal de la presente tesis es que la ET es una herramienta útil en el abordaje global de la EHGNA, dado que permitiría diagnosticar la enfermedad mediante la detección de esteatosis gracias al PAC, estratificar el riesgo de presentar una enfermedad severa a través del estadiaje no invasivo de la fibrosis y predecir la aparición de eventos clínicos de origen hepático (CHC) y extrahepático.

Para evaluar dicha hipótesis se han llevado a cabo tres estudios que exploran el papel de la ET en el manejo de la EHGNA.

El primer estudio pretende estimar la prevalencia de EHGNA y de los diferentes estadios de fibrosis en población general española usando datos de ET y PAC.

El segundo estudio investiga la capacidad de los TNI (test serológicos y la ET) de predecir el desarrollo de CHC y complicaciones asociadas en pacientes con EHGNA.

El tercer estudio evalúa la asociación entre el desarrollo de eventos clínicos y complicaciones de la DM2 con la presencia de EHGNA y fibrosis medida por ET.

3. OBJETIVOS

3. OBJETIVOS

3.1. Objetivo principal

- Evaluar la utilidad de la ET en el diagnóstico de la EHGNA, estadiaje de la enfermedad y en la predicción de eventos clínicos.

3.2. Objetivos secundarios

- Describir la prevalencia de fibrosis hepática por EHGNA en población general española.
- Investigar la correlación entre los valores de elasticidad hepática medida por ET y el grado de fibrosis histológica en la EHGNA.
- Describir la incidencia de CHC en fases cirróticas y precirróticas en pacientes con EHGNA de nuestro medio.
- Evaluar el impacto de la EHGNA sobre el control metabólico y las complicaciones micro y macrovasculares de la diabetes.

4. METODOLOGÍA

4. METODOLOGÍA

4.1. Estudio 1: Estimación de la prevalencia de fibrosis significativa por esteatohepatitis no alcohólica en España combinando elastografía de transición e histología

4.1.1. Pacientes y métodos

El estudio 1 analiza dos cohortes contemporáneas y se ha realizado en 4 pasos siguiendo el esquema de la Figura 4:

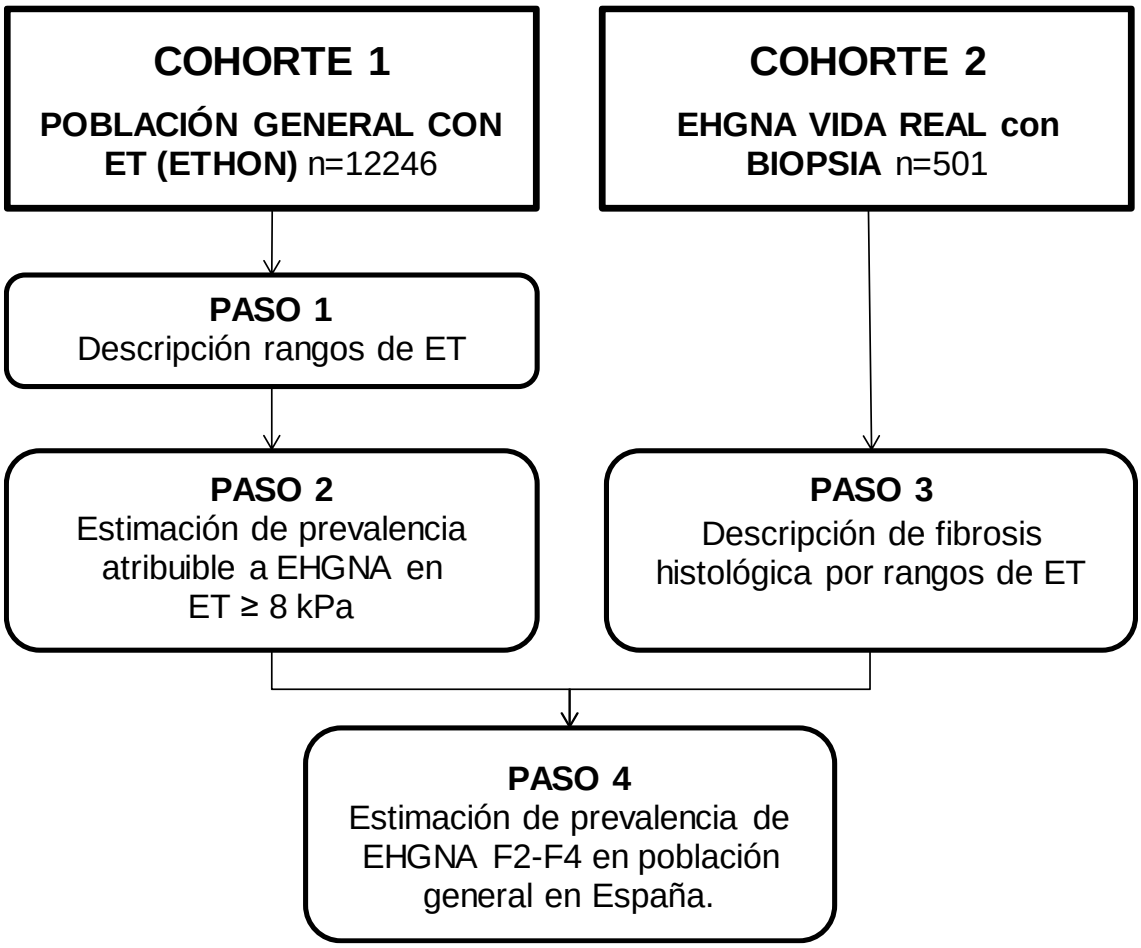

Figura 4**.** Cohortes y diseño del estudio 1. ET; Elastografía de transición; EHGNA, enfermedad hepática grasa no alcohólica.

Las cohortes que componen el estudio son las siguientes:

- Cohorte 1 (Población general con ET): La cohorte ETHON (Estudio poblacional de enfermedades hepáticas nacional) está compuesta de sujetos de entre 20 y 79 años seleccionados de forma aleatoria entre la población general de 18

centros de atención primaria pertenecientes a tres hospitales universitarios en Madrid, Valencia y Santander, entre 2015 y 2017. A todos los participantes se les practicó una ET, cuestionarios epidemiológicos, un examen físico y un análisis sanguíneo el mismo día. La cohorte ETHON se ha empleado para la estimación de valores de ET en población general (PASO 1) y para la estimación del peso atribuible a EHGNA dentro del rango de ET ≥ 8 kPa (PASO 2)

- Cohorte 2 (EHGNA comprobada por biopsia en vida real): Cohorte de práctica clínica de pacientes con EHGNA diagnosticados mediante biopsia hepática de 5 hospitales terciarios en España (Marqués de Valdecilla, Cantabria; Puerta de Hierro, Madrid; Virgen del Rocío, Sevilla; Clínico Universitario, Valladolid; y Vall d'Hebron, Barcelona). Los participantes se seleccionaron según práctica clínica habitual, en la que pacientes con alteraciones analíticas y/o esteatosis por ecografía son derivados al especialista. En la consulta de hepatología se les realizó una ET, de modo que aquellos con una ET ≥ 8 kPa se clasificaron como pacientes de riesgo. En estos pacientes se valoró la realización de una biopsia hepática para el estadiaje la fibrosis y su inclusión en ensayos clínicos cuando fuera posible. Los pacientes de la cohorte 2 fueron evaluados entre 2015 y 2020, estando la ET y la biopsia hepática realizadas en un intervalo inferior a 12 meses. Esta cohorte sirvió para evaluar la correlación entre las medidas de ET y los estadios de fibrosis hepática por histología (PASO 3) y para trasladar los resultados de ET en población general a las estimaciones de los diferentes estadios de fibrosis por EHGNA basadas en histología (PASO 4).

En ambas cohortes, la ET se realizó por personal entrenado (> 500 pruebas) usando la sonda recomendada por el aparato en cada individuo (M o XL). Se excluyeron del análisis aquellos sujetos con valores inválidos de ET o con ET < 8 kPa bajo la premisa de que estos últimos no serían referidos al especialista en práctica clínica real para la realización de una biopsia hepática.

Se consideró diagnóstico de esteatosis un valor de PAC ≥ 250 dB/m mediante ET, excepto en aquellos pacientes con ET > 20 kPa para evitar la exclusión de pacientes con cirrosis por EHGNA "quemada". Este término se refiere a la sustitución masiva de los hallazgos diagnósticos de EHGNA (como la esteatosis) por fibrosis en pacientes con cirrosis evolucionada, lo que clásicamente ha

llevado a clasificar pacientes con cirrosis por EHGNA “quemada” como cirrosis criptogénica o no filiada.

Por último, el diagnóstico de SM se basó en los criterios de *National Cholesterol Education Program Adult Treatment Panel III*.[80]

4.1.2. Análisis estadístico

Se emplearon los datos del Instituto Nacional de Estadística y de estudios locales para las comparaciones y extrapolaciones de la comorbilidad metabólica en población general de España.[81-83] Las variables cualitativas se expresaron en números (%) y las cuantitativas en media ± desviación estándar o mediana (rango intercuartílico-RIC-) según la distribución de los datos.

4.2. Estudio 2: Los test no invasivos de fibrosis hepática ayudan a predecir el desarrollo de carcinoma hepatocelular en pacientes con enfermedad hepática grasa no alcohólica

4.2.1. Pacientes y métodos

En el estudio 2 de la presente tesis doctoral se analiza retrospectivamente una cohorte prospectiva de pacientes con EHGNA evaluados en la consulta monográfica del Hospital Universitario Vall d'Hebron desde enero de 2016 hasta octubre de 2021.

Se han incluido pacientes diagnosticados de EHGNA mediante histología (esteatosis > 5%) y/o una ET con PAC ≥ 275 dB/m.[60]

El diagnóstico de CHC se realizó mediante técnicas de imagen (tomografía computarizada multifásica y/o RM mostrando hallazgos típicos de CHC)[84] y/o biopsia hepática en pacientes sin cirrosis o en aquellos con pruebas de imagen no concluyentes. Los sujetos con F4 en la biopsia hepática, alteraciones volumétricas hepáticas o signos de HP en pruebas de imagen fueron considerados como cirróticos. El estadiaje y el manejo del CHC se realizó según la Clasificación Barcelona Clinic Liver Cancer (BCLC).[85]

Se excluyeron pacientes con otras causas de enfermedad hepática y/o un consumo de alcohol > 20 y > 30 gr/día en mujeres y hombres, respectivamente. Los sujetos con un diagnóstico de CHC previo a la inclusión en la base de datos, un tiempo de seguimiento < 6 meses o que perdieron el seguimiento a lo largo del periodo del estudio fueron excluidos del análisis.

Para la estimación de los grados de fibrosis hepática mediante ET se emplearon los siguientes puntos de corte: < 8,1 kPa para F0–F1, 8,2-9,6 kPa para F2, 9,7-13,5 kPa para F3 y ≥ 13,6 kPa para F4.[58]

4.2.2. Análisis estadístico

Se realizó un análisis de supervivencia para evaluar la incidencia acumulada de aparición de CHC en función de las variables asociadas. Para el análisis de predictores de CHC en pacientes con cirrosis por EHGNA se realizó un análisis multivariado mediante un modelo de Cox.

4.3 Estudio 3: Predicción de eventos clínicos por elasticidad hepática y enfermedad renal crónica por enfermedad hepática grasa no alcohólica en pacientes con diabetes tipo 2

4.3.1. Pacientes y métodos

El estudio 3 tiene un diseño prospectivo y analiza los datos recogidos hasta octubre de 2021 de la cohorte PRECISED (*Preventing Cardiovascular Ischemic Events and Arresting Their Consequences in Type 2 Diabetic Population* -NCT02248311-). Dicha cohorte fue constituida entre 2014 y 2017 e incluyó sujetos con DM2 sin enfermedad cardiovascular previa, de entre 50 y 79 años, diagnosticados de DM2 en la consulta de Endocrinología del Hospital Vall d'Hebron y los centros de atención primaria de referencia según los criterios habituales[86] al menos 12 meses antes de la inclusión en el estudio y en seguimiento activo. Se incluyeron además controles apareados por edad.

El diagnóstico de EHGNA se estableció mediante un PAC ≥ 275 dB/m en pacientes con comorbilidad metabólica asociada.[60] Aquellos sujetos con otras causas de enfermedad hepática crónica y/o un consumo de alcohol > 20 y > 30 gr/día en mujeres y hombres, respectivamente, se clasificaron como no-EHGNA.

Se definió nefropatía diabética (ND) como la presencia de microalbuminuria > 30 mg/dl y enfermedad renal crónica (ERC) como una tasa estimada de filtrado glomerular estimado (TEFG) < 60ml/min/1,73m^2 y/o albuminuria > 30 mg/dl.[87] La enfermedad CV subclínica incluyó la presencia de un *score* de calcio arterial coronario ≥ 400 AU, una placa carotídea ≥ 3mm, y/o un grosor íntima-media > 1mm en la arteria carótida.

Los eventos cardiovasculares (ECV) englobaron el síndrome coronario agudo, el ictus y la enfermedad vascular periférica aguda. Los eventos hepáticos incluyeron una primera descompensación de la cirrosis (ascitis, encefalopatía hepática y/o hemorragia digestiva por hipertensión portal) y/o CHC. La variable compuesta de eventos clínicos globales (ECG) agrupaba la presencia de ECV, hepáticos y la mortalidad de cualquier causa.

4.3.2. Análisis estadístico

Se realizó un análisis de supervivencia para evaluar la incidencia acumulada de aparición de eventos clínicos en pacientes diabéticos según las variables asociadas. Para el análisis de predictores de eventos clínicos y ERC en sujetos con DM2 se realizó un análisis multivariado mediante un modelo de Cox.

5. RESULTADOS

5. RESULTADOS

5.1. Estudio 1: Estimación de la prevalencia de fibrosis significativa por esteatohepatitis no alcohólica en España combinando elastografía de transición e histología

Del global de pacientes de la cohorte ETHON (N=12246), 806 (6,6%) fueron excluidos por mediciones inválidas de ET. Las características basales de los pacientes de la cohorte 1 incluidos en el análisis se muestran en la Tabla 2.

COHORTE 1: POBLACIÓN GENERAL (ETHON) n=11440	
Edad, años	51 (42-60)
Varones, n (%)	4792 (41,9)
Origen geográfico, n (%)	
Cantabria	5090 (44)
Madrid	4088 (36)
Valencia	2262 (20)
Caucásicos n (%)	10058 (87,9)
Consumo alcohol riesgo, n (%)	442 (3,9)
Índice masa corporal, kg/m^2	26,1 (23,3-29,3)
Sobrepeso (≥ 25 - < 30 kg/m^2)	3597 (37,8)
Obesidad (≥ 30 kg/m^2)	2075 (21,8)
Diabetes mellitus tipo 2, n (%)	1540 (13,5)
Hipertensión arterial, n (%)	5206 (53,6)
Dislipemia, n (%)	7418 (64,8)
Síndrome metabólico, n (%)	1764 (15,4)
AST, (U/L)	22 (19-27)
ALT, (U/L)	20 (16-28)
Elasticidad hepática, (kPa)	4,5 (3,6-5,6)
PAC (dB/m) †	247 (209-293)

Tabla 2. Características basales de los pacientes de la cohorte de población general (ETHON) incluidos en el análisis. †Datos de la subcohorte de Cantabria. Valores expresados en mediana (rango intercuartílico). ETHON, Estudio poblacional de enfermedades hepáticas nacional; AST, Aspartato aminotransferasa; ALT, Alanina aminotransferasa; PAC, Parámetro de Atenuación Controlada.

Cabe destacar que un 21,8% del global de pacientes analizados de la cohorte 1 (ETHON) presentó obesidad, mientras que la prevalencia de DM2 e hipertensión

arterial se situó en 13,5% y 53,6%, respectivamente. Estos datos metabólicos son comparables a los reportados en población general española.

El estudio 1 se estructuró en cuatro pasos consecutivos (Figura 4), a saber:

5.1.1. PASO 1: Rangos de elastografía de transición en población general (COHORTE 1 -ETHON-)

En el primer paso se describió la proporción de sujetos en cada rango de ET. Un 5,61% y un 2,61% mostró una ET ≥ 8 kPa y ≥ 10 kPa, respectivamente.

Elasticidad	PACIENTES, n (%)	ET ≥ 8 kPa, n (%)
< 8 kPa	10797 (94,39)	
8-10 kPa	344 (3,00)	643 **(5,61)**
10-15 kPa	185 (1,62)	
15-20 kPa	36 (0,31)	
> 20 kPa	78 (0,68)	
TOTAL	11440 (100)	

Tabla 3. Prevalencia de los diferentes rangos de elasticidad hepática en la población general. ET, Elastografía de transición.

5.1.2. PASO 2: Estimación de la prevalencia atribuible a EHGNA dentro del subgrupo de pacientes con ET ≥ 8 kPa

El segundo paso consistió en estimar el peso relativo de la EHGNA como etiología respecto al resto de causas de hepatopatía crónica dentro del conjunto de la población con una ET ≥ 8 kPa.

Para este cálculo empleamos la subcohorte de Cantabria (n=5090), dado que contiene el 99,7% de las mediciones de PAC de la cohorte ETHON. Además, los datos metabólicos y elastográficos de la subcohorte de Cantabria son muy similares a los del global de pacientes incluidos en la cohorte ETHON (p.ej. en la subcohorte de Cantabria el 23,8% eran obesos y el 20% cumplieron criterios de SM, siendo el valor mediano de ET de 4,4 kPa). Así pues, tanto la subcohorte de Cantabria como la cohorte 1 en su conjunto son representativas de la población general española.

Como se muestra en la Figura 5, la proporción atribuible a EHGNA fue de 57,3%, calculada como la ratio de pacientes restantes después de la exclusión de sujetos con hepatitis virales, consumo de alcohol de riesgo o ausencia de esteatosis evaluada por el PAC (250 dB/m) entre el total de pacientes con ET ≥ 8 kPa y datos de PAC.

Por consiguiente, la prevalencia global de sujetos con ET ≥ 8 kPa se sitúa en un 5,61%, de los cuales un 57,3% se deben a la EHGNA, por lo que la prevalencia estimada de pacientes con ET ≥ 8 kPa por EHGNA en la población general es del 3,21% (5,61*0,573).

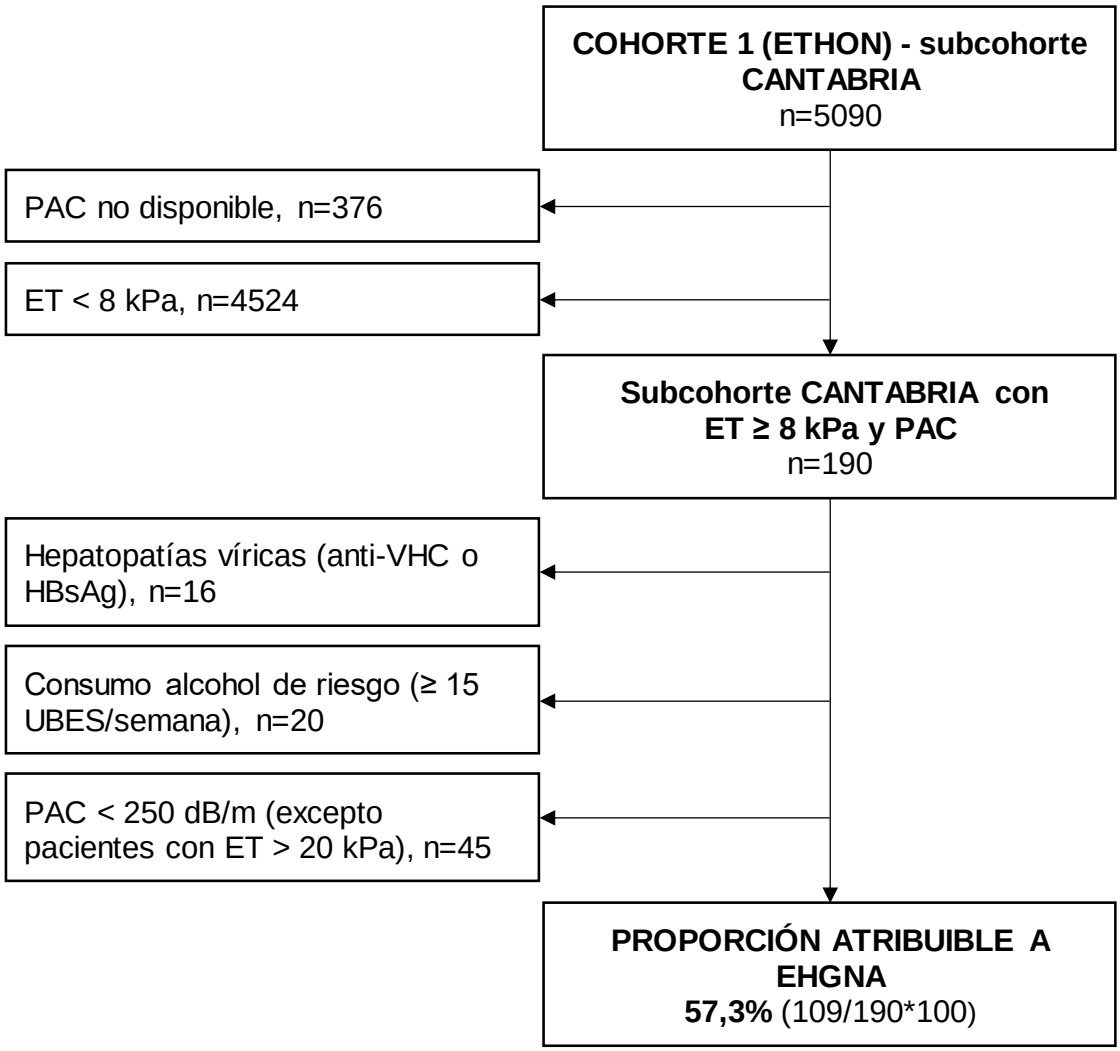

Figura 5. Diagrama de flujo del cálculo de la proporción atribuible a EHGNA en la cohorte poblacional. PAC, Parámetro de Atenuación Controlada; ET, Elastografía de transición; VHC, virus hepatitis C; HBsAg; Antígeno de superficie de virus hepatitis B; UBES, unidades de bebida estándar; EHGNA, enfermedad hepática grasa no alcohólica.

5.1.3. PASO 3: Distribución histológica de la fibrosis por EHGNA de acuerdo con los rangos de ET

La edad mediana de los pacientes incluidos en la cohorte 2 (EHGNA comprobada por biopsia en vida real) fue de 59 años y el 57% eran varones. En esta cohorte, un 66% de los sujetos presentó obesidad y un 44% DM2.

La mediana de tiempo desde la ET hasta la biopsia fue de 2,29 meses (RIC 1,12-4,41) y el tamaño mediano de la biopsia fue de 23,0 mm (RIC 19,0-28,0) con sólo un 5,1% de muestras fragmentadas en toda la cohorte. Estos datos son similares a los obtenidos al analizar específicamente las muestras de pacientes con sospecha de cirrosis (ET > 20 kPa) (tamaño mediano 21,5 mm [RIC 17-27] y un 4,5% de muestras fragmentadas).

De los 501 pacientes con EHGNA comprobada por biopsia, 112 (22,3%) presentaron una ET < 8 kPa y fueron excluidos. Entre estos pacientes, el 82% fueron F0-1, sólo cinco (4,5%) fueron F3 y ningún paciente presentó cirrosis histológica.

La distribución de los 389 pacientes restantes con una ET ≥ 8 kPa se detalla en la Tabla 4 y se representa en la Figura 6.

	RANGOS DE ET, n (%)				
FIBROSIS	**8-10 kPa**	**10,1-15 kPa**	**15,1-20 kPa**	**> 20 kPa**	**ET ≥ 8 kPa**
F0	25 (22,32)	23 (14,84)	3 (5,66)	1 (1,44)	52 (13,37)
F1	37 (33,04)	41 (26,45)	6 (11,32)	7 (10,15)	91 (23,39)
F2	28 (25,00)	28 (18,07)	7 (13,20)	7 (10,15)	70 (18,00)
F3	19 (16,96)	43 (27,74)	17 (32,08)	12 (17,39)	91 (23,39)
F4	3 (2,68)	20 (12,90)	20 (37,74)	42 (60,87)	85 (21,85)
TOTAL	112 (100)	155 (100)	53 (100)	69 (100)	389 (100)

Tabla 4. Distribución de la fibrosis histológica según los rangos de elasticidad hepática en la cohorte de EHGNA comprobada por biopsia. ET, elastografía de transición.

Merece la pena destacar que un 37% de los pacientes con ET ≥ 8 kPa presentó una fibrosis grado 0-1 mientras que, del total de pacientes con ET ≥ 10 kPa, hasta un 44% no mostró fibrosis avanzada (F3-4) en la histología.

Como se muestra en la Figura 6, la proporción de pacientes con fibrosis avanzada aumenta en cada intervalo de ET, pero incluso en el rango de pacientes con ET > 20 kPa hay un porcentaje relevante de pacientes sin cirrosis (39%).

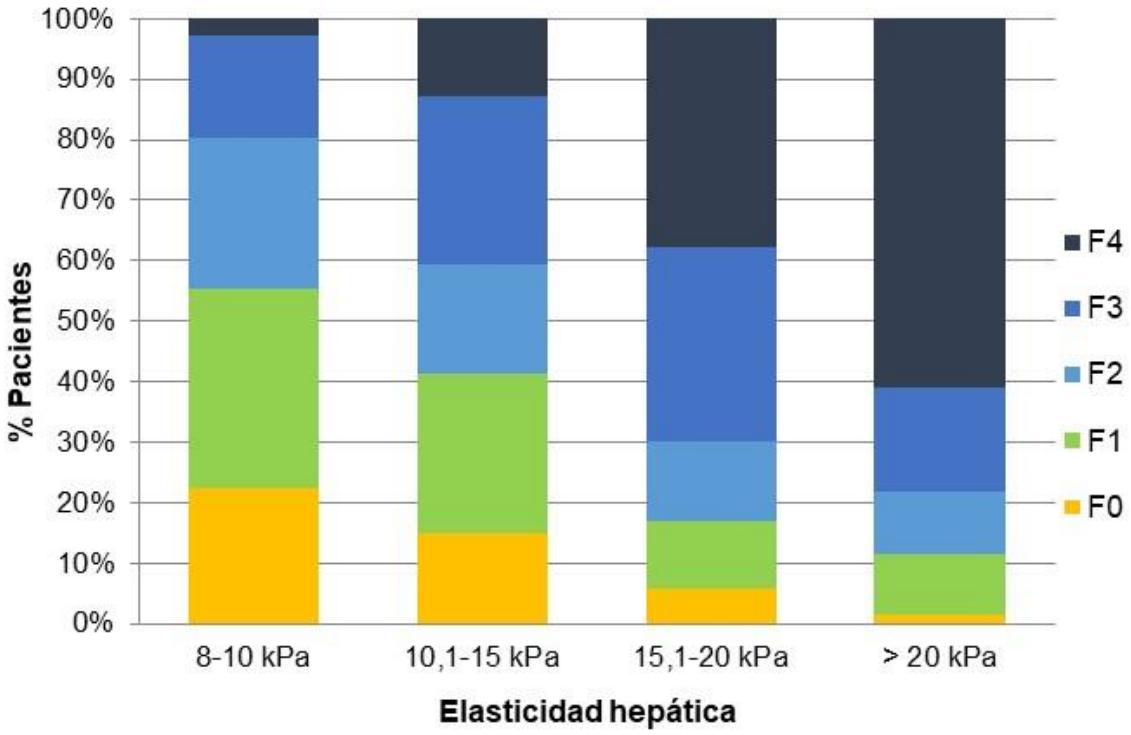

Figura 6. Distribución de la fibrosis hepática según rangos crecientes de elasticidad hepática en la cohorte de EHGNA comprobada por biopsia.

5.1.4. PASO 4. Estimación de la prevalencia de los estadios de fibrosis por EHGNA en población general de España (2015-2020)

El resultado principal del estudio 1 se obtuvo al combinar los resultados obtenidos en los pasos previos (Tabla 5).

La prevalencia de los diferentes estadios de fibrosis por EHGNA se calculó cruzando la prevalencia del total de pacientes con ET ≥ 8 kPa por EHGNA de la cohorte ETHON obtenida tras aplicar los PASOS 1 y 2 (3,21%) con las proporciones correspondientes de cada estadio de fibrosis evaluada por histología en el rango de pacientes con ET ≥ 8 kPa observadas en la cohorte de vida real de EHGNA comprobada por biopsia (PASO 3, Tabla 4).

	RESULTADO TABLA 4	**PREVALENCIA FIBROSIS POR EHGNA (%)**			
FIBROSIS	**ET ≥ 8 kPa (%)**	**ET ≥ 8 kPa (IC 95%)**	**FIBROSIS SIGNIFICATIVA (F2-F4)**	**ESTADIOS INTERMEDIOS (F2-F3)**	**CIRROSIS (F4)**
F0	13,37	0,43 (0,04-4,49)			
F1	23,39	0,75 (0,11-5,04)			
F2	17,99	0,58 (0,07-4,75)		**1,33** (0,29-5,98)	
F3	23,39	0,75 (0,11-5,04)	**2,03** (0,56-7,05)		
F4	21,85	0,70 (0,10-4,95)			**0,70** (0,10-4,95)
TOTAL	100	**3,21 (1,13-8,75)**			

Tabla 5. Estimaciones de prevalencia de los diferentes grados de fibrosis por EHGNA en población general de España. IC, Intervalo de confianza.

Finalmente, se estimó que el 2,03% (IC 95% 0,56-7,05) de la población española presenta una fibrosis significativa por EHGNA mientras que la prevalencia de cirrosis se situó en un 0,70% (IC 95% 0,10-4,95).

Por último, siguiendo la metodología previamente descrita, se calcularon las estimaciones de prevalencia de fibrosis por EHGNA en población general de España empleando el valor de PAC ≥ 275 dB/m, recientemente recomendado como punto de corte más preciso para el diagnóstico de esteatosis[60] (Tabla 6).

PREVALENCIA FIBROSIS POR EHGNA (%) usando PAC ≥ 275 dB/m	
	ET ≥ 8 kPa (IC 95%)
FIBROSIS SIGNIFICATIVA (F2-4)	1,75 (0,45-6,62)
ESTADIOS INTERMEDIOS (F2-3)	1,15 (0,22-5,69)
CIRROSIS (F4)	0,60 (0,07-4,78)

Tabla 6. Estimaciones de prevalencia de los estadios de fibrosis por EHGNA en población general de España usando el punto de corte de PAC 275 dB/m. PAC, Parámetro de Atenuación Controlada; IC, Intervalo de confianza.

5.2. Estudio 2: Los test no invasivos de fibrosis hepática ayudan a predecir el desarrollo de carcinoma hepatocelular en pacientes con enfermedad hepática grasa no alcohólica

En el estudio 2 se incluyeron 996 pacientes con EHGNA, de los cuales 26 (2,6%) desarrollaron CHC durante una mediana de seguimiento global de 2,5 años (RIC 1,9-3,6).

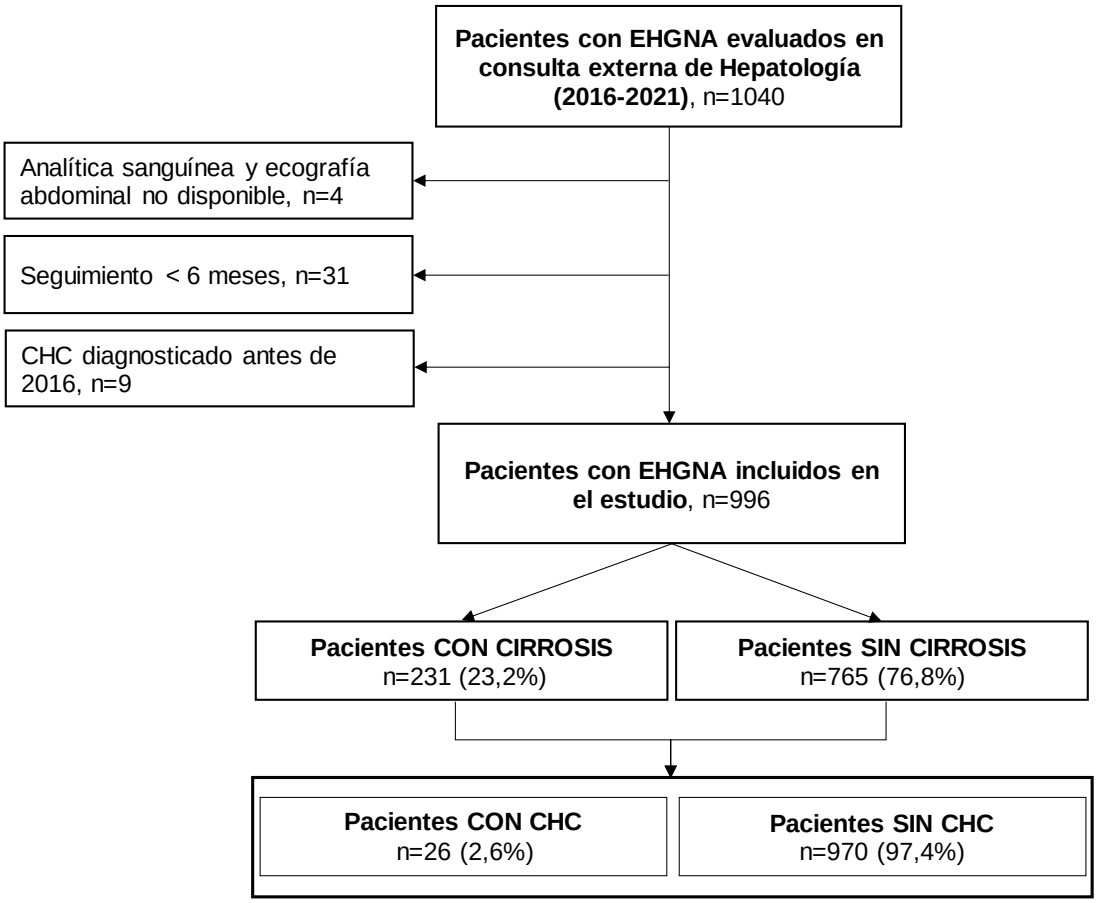

Figura 7. Diagrama de flujo de los pacientes incluidos en el estudio 2. EHGNA, Enfermedad hepática grasa no alcohólica; CHC, Carcinoma hepatocelular.

Los pacientes que desarrollaron CHC presentaron una edad mediana más alta y fueron mayoritariamente varones (Tabla 7). Los TNI de fibrosis (FIB-4 *index* y ET) mostraron unos valores significativamente más elevados en pacientes con CHC, lo que concuerda con las mayores tasas de fibrosis avanzada (F3-4) obtenidas en el estudio histológico (90,9% vs 53,8%; p=0,03).

De forma concomitante, se observó un deterioro de los parámetros de función hepática y una mayor mortalidad global en los pacientes con CHC en

comparación con aquellos sin CHC (30,8% frente a 4,4%; p<0,001). La insuficiencia hepática y las infecciones fueron las causas más frecuentes de muerte globalmente, mientras que el CHC fue responsable directo del fallecimiento de 2 pacientes (4,0%).

Variables	**No CHC N=970**	**CHC N=26**	**Valor p**
Edad (años)	60 (51-68)	69 (60-72)	0,001
Mujeres, n (%)	505 (52,1)	2 (7,6)	<0,001
Índice masa corporal (kg/m²)	30,9 (27,7-34,7)	29,8 (27,5-35,0)	0,4
Hipertensión arterial, n (%)	530 (54,6)	10 (38,5)	0,10
Diabetes mellitus 2, n (%)	396 (40,8)	19 (73,1)	0,001
Dislipemia, n (%)	595 (61,3)	8 (30,8)	0,002
AST (U/L)	32 (24-45)	48 (33-57)	0,002
ALT (U/L)	36 (24-54)	40 (28-45)	0,9
Plaquetas, x10⁹/L	228 (176-278)	102 (72-139)	<0,001
Bilirrubina, mg/dL	0,61 (0,49-0,85)	1,10 (0,86-1,83)	<0,001
Albúmina, g/L	4,3 (4,1-4,5)	3,8 (3,3-4,2)	<0,001
FIB-4 *index*	1,3 (0,9-2,1)	5,5 (2,6-7,5)	<0,001
Elasticidad hepatica (kPa)	7,8 (5,4-12,6)	32,0 (17,8-58,2)	0,001
Estadio de fibrosis por histología, n (%)	N=238	N=11	0,009
F0	29 (12,1)	1 (9,1)	
F1	47 (19,8)	0	
F2	34 (14,3)	0	
F3	43 (18,1)	0	
F4	85 (35,7)	10 (90,9)	
Mortalidad, n (%)	43 (4,4)	8 (30,8)	<0,001
Causa mortalidad, n (%)			
Insuficiencia hepática	12 (27,9)	1 (12,5)	0,005
CHC	0	2 (25)	
Cardiovascular	6 (13,9)	0	
Neoplasia extrahepática	8 (18,7)	4 (50)	
Infecciosa	12 (27,9)	1 (12,5)	
Otras causas	5 (11,6)	0	

Tabla 7. Características basales y eventos de la cohorte completa incluida en el estudio 2 según la presencia de carcinoma hepatocelular. Valores expresados en mediana (RIC). AST, Aspartato aminotransferasa; ALT, Alanina aminotransferasa; CHC, Carcinoma hepatocelular

Un 23,2% de los pacientes fueron clasificados como cirróticos al inicio del seguimiento, siendo la presencia de cirrosis significativamente más frecuente entre los sujetos que desarrollaron CHC (92,3% vs 21,3%; p<0,001). Al comparar las características basales de pacientes cirróticos, observamos que los que desarrollaron CHC presentaron menores cifras de plaquetas (99,5 [71,5–126] vs 139 [94–208]; p=0,01) y albúmina (3,7 [3,2–4,1] vs 4,1 [3,8–4,4]; p=0,001), así como un mayor valor mediano de bilirrubina (1,2 [0,8–1,9] vs 0,8 [0,6–1,1]; p<0,001), lo que se tradujo en puntuaciones más elevadas en la escala MELD (Model for End-stage Liver Disease) y la clasificación Child-Pugh. El 17,7% de los cirróticos había presentado una descompensación clínica anteriormente a su inclusión en el estudio. No se encontraron diferencias en la incidencia de CHC entre cirróticos compensados y descompensados (p=0,65) ni respecto a la presencia de signos de HP entre los pacientes con y sin CHC. Respecto a la evolución de la cirrosis durante el seguimiento, los sujetos con CHC presentaron mayores tasas de descompensación clínica, TH y mortalidad global (Tabla 8).

Variables	**Cirrosis No CHC N=207**	**Cirrosis CHC N=24**	**Valor p**
Clasificación Child-Pugh, n (%)			0,001
Clase A	175 (90,9)	15 (62,5)	
Clase B	15 (8,6)	8 (33,3)	
Clase C	1 (0.5)	1 (4.1)	
MELD	7,7 (6,7–9,4)	9,4 (7,5–12,0)	0,004
Descompensación clínica, n (%)	59 (29,6)	15 (71,4)	<0,001
Ascitis, n (%)	48 (31,1)	9 (37,5)	0,5
Encefalopatía hepática, n (%)	26 (16,8)	10 (41,6)	0,005
Hemorragia digestiva por HP, n (%)	22 (14,2)	5 (20,8)	0,4
Trasplante hepático, n (%)	1 (0,4)	9 (37,5)	<0,001
Mortalidad global, n (%)	37 (17,8)	8 (33,3)	0,09

Tabla 8. Escalas de función hepática y eventos de pacientes cirróticos a lo largo del seguimiento de acuerdo con la presencia de CHC. Valores expresados en mediana (rango intercuartílico). CHC, Carcinoma hepatocelular; HP, Hipertensión portal; MELD, Model for End-stage Liver Disease.

La tasa de incidencia de CHC de toda la cohorte fue de 9,49 (IC 95% 6,4–13,9) por 1000 personas-año. Al estratificar a los pacientes según la presencia de cirrosis, la incidencia se situó en 41,2 (IC 95% 27,6–61,6) y 0,93 (IC 95% 0,23–3,70) por 1000 personas-año para los pacientes con y sin cirrosis, respectivamente. Como se muestra en la Figura 8, la incidencia acumulada de CHC fue significativamente mayor en pacientes con cirrosis y puntuaciones elevadas en los TNI de fibrosis (ET y FIB-4 *index* ajustado por edad).

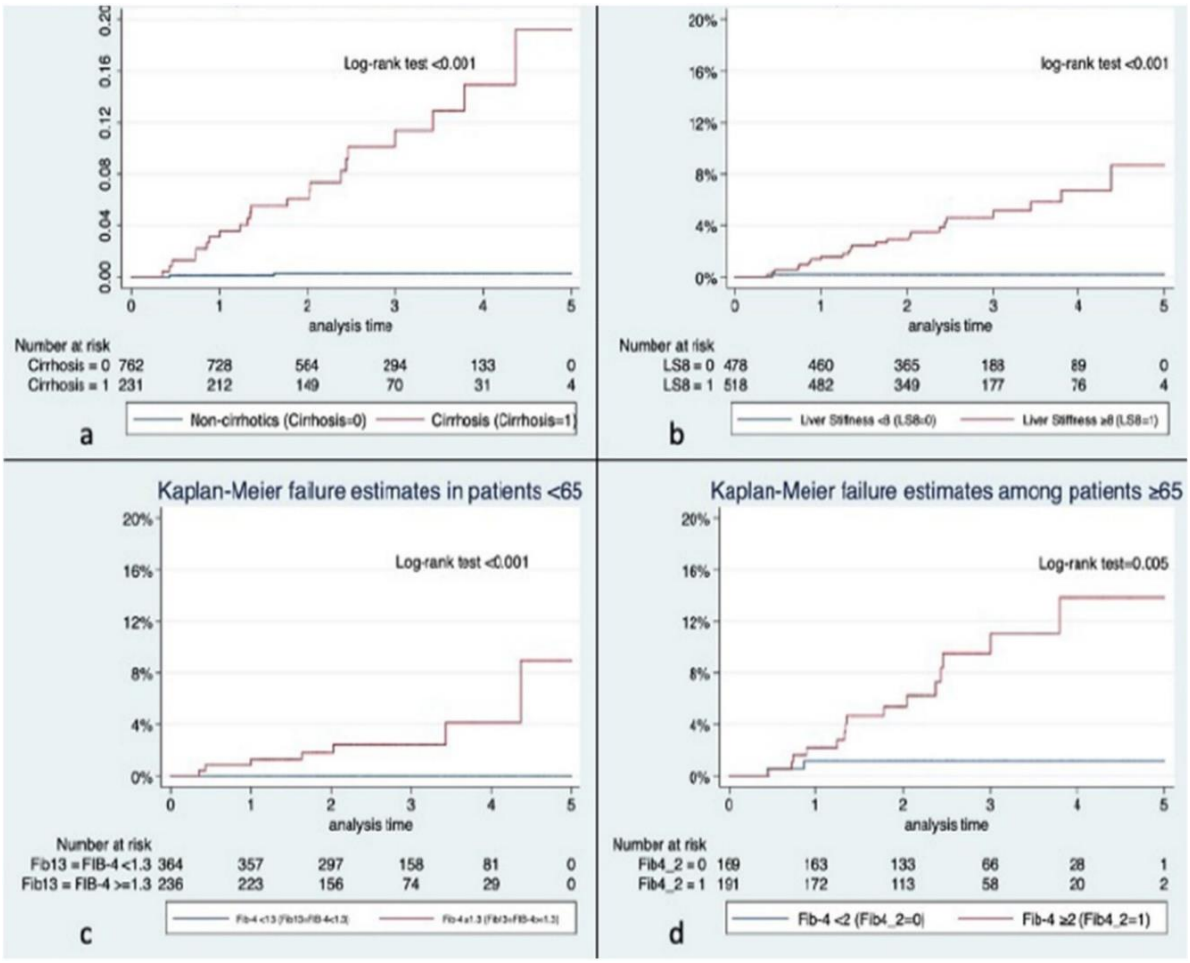

Figura 8. Incidencia acumulada de carcinoma hepatocelular de acuerdo con la presencia de cirrosis (a), elasticidad hepática (b), y FIB-4 *index* ajustado por edad (c, d).

Al analizar a los 249 sujetos con biopsia hepática, no se encontraron diferencias significativas en la incidencia de CHC según la presencia de esteatosis (p=0,06), inflamación lobulillar (p=0,4) o balonización (p=0,1). Por el contrario, los pacientes con fibrosis avanzada (F3-4) tuvieron una incidencia significativamente mayor de CHC en comparación con pacientes en estadios precoces (F0-2) (10/138 [7,25%] frente a 1/111 [0,9%]; p=0,02) (Figura 9).

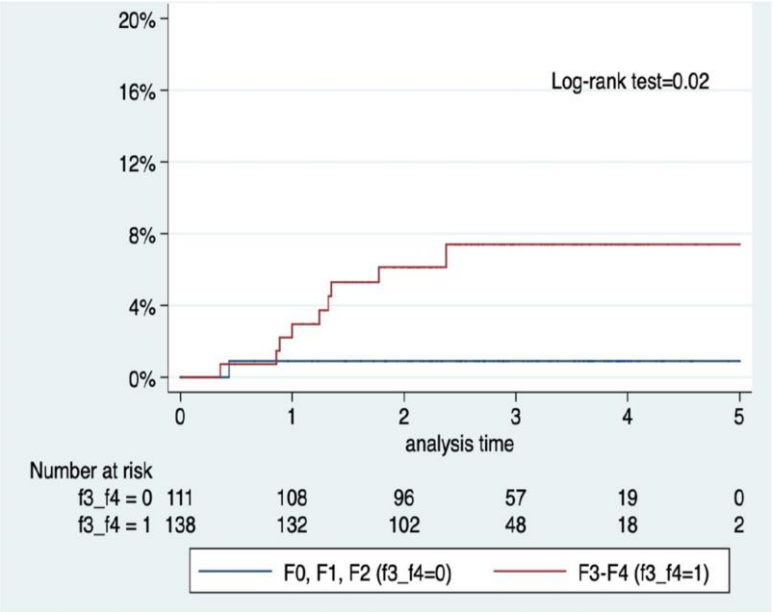

Figura 9. Incidencia acumulada de carcinoma hepatocelular según la presencia de fibrosis avanzada en la biopsia hepática (n = 249).

El 80,7% de los pacientes con CHC (21/26) fueron diagnosticados en un estadio BCLC 0 o A y recibieron un tratamiento con intención curativa (resección quirúrgica, TH o ablación por radiofrecuencia). La mediana de tamaño de las lesiones fue de 30 mm (RIC 16-35) y el 73,1% (19/26) de los pacientes presentaron una lesión única al diagnóstico. Un 30,7% (8/26) de los pacientes presentaron una recidiva del CHC, siendo preciso el tratamiento con quimioembolización transarterial y terapia sistémica en 7 y 2 ocasiones, respectivamente.

El análisis multivariante en pacientes cirróticos identificó como variables predictoras de desarrollo de CHC el recuento de plaquetas (HR=0,98, IC 95% 0,98-0,99; p=0,001) y la albúmina (HR=0,34, IC 95% 0,13-0,87; p=0,024), cuyos valores crecientes suponen un riesgo menor de CHC, así como aumentos progresivos de elasticidad hepática (HR=1,03, IC 95% 1,00-1,06; p=0,016), los cuales se asocian con un mayor riesgo de CHC con el tiempo.

Finalmente, se calculó la capacidad diagnóstica para CHC de los TNI de fibrosis, mostrando el FIB-4 un área bajo la curva de 0,87 (IC 95% 0,78–0,96), por un 0,85 (IC 95% 0,77–0,93) en el caso de la ET.

5.3. Estudio 3: Predicción de eventos clínicos por elasticidad hepática y enfermedad renal crónica por enfermedad hepática grasa no alcohólica en pacientes con diabetes tipo 2

En el estudio 3 se incluyeron el 93,4% del total de sujetos de la cohorte PRECISED, de los cuales 186 eran diabéticos y 57 controles (Figura 10). Entre los diabéticos, la prevalencia de EHGNA fue del 66,6% (124/186).

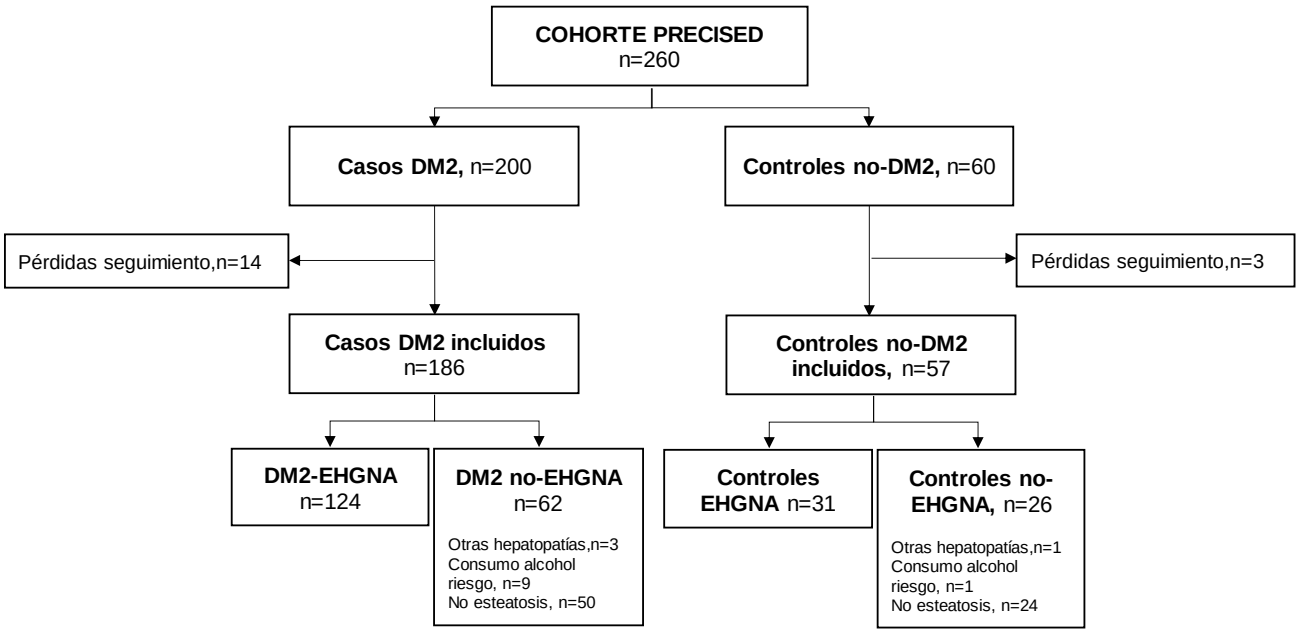

Figura 10. Diagrama de flujo de los pacientes incluidos en el estudio 3. DM2, Diabetes mellitus tipo 2; EHGNA, Enfermedad hepática grasa no alcohólica.

Basalmente, los pacientes con DM2 y EHGNA mostraron una mayor carga metabólica que aquellos sin EHGNA, con tasas más elevadas de obesidad y dislipemia, así como un peor control de la DM2 (Tabla 9).

Los pacientes con EHGNA presentaron valores de transaminasas y elasticidad hepática significativamente más elevados que aquellos diabéticos sin EHGNA, así como una peor función renal estimada tanto por el valor de creatinina como por TEFG. El tiempo mediano desde el diagnóstico de la DM2 fue similar entre grupos (11,0 años [RIC 6,0-20,0] vs 12,5 años [RIC 7,0-22,5]; p=0,64). Además, cabe destacar que un 10,8% del global de sujetos con DM2 (20/186) presentó una ET ≥ 10 kPa.

Variables	DM2 EHGNA (N=124)	DM2 no-EHGNA (N=62)	Valor p
Edad (años)	65,4±6,6	66,2±6,0	0,41
Mujeres, n (%)	67 (54,0)	40 (64,5)	0.17
Índice masa corporal (kg/m^2)	31,1 (28,2-34,5)	27,2 (25,0-30,0)	<0,001
Obesidad (≥ 30 kg/m^2)	76 (61,3)	18 (29,0)	<0,001
Hipertensión arterial, n (%)	94 (75,8)	40 (64,5)	0,10
Dislipemia, n (%)	104 (83,9)	44 (71,0)	0,04
HbA1c (%)	7,5±1,1	7,1±1,1	0,04
Creatinina (mg/dL)	0,85±0,26	0,76±0,17	0,012
TEFG (mL/min)	80,2±17,4	84,8±13,1	0,045
AST (U/L)	25,9±14,6	24,8±17,5	0,64
ALT (U/L)	27,7±17,3	22,3±15,3	0,041
Elasticidad hepatica (kPa)	5,6 (4,5-7,3)	4,8 (4,2-5,8)	0,004

Tabla 9. Características basales de los sujetos diabéticos con y sin EHGNA. Valores expresados en mediana (rango intercuartílico) o media (desviación estándar) según la distribución de cada variable. ALT, Alanina aminotransferasa; AST, Aspartato aminotransferasa; DM2, Diabetes mellitus tipo 2; EHGNA, Enfermedad hepática grasa no alcohólica; HbA1c, Hemoglobina glicosilada; TEFG, Tasa estimada de filtrado glomerular.

Durante una mediana de seguimiento de 5,6 años (RIC 4,8-6,4), considerando toda la cohorte PRECISED, 23 diabéticos (12,4%) desarrollaron un ECV en comparación con un evento (1,8%) en el grupo control (p=0,019). El único evento hepático (ascitis) lo desarrolló un sujeto con DM2 y el 8,1% de los pacientes con DM2 fallecieron en comparación con el 5,3% de los controles (p=0,48). La tasa de eventos clínicos globales (ECG) fue significativamente mayor entre los DM2 en comparación con los controles (17,7% frente a 7,0%; p=0,049)

Al comparar pacientes diabéticos con y sin EHGNA, no encontramos diferencias en la tasa de desarrollo de ECV en su conjunto (12,9% frente a 11,5%; p=0,75), siendo el síndrome coronario agudo el evento más reportado entre el total de pacientes con DM2 (16/186). Un sujeto con EHGNA presentó ascitis y fallecieron 8 pacientes con EHGNA (6,5%), un 25% de los cuales como consecuencia de ECV y ninguno por causa hepática. No se encontraron diferencias en mortalidad entre grupos (p=0,25). Finalmente, la tasa de ECG fue similar entre los pacientes diabéticos con y sin EHGNA (16,9% vs 19,4%; p=0,68).

En el análisis de supervivencia realizado en pacientes con DM2 se objetivó que los pacientes varones y aquellos con una ET ≥ 10 kPa desarrollaron significativamente más eventos clínicos globales durante el período del estudio (Figura 11).

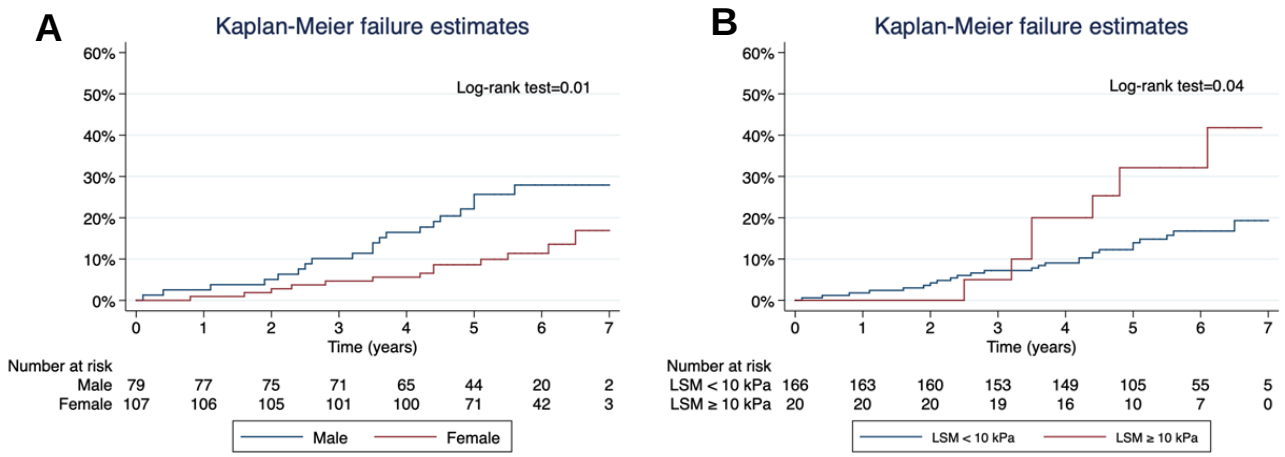

Figura 11. Incidencia acumulada de eventos clínicos globales en pacientes diabéticos según: a) Sexo; B) Elasticidad hepática.

Los factores de riesgo asociados al desarrollo de ECG, tanto entre el total de pacientes diabéticos como entre aquellos sujetos con DM2 y EHGNA, fueron el sexo masculino, la edad avanzada y la elasticidad hepática medida por ET.

	Total DM2 (n=186)		**DM2-EHGNA (n=124)**	
	HR (IC 95%)	**Valor p**	**HR (IC 95%)**	**Valor p**
Edad	1,09 (1,02-1,17)	0,009	1,10 (1,02-1,20)	0,003
Sexo masculino	2,55 (1,19-5,43)	0,015	4,82 (1,70-13,64)	0,015
Obesidad (IMC≥ 30 kg/m^2)	1,13 (0,54-2,38)	0,73	1,05 (0,40-2,78)	0,91
Elasticidad hepática (por aumento de 1 kPa)	1,03 (1,01-1,05)	0,002	1,05 (1,01-1,08)	0,009
ECV subclínica	0,76 (0,34-1,71)	0,51	0,52 (0,19-1,44)	0,21
EHGNA	1,04 (0,48-2,27)	0,90		

Tabla 10. Factores de riesgo asociados con el desarrollo de eventos clínicos globales. Regresión multivariante. DM2, Diabetes mellitus tipo 2; HR, Hazard ratio; ECV, Enfermedad cardiovascular; EHGNA, Enfermedad hepática grasa no alcohólica.

No se encontraron diferencias significativas entre pacientes diabéticos con y sin EHGNA al evaluar la tasa de complicaciones microvasculares (nefropatía diabética [p=0,07], retinopatía diabética [p=0,24] y neuropatía periférica [p=0,59]) ni al estudiar la presencia enfermedad CV subclínica (p=0,67).

Por el contrario, los pacientes diabéticos con EHGNA sí mostraron una mayor tasa de ERC (45,1% vs 24,6%; p=0,007) que aquellos sin EHGNA, asociándose la presencia de EHGNA a la ERC en el análisis multivariante (Odds Ratio=2,29, IC 95% 1,07-4,87; p=0,031), independientemente de factores de riesgo conocidos de disfunción renal, como la hipertensión arterial (OR=5,03, IC 95% 2,09-12,08; p<0,001), la presencia de dislipemia (OR=3,33 IC 95% 1,31-8,44; p=0,011) o el sexo masculino (OR sexo femenino=0,40, IC 95% 0,20-0,80; p=0,009).

6. DISCUSIÓN

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En la presente tesis doctoral se investiga la utilidad de la ET en el abordaje integral de la EHGNA. El primer estudio demuestra la capacidad de la ET para diagnosticar y estratificar la severidad de la enfermedad, aportando estimaciones precisas sobre la prevalencia de fibrosis por EHGNA a nivel poblacional. El segundo y tercer estudio evidencian que la ET es capaz de predecir eventos clínicos en poblaciones de riesgo, como sujetos con cirrosis por EHGNA y personas con DM2, respectivamente.

6.1. Prevalencia de EHGNA y papel de la ET en el diagnóstico y estadiaje de la fibrosis

La EHGNA se considera una de las epidemias del siglo XXI, afectando al 25-35% de adultos de la población general. Dado que la presencia y severidad de la fibrosis condiciona la historia natural de la enfermedad y el pronóstico de los pacientes, conocer la prevalencia exacta de los estadios de fibrosis por EHGNA tiene un gran interés científico.

En el estudio 1, a través de estimaciones de fibrosis mediante ET en la cohorte poblacional más extensa reportada en Europa hasta la fecha (> 12.000 pacientes), sumado a datos histológicos de una cohorte contemporánea con más de 500 biopsias, se ha estimado que el 5,61% de la población general española presenta una ET ≥ 8 kPa, siendo en el 57,3% de los casos atribuible a la EHGNA, lo que representa que un 3,21% de la población general española tendría una ET ≥ 8 kPa como consecuencia de la EHGNA. Finalmente, tras cruzar estos datos con la probabilidad de presentar cada estadio de fibrosis evaluada por histología, la prueba *gold standard* para la estratificación de la fibrosis, estimamos que el 2,03% de la población general adulta española presenta una EHGNA con fibrosis significativa, de la cual un 1,33% se encuentra en estadios intermedios (F2-3) y un 0,70% en fase de cirrosis.

En el presente trabajo, el diagnóstico de esteatosis se ha realizado a través del uso de PAC en > 5000 pacientes, lo que sumado a la exclusión de otras causas de enfermedad hepática crónica mediante datos analíticos y demográficos, permiten estimar un peso relativo del 57,3% atribuible a la EGHNA. Estos

resultados difieren de los presentados en estudios previos,[88-90] en los que la atribución de EHGNA carecía de evaluación concomitante de esteatosis. Por consiguiente, concluimos que el PAC de la ET es capaz de identificar la presencia de esteatosis en pacientes con sospecha de enfermedad hepática, permitiendo realizar estimaciones precisas del peso relativo de la EHGNA y convirtiendo a la ET una herramienta potencialmente útil para el cribado de EHGNA.

El segundo aspecto a resaltar del estudio 1 es la menor prevalencia de fibrosis significativa observada en comparación con resultados previos de cohortes europeas similares.[89-91] Por un lado, los datos de fibrosis están influenciados por el peso relativo de cada etiología, en particular de la enfermedad hepática por alcohol, sobre la que se han descrito diferencias significativas entre países de nuestro entorno.[91] Por otra parte, la mayor precisión obtenida en las presentes estimaciones gracias al importante tamaño muestral de ambas cohortes, sumado a la verificación histológica realizada en el estudio 1, sugiere que las estimaciones previas sobreestimaban la prevalencia y la severidad de la fibrosis.

Finalmente, podría resultar llamativo que hasta un 40% de los pacientes con ET > 20 kPa no presentaron una cirrosis histológica. Si bien es cierto que se ha descrito cierta variabilidad en la interpretación de las biopsias de pacientes con EHGNA,[92] estos datos concuerdan con estudios previos que subrayan el bajo VPP de la ET para diagnosticar pacientes con EHCAC a nivel poblacional.[58,62]

La información obtenida en el estudio 1 proporciona una idea fidedigna de la magnitud del problema de salud pública que representa la EHGNA, permitiendo una planificación dirigida de los esfuerzos y estrategias necesarias para tratar esta epidemia. Así mismo, queda patente que la ET es una herramienta útil en el diagnóstico y estratificación de la severidad en pacientes con EHGNA, lo que la posiciona como una técnica diagnóstica y de cribado idónea, aplicable no sólo a nivel poblacional, sino potencialmente también en grupos de riesgo o en poblaciones con características especiales (p.ej. pacientes con VIH, enfermedades inmunomediadas, síndrome de ovario poliquístico o población penitenciaria).[93]

6.2. Impacto de la EHGNA en grupos de riesgo y rol de la ET en la predicción de eventos clínicos

La EHGNA presenta un curso clínico ampliamente variable que depende en gran medida de la severidad de la enfermedad hepática y de la presencia de determinadas comorbilidades, como la DM2, las cuales predisponen a desarrollar una enfermedad más agresiva y aumentan la probabilidad de sufrir complicaciones. Los estudios 2 y 3 investigan la probabilidad de presentar eventos clínicos en poblaciones de riesgo y el papel de la ET en la predicción de los mismos.

La progresión a cirrosis y la aparición de descompensaciones clínicas y de CHC empeoran de forma dramática el pronóstico de los enfermos, por lo que resulta de vital importancia: a) identificar a los pacientes en fases precirróticas; b) prevenir el desarrollo de HP y eventos clínicos en pacientes con EHCAC o cirrosis; c) tratar de forma precoz las complicaciones hepáticas.

En este sentido, la ET podría ser una técnica a tener en cuenta en el diseño e implementación en práctica clínica de modelos predictivos que permitan la identificación no invasiva de pacientes con EHCAC susceptibles de desarrollar eventos. En la EHGNA este planteamiento es especialmente relevante, ya que la probabilidad de desarrollar eventos hepáticos, como el CHC, podría ser significativa también en fases previas a la cirrosis, lo cual es infrecuente en otras causas de enfermedad hepática crónica y condicionaría el manejo y el seguimiento los pacientes con EHGNA.[33,71]

Los resultados del estudio 2 muestran una incidencia de CHC en pacientes con EHGNA de 9,49 (IC 95% 6,4–13,9) por 1000 personas-año, asentando el 92,3% de los CHC sobre hígados cirróticos, siendo la prevalencia de CHC en fases precirróticas inferior a la descrita previamente.[94,95] Sin embargo, los datos histológicos reportados en estudios previos son limitados, lo que podría condicionar una clasificación errónea de los estadios de fibrosis. Esta situación sería particularmente significativa en el caso de la EHGNA, donde se ha descrito que los pacientes con F3 muestran un riesgo sustancialmente mayor de presentar CHC que aquellos F0-2,[33,96] tal y como se ha confirmado en el presente estudio (Figura 9).

Los pacientes con CHC presentaron una peor evolución que aquellos sin CHC. La tasa de mortalidad global entre pacientes con CHC fue del 5,1%, mientras que 74 sujetos presentaron una descompensación clínica (todos ellos cirróticos). Estas cifras son superiores a los descritos por Sanyal,[33] probablemente debido al mayor porcentaje de cirróticos en nuestra cohorte (23,2% *versus* 9,4%) y al mayor número de pacientes con CHC (26/996 *versus* 9/1761)

Finalmente, los factores predictores de CHC en pacientes con cirrosis por EHGNA fueron la elasticidad hepática medida por ET, la albúmina y el recuento de plaquetas, variables consistentemente relacionadas con el desarrollo de eventos hepáticos y habitualmente incluidas en modelo predictivos validados de pacientes con EHCAC o cirrosis.[71,97,98]

Por todo lo anterior, la ET constituye un método útil en la predicción de CHC en pacientes con cirrosis por EHGNA. Los resultados del estudio 2 subrayan la importancia de incorporar la ET en el seguimiento habitual de pacientes con EHCAC o cirrosis, permitiendo una mejor predicción del riesgo incidente de presentar eventos, en particular CHC, con mayores tasas de diagnóstico en fases iniciales y un manejo personalizado con mayores posibilidades de recibir tratamientos con intención curativa.

En definitiva, el manejo de las complicaciones y de la propia cirrosis por EHGNA continúa siendo un desafío que involucra a clínicos e investigadores,[99] y afecta directamente a los pacientes, sobre los que debe girar la atención clínica y la toma de decisiones.[100] Por último, se han de tener en cuenta factores ajenos a la enfermedad hepática potencialmente capaces de modular la historia natural de la cirrosis, como la reciente pandemia por el virus SARS-CoV-2,[101] así como el impacto de la cirrosis sobre el curso evolutivo y el manejo de patologías de origen extrahepático.[102]

Un segundo grupo de riesgo de la EHGNA son aquellos pacientes que presentan comorbilidad metabólica. La asociación de la EHGNA con las diferentes entidades del SM está ampliamente establecida.[103] Los pacientes con DM2 u obesidad representan una población vulnerable para la EHGNA, con un mayor riesgo de complicaciones y un peor pronóstico hepático, metabólico y global.[104]

El estudio 3 confirma el riesgo aumentado de presentar eventos clínicos en pacientes con DM2. Sin embargo, los sujetos con DM2 y EHGNA no presentaron proporcionalmente más eventos clínicos ni complicaciones de la DM2 que aquellos sin EHGNA. Estos resultados están condicionados por el relativamente bajo número de eventos ocurridos durante el seguimiento, inferior al publicado en la literatura[105,106] y que se relaciona con el buen control glicémico de nuestra cohorte, la pequeña proporción de pacientes con fibrosis avanzada o cirrosis (sólo un 2,4% presentó una ET ≥ 20 kPa) y al bajo riesgo cardiovascular basal comparado con estudios previos.[107-109]

Los pacientes diabéticos con EHGNA presentaron, no obstante, un mayor riesgo de ERC con similares tasas de nefropatía diabética, lo que apoya la hipótesis del daño renal mediado por la enfermedad hepática independientemente de la DM2.[110,111]

La elasticidad hepática por ET, la edad y el género masculino se asociaron al desarrollo de eventos clínicos tanto en el global de diabéticos como en aquellos con EGHNA. Estos resultados confirman la influencia decisiva de la fibrosis en la fisiopatología de la enfermedad extrahepática en pacientes con EHGNA, así como la necesidad de establecer programas de cribado con TNI sobre población metabólica vulnerable a nivel comunitario[112] y en atención especializada de pacientes con alta incidencia de SM (p.ej. consultas de diabetes, unidades de obesidad o de riesgo cardiovascular)

6.3. Limitaciones

La principal limitación del estudio 1 es el riesgo de sobreestimación de la prevalencia de fibrosis en estadios avanzados. En primer lugar, las 2 cohortes no están sistemáticamente ligadas, lo que conlleva un riesgo inevitable de incurrir en un sesgo de referencia. Por otra parte, se trata de un estudio observacional retrospectivo en el que la indicación de la biopsia hepática no estaba protocolizada y su interpretación no se centralizó. Por último, el cálculo del peso relativo de EHGNA como etiología se basó en el PAC, para cuyo punto de corte no había un claro consenso en la literatura en el momento de la realización del estudio. Se optó por un punto de corte conservador (250 dB/m) con la intención de identificar al mayor número de sujetos con esteatosis en una población de

baja prevalencia como la población general. No obstante, se aportan estimaciones alternativas usando 275 dB/m. Además, el peso específico de la EHGNA se ha de ajustar según el consumo relativo de alcohol y la diversidad étnica en cada población.

En el estudio 2 sólo se analizaron los pacientes incluidos en la cohorte de la consulta monográfica de EHGNA, por lo que potencialmente existirían casos de CHC que no se recogieron en el análisis. Por otra parte, la fecha de diagnóstico de la cirrosis es desconocida en algunos pacientes, lo que influye en el riesgo cumulativo de presentar eventos. Por último, los resultados están influenciados por el escaso número de eventos recogidos y el carácter retrospectivo del estudio por lo que deben interpretarse con cautela.

El número de eventos clínicos del estudio 3 es relativamente pequeño pese al largo periodo de seguimiento, lo que limita la interpretación de los resultados, particularmente en lo referente al papel de la enfermedad CV silente como promotor de eventos en sujetos con DM2 y EHGNA. Por otra parte, la relación entre la EHGNA, el desarrollo de eventos clínicos y la prevalencia de complicaciones de la DM2 está condicionada por el tamaño muestral y la ausencia de confirmación histológica de la EHGNA y del grado de fibrosis. No obstante, en su lugar se empleó el PAC de la ET (275 dB/m), que ha mostrado una alta sensibilidad y VPP para el diagnóstico de esteatosis, especialmente en poblaciones con alta prevalencia de EHGNA como los sujetos con DM2.

Finalmente, una potencial limitación de la aplicabilidad práctica de los resultados de los 3 estudios que componen la presente tesis es la necesidad de disponer de un aparato de ET. Esta herramienta está ampliamente implementada a nivel hospitalario y en centros de referencia de patología hepática, siendo su accesibilidad más limitada en atención primaria. Además, se ha de tener en cuenta que los valores de ET se ven influenciados por la presencia de inflamación, congestión hepática u obstrucción biliar.

7. CONCLUSIONES

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- La ET es capaz de realizar estimaciones precisas de la prevalencia de fibrosis por EHGNA a nivel poblacional, que en España se estima en 2,03% y 0,7% para fibrosis significativa y cirrosis, respectivamente.
- La presencia de fibrosis avanzada medida por histología o por ET se asocia al desarrollo de CHC en pacientes con EHGNA, siendo infrecuente la aparición CHC en estadios precirróticos en nuestra cohorte.
- La presencia de EHGNA y la elasticidad hepática medida por ET se relacionan de forma independiente con la ERC y con el riesgo incidente de presentar eventos clínicos en pacientes con DM2.
- En resumen, la ET es una herramienta útil en el abordaje global de la EHGNA ya que permite identificar correctamente la presencia de esteatosis, estadiar la severidad de la enfermedad y predecir la aparición de eventos clínicos.

8. LÍNEAS DE FUTURO

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8.1. Evaluación de la EHGNA mediante ET en poblaciones especiales

Los resultados aportados en la presente tesis muestran que el PAC de la ET es capaz de discriminar correctamente la presencia de esteatosis, lo que convierte a la ET en una herramienta útil para el cribado de EHGNA, también en poblaciones especiales o con una menor prevalencia de la enfermedad *a priori*, sobre las que existe menos evidencia.

Tienen especial interés aquellos sujetos afectos de patología con base inmune (p.ej. enfermedad inflamatoria intestinal, psoriasis o lupus), enfermedades infecciosas como el VIH, presencia de polimorfismos genéticos que predisponen a EHGN, como los genes *patatin-like phospholipase domain-containing protein 3* y *transmembrane 6 superfamily member 2,* o el estudio de subgrupos vulnerables con un mayor riesgo de desarrollar comorbilidad metabólica como la población penitenciaria o psiquiátrica.

Por otro lado, es preciso ahondar en la fisiopatología e historia natural de la esteatosis mixta por EHGNA y enfermedad hepática por alcohol, en tanto que se ha descrito un efecto deletéreo aditivo con una peor evolución de la enfermedad hepática global. Además, dicho fenotipo es el más habitual en pacientes que consultan por sospecha de esteatosis en práctica real, lo que confiere a esta línea de trabajo una relevancia clínica extraordinaria. Por todo lo anterior, se ha desarrollado un proyecto de investigación que busca caracterizar los principales mecanismos moleculares que definen la esteatosis mixta con intención de describir futuras dianas terapéuticas (FIS PI22/01770).

Así pues, los potenciales resultados de esta línea de investigación aportarían datos novedosos sobre poblaciones menos estudiadas y con un especial interés desde el punto de vista clínico, sobre las que no se conoce la prevalencia exacta y el grado de severidad de la EHGNA.

8.2. Caracterización de la EHCAC y la HP en la EHGNA

Los estudios 2 y 3 han investigado la capacidad de la ET de predecir eventos clínicos en pacientes con EHGNA, quedando, no obstante, múltiples cuestiones por resolver.

En primer lugar, se requieren estudios prospectivos con largos periodos de seguimiento que abarquen el espectro global de la EHGNA y a la totalidad de los casos de CHC independientemente del estadio para conocer la incidencia real de CHC en fases precirróticas. Por otra parte, es necesario profundizar en la historia natural de la EHCAC por EHGNA y en el riesgo incidente de desarrollar descompensaciones clínicas de la cirrosis (ascitis, encefalopatía o hemorragia por hipertensión portal) o fallo hepático agudo sobre crónico.

El desarrollo de HP supone un hito en la historia natural de la EHCAC y tiene importantes implicaciones pronósticas. Sin embargo, la fisiopatología de la HP en sujetos con obesidad y EHGNA no está completamente caracterizada, habiéndose sugerido que alteraciones dinámicas funcionales y estructurales a nivel sinusoidal, independientes de la presencia de fibrosis, podrían jugar un papel en el desarrollo y evolución de la HP en la EHGNA.[113,114]

Así pues, de la presente tesis doctoral se desprende la necesidad de realizar estudios que permitan dilucidar la historia natural de la EHCAC y el comportamiento de la HP en la EHGNA. De igual manera, se requieren más datos sobre el papel de la obesidad en la fisiopatología de la HP y acerca de la capacidad diagnóstica de los tests invasivos y no invasivos de HP en sujetos con EHGNA, de cara a definir puntos de corte capaces de identificar pacientes con HP y HPCS y predecir la aparición de complicaciones.

En este sentido, nuestro grupo de trabajo está diseñando un modelo predictivo de eventos clínicos basado en la ET en sujetos con EHCAC por EHGNA. Además, en el marco de esta línea de investigación se está desarrollando un proyecto multidisciplinar (FIS PI22/008) que pretende evaluar la precisión de diferentes técnicas invasivas y no invasivas para el diagnóstico de HP en pacientes con obesidad mórbida y EHGNA, así como el efecto de una potencial intervención sobre la HP.

8.3. Impacto de potenciales tratamientos para la EHGNA y monitorización de la enfermedad

Pese a disponer de métodos diagnósticos que permiten identificar sujetos de riesgo de forma precoz, el abordaje terapéutico del grueso de pacientes con EHGNA es subóptimo dada la ausencia de fármacos aprobados específicamente para la enfermedad.

Sin embargo, ciertos fármacos aprobados para otra indicación se han ensayado para la EHGNA con resultados positivos. Los análogos del péptido similar al glucagón 1 y los inhibidores del transportador de sodio-glucosa 2 se emplean en práctica clínica habitual de pacientes con DM2 y han mostrado un potencial beneficio sobre la EHGNA,[74,115] que podría ser todavía mayor si se administraran en combinación.

Estudiar el impacto de dichos fármacos sobre pacientes de vida real con EHGNA y DM2 sería de gran interés puesto que permitiría expandir las limitadas opciones terapéuticas disponibles actualmente, sobre todo si tenemos en cuenta que la aprobación definitiva de los compuestos testados en ensayos clínicos puede prolongarse años.

Además, la realización de estudios prospectivos en vida real nos permitiría evaluar el papel de la ET en la monitorización y la respuesta a eventuales tratamientos, así como correlacionar la evolución elastográfica con los cambios ponderales, metabólicos y bioquímicos.

9. BIBLIOGRAFÍA

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10. ANEXOS

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10.1. Anexo I: Publicaciones de la tesis

10.1.1. Estudio 1

Calleja JL, Rivera-Esteban J, Aller R, Hernández-Conde M, Abad J, Pericàs JM, Benito HG, Serra MA, Escudero A, Ampuero J, Lucena A, Sánchez Y, Arias-Loste MT, Iruzubieta P, Romero-Gómez M, Augustin S, Crespo J. Prevalence estimation of significant fibrosis because of NASH in Spain combining transient elastography and histology. Liver Int. 2022 Aug;42(8):1783-1792.

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ORIGINAL ARTICLE

Prevalence estimation of significant fibrosis because of NASH in Spain combining transient elastography and histology

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Abstract

Background & Aims: Non-alcoholic fatty liver disease (NAFLD) has become a major public health problem, but the prevalence of fibrosis associated with non-alcoholic steatohepatitis (NASH) is largely unknown in the general population. This study aimed to provide an updated estimation of the prevalence of NASH fibrosis in Spain.

Methods: This was an observational, retrospective, cross-sectional, population-based study with merged data from two Spanish datasets: a large ($N = 12\,246$) population-based cohort (ETHON), including transient elastography (TE) data, and a contemporary multi-centric biopsy-proven NASH cohort with paired TE data from tertiary centres ($N = 501$). Prevalence for each NASH fibrosis stage was estimated by crossing TE data from ETHON dataset with histology data from the biopsy-proven cohort.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; CAP, controlled attenuation parameter; CI, confidence interval.; GGT, gamma-glutamyl transferase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HDL, high-density lipoprotein; INE, Instituto Nacional Estadística; LDL, low-density lipoprotein; LSM, liver stiffness measurement; NAFLD, Non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NCEP-ATP III, National Cholesterol Education Program-Adult Treatment Panel III; TE, transient elastography.

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Results: From the patients with valid TE in ETHON dataset (*N* = 11440), 5.61% (95% confidence interval [95% CI]: 2.53-11.97) had a liver stiffness measurement (LSM) ≥ 8 kPa. The proportion attributable to NAFLD (using clinical variables and Controlled Attenuation Parameter) was 57.3% and thus, the estimated prevalence of population with LSM ≥ 8 kPa because of NAFLD was 3.21% (95% CI 1.13–8.75). In the biopsy-proven NASH cohort, 389 patients had LSM ≥ 8 kPa. Among these, 37% did not have significant fibrosis (F2-4). The estimated prevalence of NASH F2-3 and cirrhosis in Spain's adult population were 1.33% (95% CI 0.29–5.98) and 0.70% (95% CI 0.10–4.95) respectively.

Conclusions: These estimations provide an accurate picture of the current prevalence of NASH-related fibrosis in Spain and can serve as reference point for dimensioning the therapeutic efforts that will be required as NASH therapies become available.

KEYWORDS
hepatic fibrosis, liver biopsy, non-alcoholic steatohepatitis, transient elastography

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become a major public health problem. Both its prevalence and incidence have risen sharply in the last decades.[1–5] and, by 2030, cirrhosis and hepatocellular carcinoma because of NAFLD are expected to increase further worldwide.[6–8] This threat is aggravated by the fact that there are currently no approved pharmacologic therapies for non-alcoholic steatohepatitis (NASH). Accurate and geographically-specific estimations of NASH prevalence by fibrosis stage are paramount for the design and implementation of public health measures and therapeutic strategies.

Nonetheless, the exact prevalence of NAFLD and for the different fibrosis stages of NASH remains unknown to date because previous calculations are subject to various problems. First, reports on NAFLD prevalence base their predictions on non-invasive diagnostic tests,[9–13] registry diagnostic codes[14] or on indirect extrapolations from basic metabolic demographics.[15] All these methods have suboptimal sensitivity and probably underestimate the real prevalence of NAFLD.[2,16] In recent years, Controlled Attenuation Parameter (CAP) by transient elastography (TE) has been proven more sensitive for the detection of steatosis than ultrasound or serum-based scores[10,17–20] but reports on NAFLD at the general population level using CAP are still scarce and small-scale.[21] Second, estimations of NASH at population level have been extrapolated from either small autopsy or living-donor series[22] or through back-calculations crossing population-scale estimates for NAFLD with non-contemporary NASH biopsy series[23–25] subject to selection and ascertainment biases, leading to overrepresentation of more advanced stages of NASH. An intermediate approach consists of the use non-invasive tests for estimations of liver fibrosis (with transient elastography and/or serum scores) as proxy for NASH in different population-based studies.[26,27] However, stratification of patients by fibrosis stage with these methods has been proven suboptimal and histological confirmation of fibrosis predictions is scarce.[9,10] Finally, the attribution of NAFLD causality in those fibrosis-based population studies has been indirect (basically based on comorbidities, without concurrent measurement of hepatic steatosis).

Lay summary

Non-alcoholic steatohepatitis (NASH) has become a major public health issue worldwide, but the exact prevalence in the general population of the different stages of liver fibrosis associated to NASH is largely unknown. In the present study, we merged data from a large general population-based dataset and a contemporary multicentric biopsy-proven NASH cohort to provide updated prevalence estimates for NASH fibrosis in Spain. These estimates might be leveraged for designing future interventions for NASH.

The aim of the present study was to provide an updated, accurate, real-life estimate of the prevalence of NASH-related fibrosis in Spain. For that purpose, we merged data from a large study on the use of TE for screening of liver disease in the general population of Spain with contemporary data from a multi-centre cohort of biopsy-proven NASH from real practice from our country.

2 | METHODS

2.1 | Aim and study design

This was an observational, retrospective, cross-sectional, population-based, epidemiological study. The main objective of the study was to provide updated estimates for the prevalence of NASH-related

fibrosis in the general population in Spain, with a special focus on those stages at higher risk of complications and that would be eventually amenable to receive pharmacologic therapy under the current regulatory framework, that is F2-4 fibrosis stages.

The general plan for the study (Figure 1) consisted of four steps:

- STEP 1: To estimate the prevalence and distribution of liver fibrosis through clinically relevant TE ranges in Spain's general population from a large population-based cohort (ETHON).
- STEP 2: To estimate the prevalence of NAFLD within the subset of patients with liver stiffness measurements (LSM) ≥ 8 kPa in the same cohort. For this estimation, we used the subcohort from Cantabria, which was the largest subcohort ($N = 5090$) and contained 99.7% of all valid CAP measurements from the whole ETHON dataset. Patients with viral hepatitis (positive anti-HCV or HBsAg), high-risk alcohol consumption (≥ 15 units/week) assessed by AUDIT test and those with the absence of steatosis defined as CAP < 250 dB/m (except for patients with LSM ≥ 20 kPa in order to avoid "burn-out" NASH cirrhosis exclusion) were excluded. The proportion attributable to NAFLD was calculated as the ratio of the remaining patients after these exclusions over the total of patients with LSM ≥ 8 kPa (Figure 2).
- STEP 3: To describe the distribution of the different liver fibrosis stages in a Spanish multi-centre cohort of biopsy-proven NASH with paired TE (LSM and CAP) data, contemporary to the population-based cohort.
- STEP 4: To generate estimations of the real prevalence for the different NASH-related fibrosis stages for Spain's general population by crossing the estimated prevalence of NAFLD drawn from ETHON cohort with the probability of each specific stage of fibrosis observed in the biopsy-proven NASH cohort in patients with LSM ≥ 8 kPa.

As secondary outcome, using the same step by step approach detailed above, we aimed at describing prevalence estimates using the LSM 9.1 kPa cut-off, which has been recently suggested as the most cost-efficient LSM threshold for theoretical population-level screening.[28]

The paper has been performed and written following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[29]

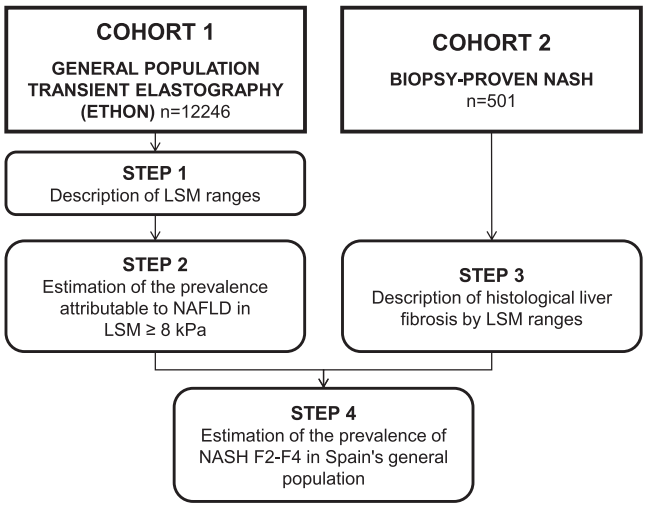

FIGURE 1 Study design and cohorts.

2.2 | Patients

The study integrates data from two large cohorts:

1. Population-based cohort (ETHON cohort). The PREVHEP-ETHON is a Spanish dataset composed of subjects aged 20–79 years selected from the general population of 18 primary care centres belonging to three university hospitals from 2015 to 2017. Participants were randomly selected and stratified by socioeconomical status, rural/urban setting and age. The geographical location of Santander, Madrid and Valencia (north, centre and east, respectively) determinates substantial differences in terms of weather, lifestyle and food habits, appropriately showing a real-life picture of Spain at population level. Thus, this cohort is considered representative of Spain's general population and has served as a reference for other observational, cross-sectional population-based studies,[30] even though the initial study was aimed to investigate the prevalence of HCV in the general population.[31]

This cohort was the basis for the estimation of TE values for the Spanish general population as well as for the estimation of the attributable causal weight of NAFLD within the range of LSM ≥8 kPa.

2. Biopsy-proven NASH cohort. This dataset was a cross-sectional, retrospective cohort of real clinical practice patients with biopsy-proven NASH from two hospitals from the ETHON cohort (Marqués de Valdecilla, Cantabria and Puerta de Hierro, Madrid) and three additional tertiary centres (Virgen del Rocío, Seville; Clínico Universitario, Valladolid; and Vall d'Hebron, Barcelona). These centres had been collecting data prospectively from NAFLD patients for several studies and for Spain's NAFLD National Registry (Hepamet).[32] Patient selection was based on what is estimated as current clinical practice in tertiary centres in Spain, where patients are referred to liver clinics based on a combination of altered liver function tests and/or finding of steatosis in liver ultrasound. Once in the clinics, patients with LSM < 8 kPa are considered low risk and are less likely to undergo liver biopsy, and in patients with LSM ≥ 8 kPa liver biopsy is individually decided for disease staging and for inclusion consideration in clinical trials. For the present study, we only included consecutive patients with paired data from liver biopsy and TE within a < 12-month period obtained with Fibroscan devices equipped with M

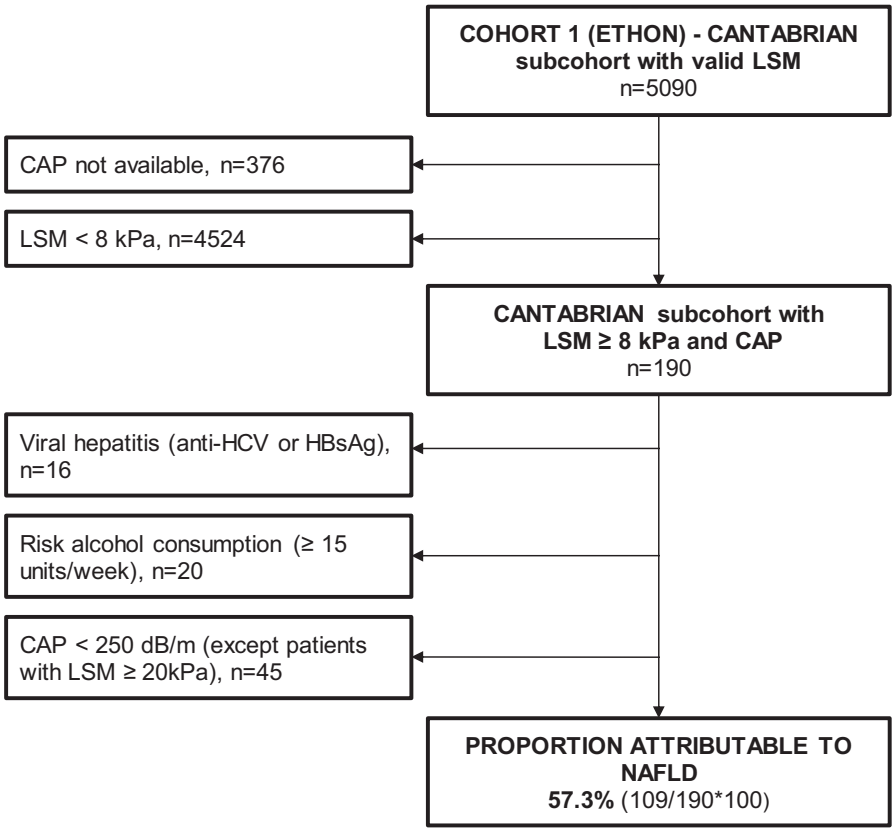

FIGURE 2 Flow chart for the calculation of the proportion attributable to non-alcoholic fatty liver disease (NAFLD) in the population-based cohort. CAP, controlled attenuation parameter; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; LSM, liver stiffness measurements; NAFLD, non-alcoholic fatty liver disease.

and XL probes, spanning from 2015 to 2020. Only liver samples clearly interpretable for the pathologists were included in the study.

The biopsy-proven cohort was used to translate TE estimations for the general Spanish population into histology-based estimations for NASH diagnosis and its different fibrosis stages.

2.3 | Procedures

TE measurements, liver biopsy and histological evaluation were performed according to current standards.[33,34] A detailed description of procedures is provided in Supplementary Material.

2.4 | Statistical analysis

For demographic extrapolations and comparisons with the Spanish general population, publicly available data from Instituto Nacional de Estadística (INE) were used. INE is a legally independent administrative institution which serves as main repository of demographic data for Spain, including key health indicators. Patients with non-valid LSM and those with LSM < 8 kPa from both cohorts will be excluded from final analyses under the assumption that these patients would not be referred to tertiary centres to undergo liver biopsy in real clinical practice.

Categorical data are presented as number (percentage). Continuous data are presented as mean ± standard deviation and median (interquartile range). A $p < .05$ was considered statistically significant. Missing values were kept as missing, and no specific statistical procedures were used for imputations. Data were collected and edited using Microsoft Excel (version Microsoft Office Pro 2019). Statistical analyses were performed using PAWS Statistics (version 19.0; SPSS Inc., Hong Kong) software.

3 | RESULTS

3.1 | Patients characteristics from the population-based transient elastography cohort (ETHON cohort)

From the complete ETHON dataset of 12 246 individuals, 806 (6.6%) were excluded because of non-valid or indeterminate LSM.

For the 11 440 patients included in the analysis (Table 1) median age was 51 years, 58% were women and 88% were Caucasian. Median BMI was 26.1 kg/m^2 and 60% were either overweight or

TABLE 1 Baseline characteristics of the patients from the general population (ETHON) cohort included in the analysis

General population cohort	*N* = 11 440
Age, years	**51 (42–60)**
Male, *n* (%)	4792 (41.9)
Geographic subcohort, *n* (%)	
Cantabria	5090 (44)
Madrid	4088 (36)
Valencia	2262 (20)
Caucasian, *n* (%)	10 058 (87.9)
Alcohol risk consumption, *n* (%)	442 (3.9)
Body mass index (BMI), kg/m^2	26.1 (23.3–29.3)
Weight, *n* (%)	
Normal weight (<25 kg/m^2)	3846 (40.4)
Overweight (≥25–<30 kg/m^2)	3597 (37.8)
Obesity (≥30 kg/m^2)	2075 (21.8)
Waist circumference, cm	90 (80–99)
Type 2 diabetes, *n* (%)	1540 (13.5)
Arterial hypertension, *n* (%)	5206 (53.6)
Dyslipidemia, *n* (%)	7418 (64.8)
Metabolic syndrome, *n* (%)	1764 (15.4)
Fasting glucose, (mg/dl)	86 (79–96)
Total cholesterol, (mg/dl)	197 (174–222)
HDL, (mg/dl)	57 (47–68)
LDL, (mg/dl)	113 (91–135)
Triglycerides, (mg/dl)	114 (78–172)
Creatinine, mg/dl	0.78 (0.67–0.91)
AST, (U/L)	22 (19–27)
ALT, (U/L)	20 (16–28)
ALP, (U/L)	68 (56–83)
GGT, (U/L)	20 (14–33)
Bilirubin, mg/dl	0.50 (0.40–0.65)
Albumin, g/dl	4.5 (4.3–4.6)
Platelets, x10E9/L	241 (205–282)
FIB–4 index	0.99 (0.72–1.36)
HBsAg positive, *n* (%)	90 (0.8)
Anti-HCV positive, *n* (%)	143 (1.3)
Liver stiffness, (kPa)	4.5 (3.6–5.6)
CAP, (dB/m)[a]	247 (209–293)

Note: Risk alcohol consumption: ≥15 units of alcohol/week. Hypertension: ≥ 140/90 mmHg or requiring treatment; type 2 diabetes: as a fasting plasma glucose ≥126 mg/dl or a non-fasting plasma glucose ≥180 mg/dl or requiring treatment.; dyslipidemia: serum triglycerides ≥150 mg/dl and/or total cholesterol >200 mg/dl, LDL >130 mg/dl, HDL < 40 mg/dl in men and < 50 mg/dl in women or requiring treatment.
[a]Data from Cantabrian subcohort, *N* = 4714.

obese. Diabetes prevalence was 13.5% and 5206 patients (53.6%) had arterial hypertension. Almost 16% of the population met NCEP-ATP III criteria for metabolic syndrome.[35]

TABLE 2 Prevalence of different ranges of LSM in the general population (ETHON) cohort

LSM ranges	Patients, *n* (%)		
< 8 kPa	10 797 (94.39)	LSM ≥ 8 kPa, *n* (%)	
8–10 kPa	344 (3.00)	643 (5.61)	LSM ≥ 10 kPa, *n* (%)
10–15 kPa	185 (1.62)		299 (2.60)
15–20 kPa	36 (0.31)		
≥ 20 kPa	78 (0.68)		
Total	11 440 (100)		

Abbreviation: LSM, liver stiffness measurements.

Subjects from Cantabria represented 44% of ETHON dataset. Within this subcohort, 23.4% were obese, 61.4% presented a BMI ≥ 25 kg/m^2 and 20% met metabolic syndrome criteria. Median LSM and CAP values from Cantabria were 4.4 kPa and 247 dB/m respectively. As shown, baseline features between the Cantabrian subcohort and the whole ETHON dataset were comparable.

Metabolic estimates from ETHON cohort were consistent with available data from Spain's general population, where 22.9%, 13.8% and 42.6% have been estimated to present obesity, diabetes and arterial hypertension respectively.[36–39]

3.2 | STEP 1: TE ranges

The first step consisted of the estimation of LSM ranges in the general population. Out of the 11 440 individuals with reliable TE from ETHON cohort, 5.61% (95%CI 2.53–11.97) had LSM ≥ 8 kPa and 2.60% (95%CI 0.83–7.88) presented LSM ≥ 10 kPa (Table 2). Within the LSM ≥ 8 kPa subgroup, 53% (344/643*100) of patients fell in the 8–10 kPa range.

3.3 | STEP 2: Estimation of the prevalence attributable to NAFLD within the LSM ≥8 kPa subcohort

The second step was to estimate the aetiologic relative weight of NAFLD within the LSM ≥ 8 kPa population. For this specific purpose, as mentioned above, we used the subcohort from Cantabria, which was the largest subcohort and contained 4714/4728 (99.7%) CAP measurements from the whole ETHON dataset.

As seen in Figure 2, the proportion attributable to NAFLD was 57.3% (109/190*100), calculated as the ratio of the remaining subjects after exclusion of patients with viral hepatitis, high-risk alcohol consumption and those with the absence of steatosis assessed by CAP, over the total of patients with LSM ≥ 8 kPa and available CAP. Thus, the prevalence of individuals with LSM ≥ 8 kPa because of NAFLD in the general population was estimated to be 3.21% (5.61*0.573).

3.4 | Patient characteristics from the biopsy-proven NASH cohort

The real clinical practice dataset was composed of 501 consecutive patients with histological confirmation of NASH and paired TE measurements from five tertiary hospitals in Spain. The main clinical features were usual in European NASH cohorts. Median age was 59 years and 57% were males. Median BMI was 32 kg/m^2, 66% of patients had obesity and 44% type 2 diabetes. Median time between TE and liver biopsy was 2.29 months (IQR 1.12–4.41) and only 12.5% (63/501) of patients presented a time interval > 6 months between procedures. A detailed description of the baseline characteristics from the biopsy-proven NASH cohort and the comparison between both study cohorts are provided in Table S1.

3.5 | STEP 3: Description of the distribution of histological NASH-related fibrosis according to different LSM ranges

Among the 501 patients of the whole biopsy-proven NASH cohort, 112 patients had LSM < 8 kPa. Among these patients, 92 (82%) were F0-1, 15 presented F2, only 5 were F3 and no patients had cirrhosis on histology.

The distribution of fibrosis by different LSM ranges for the remaining 389 patients are shown in Figure 3 and Table 3. Among patients with LSM ≥ 8 kPa, 37% (143/389) had F0-1 and, for those patients with LSM ≥ 10 kPa, as much as 44% (123/277) did not show advanced fibrosis (F3-4) at histology. As seen, the proportion of patients with advanced fibrosis increased at each LSM interval, but even at the highest interval (LSM ≥ 20 kPa), there was a substantial proportion of patients without cirrhosis (39%).

Regarding liver biopsy quality parameters, median tissue length among patients with LSM ≥ 8 kPa was 23.0 mm (IQR 19.0–28.0), and only 5.1% of samples were fragmented into more than two pieces. Of note, the subset of patients within the highest LSM interval (≥ 20 kPa) showed similar quality data (21.5 mm median size -IQR 17.0–27.0- and 4.5% fragmentation respectively).

3.6 | STEP 4: Prevalence estimation of NASH-related fibrosis stages for Spain's general population (2015–2020)

The main study outcome was calculated by merging the main results from the two cohorts. The prevalence for each NASH-related fibrosis stage in Spain's general population was estimated by crossing the

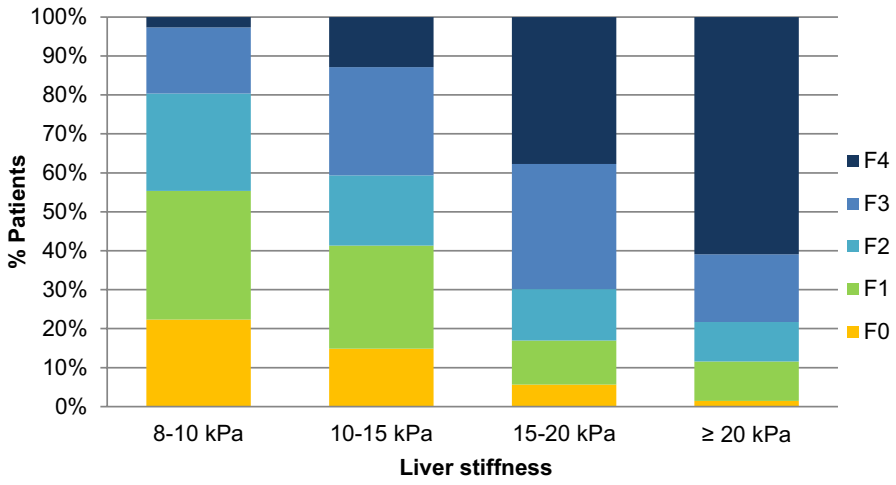

FIGURE 3 Distribution of liver fibrosis within increasing ranges of LSM in the biopsy-proven cohort of patients with non-alcoholic steatohepatitis (NASH)

	LSM ranges, *n* (%)					
Fibrosis	8–10 kPa	10–15 kPa	15–20 kPa	≥ 20 kPa	LSM ≥ 8 kPa	LSM ≥ 10 kPa
F0	25 (22.32)	23 (14.84)	3 (5.66)	1 (1.44)	52 (13.37)	27 (9.75)
F1	37 (33.04)	41 (26.45)	6 (11.32)	7 (10.15)	91 (23.39)	54 (19.49)
F2	28 (25.00)	28 (18.07)	7 (13.20)	7 (10.15)	70 (18.00)	42 (15.16)
F3	19 (16.96)	43 (27.74)	17 (32.08)	12 (17.39)	91 (23.39)	72 (26.00)
F4	3 (2.68)	20 (12.90)	20 (37.74)	42 (60.87)	85 (21.85)	82 (29.60)
Total	112 (100)	155 (100)	53 (100)	69 (100)	389 (100)	277 (100)

TABLE 3 Distribution of histological liver fibrosis according to LSM in the biopsy-proven NASH cohort

Abbreviation: LSM, liver stiffness measurements.

estimated prevalence of NAFLD with LSM ≥ 8 kPa (3.21%) drawn from ETHON dataset with the different fibrosis stages probabilities observed in the biopsy-proven NASH cohort (detailed in Table 3—LSM ≥ 8 kPa column). The final calculations from our study are shown in Table 4 (i.e., prevalence of F2 fibrosis stage resulted in 0.58% = 3.21*0.18). Finally, the estimated prevalence of NASH with significant fibrosis F2-4 in Spain was 2.03 (95% CI 0.56–7.05), 1.33 (95% CI 0.29–5.98) of the Spanish population aged 20–79 years presented NASH F2-3 and 0.70 (95% CI 0.10–4.95) was estimated to have NASH cirrhosis.

Additional estimations using the LSM ≥ 10 kPa threshold and alternative approximations to the proportion attributable to NAFLD using more or less conservative CAP thresholds (275 or 220 dB/m, respectively) are provided in Tables S2–S5.

3.7 | Prevalence estimates with LSM 9.1 kPa threshold

The LSM ≥ 9.1 kPa threshold has been suggested as the most cost-effective cut-off for a theoretical TE-based screening programme of liver fibrosis in populations with lower prevalence of risk alcohol consumption, such as Spain.[28] Applying the same methodology used for the 8 kPa threshold we found that in ETHON cohort, prevalence of LSM ≥ 9.1 kPa was 3.36%. In our biopsy-proven cohort, 66.5% (223/335) of patients had significant fibrosis (F2-4) and 49.8% (167/335) presented advanced fibrosis (F3-4). The proportion attributable to NAFLD resulted in 55.2% of cases of all Spain's population with LSM ≥ 9.1 kPa, and thus 1.85% of Spain's population would be assumed to have NAFLD, with 1.10% estimated to have F2-4.

Of note, raising the threshold from 8.0 to 9.1 kPa in a hypothetical screening plan would represent a 39.9% relative reduction in the proportion of patients that would be targeted. However, according to our biopsy-proven cohort, in the subcohort of 54 patients with LSM 8–9.1 kPa, 9 (16.6%) had advanced fibrosis. The advantages and risks of using the 9.1 kPa threshold should be weighted in dedicated cost-effective studies.

4 | DISCUSSION

In the present study, we provide an updated estimation of the prevalence of NASH-related fibrosis in Spain's general population, by combining TE data from a large population-based screening cohort, and histological information from a contemporary multi-centre cohort of biopsy-proven NASH from Spain. We observed that 5.61% of the general population had LSM ≥ 8 kPa, 57.3% of which (3.21%) was attributable to NAFLD. By crossing these estimates with the corresponding probabilities for the different fibrosis stages by LSM intervals drawn from the biopsy-proven cohort, we estimated that the current prevalence of NASH with significant fibrosis in Spain was 2.03% (1.33% NASH F2-3 and 0.70% NASH cirrhosis).

The estimates on NASH with significant fibrosis from our study are lower than those provided in previous epidemiological reports from other European cohorts,[28,40–42] even though the prevalence of LSM ≥ 8 kPa in the ETHON population is almost the same. The most likely reason for these differences stems from the fact that fibrosis estimations in those studies were fundamentally TE-based (backed up by the absence of liver biopsies or a relatively small number of them) and, in the present study, TE estimations were back-tested against a large contemporary histological dataset, which clearly suggests that current TE-based definitions are overestimating the prevalence of fibrosis in NASH. In fact, 37% of the patients in our study with LSM ≥ 8 kPa did not have "significant fibrosis," and 44% of patients with LSM ≥ 10 kPa did not have "advanced fibrosis" at histology. A recent paper,[28] integrating population-based TE data with histological data from 6 independent cohorts, with 6300 patients and 350 biopsies, showed an estimated prevalence of F2-4 in the general population (all aetiologies combined) of 3.9%. In our study, there were more than 12000 patients in the population-based cohort and more than 500 biopsies, which served at narrowing confidence intervals for the different degrees of fibrosis that could be expected at each TE interval, providing more precise references for the population-based estimates. As a practical consequence, we firmly believe that current TE-based definitions of "significant" and "advanced" fibrosis (LSM ≥ 8 kPa and 10 kPa, respectively) should be avoided, since they provide unrealistic overestimations of the actual prevalence of the corresponding fibrosis stages.

TABLE 4 Prevalence estimation of the different NASH fibrosis stages in Spain's general population

	Nash fibrosis prevalence (%)			
Fibrosis	LSM ≥ 8 kPa (95%CI)	Significant fibrosis (F2-F4)	Intermediate stages (F2-F3)	Cirrhosis (F4)
F0	0.43 (0.04–4.49)			
F1	0.75 (0.11–5.04)			
F2	0.58 (0.07–4.75)	2.03 (0.56–7.05)	1.33 (0.29–5.98)	
F3	0.75 (0.11–5.04)			
F4	0.70 (0.10–4.95)			0.70 (0.10–4.95)
Total	3.21 (1.13–8.75)			

Abbreviations: CI, confidence interval; LSM, liver stiffness measurements; NASH, non-alcoholic steatohepatitis.

The second reason for the different prevalence of NASH F2-4 is the more comprehensive attribution of aetiology made in our study, where we estimated that approximately 60% of patients with LSM ≥ 8 kPa from the general population had NAFLD. In the paper by Caballeria et al.,[40] attribution of NAFLD aetiology was made by exclusion of with viral hepatitis and patients with high-risk alcohol consumption, resulting in a remarkably high aetiologic weight of NAFLD (near 90%). On the other hand, in the other two large European studies,[41,42] NAFLD was suspected basically on clinical grounds, yielding a much lower estimated prevalence (32%–42% for the whole LSM spectrum). By contrast, to estimate the proportion attributable to NAFLD we added the use of CAP in nearly 5000 patients from a representative subcohort, enabling the extrapolation of the results to the whole dataset and, thus, to the general population. We believe that the combination of CAP along clinical and laboratory values in the present study likely provides the most accurate estimation for the attribution of aetiology in population-based studies to date.

The study has nonetheless some limitations that should be taken into account when interpreting the transferability of our results to real practice decisions. The main limitation is the risk of overestimation of the prevalence of F2-4 stages, as consequence of unavoidable referral and selection bias. It has been consistently shown that NASH prevalence is overestimated in tertiary settings.[2] Local initiatives for structured early detection and referral have been developed in recent years in the biopsy-proven cohort centres and may have helped at buffering that risk. However, the efficiency of systematic referral is still suboptimal, and thus mitigation of referral bias is probably modest. The risk of selection bias is also unavoidable in this kind of retrospective studies since decision for biopsy was not standardized and could have been influenced by other criteria different from LSM. However, it should be noted that current practice in all participating centres is quite homogenous and representative of what is done in daily clinical practice in Spain. In fact, the 2 main centres recruiting for the ETHON cohort were also part of the contemporary biopsy-proven cohort (Cantabria, Madrid). In any case, the ideal approach to minimize these biases would be the implementation of prospective large-scale screening programmes, with per-protocol liver biopsies above a pre-specified LSM threshold. Such a study is already ongoing (LiverScreen, H2020-EU ID 847989), but results of such large efforts will take long. Thus, until this sort of large-scale prospective data becomes available, the estimations provided in the present study remain as the most accurate approximations to the prevalence of NASH with significant fibrosis in a European population.

Other limitations should be considered. Biopsy reading was not centralized and could be subject of interobserver variability, although we believe this is a reflection of what should be expected in real-life situations. We acknowledge that the attribution of the relative weight of NAFLD as aetiology was imperfect. The choice of the CAP threshold was arbitrary, since there is no clear consensus in the literature and there are no clear published reports on the specificity and positive predictive values of CAP estimates for patients with higher LSM.[10] It can be also argued about the positive predictive value of 250 dB/m CAP threshold to identify steatosis at general population level. This issue can be partially mitigated in two ways. First, by adjusting CAP values according to influencing covariables, such as type-2 diabetes, BMI and liver aetiology.[18] Second, as provided in the present study, by generating alternative estimations using different CAP cutoffs with greater or lesser sensitivity (including the 275 dB/m threshold suggested in the latest clinical practice guideline).[17] Moreover, the estimation on the relative weight of NAFLD would require adjustment by the alcoholic liver disease burden in each country. There will be also patients with other liver aetiologies, although their reported frequencies in primary care series are very low (<2%) and should not distort significantly large-scale epidemiological estimations.[43] Finally, the ethnic background in our study is predominantly Caucasian, so extrapolation to populations with more diverse ethnicities should be done with caution.

In conclusion, our large-scale epidemiological study, merging population-based TE data with histological data from real-world practices, provides accurate and updated estimates of the current prevalence of NASH-related fibrosis in Spain, with a special focus on those stages that should be the target of early detection and referral policies to identify high-risk patients who will benefit more from the pharmacological therapies under development.

Until large-scale prospective epidemiological data with biopsy confirmation becomes available, our results could serve as reference points for both assessing the current burden of disease and for modelling changes in time, so health policies can be designed and adapted accordingly, and besides for dimensioning the therapeutic efforts that will be required as the time when we have effective treatments to treat NASH approaches.

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CONFLICT OF INTEREST

JLC received consulting and lecture fees for Intercept, Echosens and Gilead Sciences. JMP received consulting, educational or research grants for Gilead, NovoNordisk, Novartis, Boehringer-Ingelheim, BMS, Pfizer, Accelerate, Astellas, ViiV, Janssen, MSD, Abbie. MRG received consulting fees from Alpha-sigma, Allergan, BMS, Boehringer-Ingelheim, Gilead, Intercept, Kaleido, MSD,

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ETHICS STATEMENT

The study protocol was approved by Vall d'Hebron Ethics Committee for Clinical Research (PR[AG]655/2020) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

PATIENT CONSENT FOR PUBLICATION

Not required.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

The material in this paper is original and does not come from other sources.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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10.1.2. Estudio 2

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Non-Invasive Tests of Liver Fibrosis Help in Predicting the Development of Hepatocellular Carcinoma among Patients with NAFLD

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Abstract: Background: The potential role of non-invasive tests (NITs) for liver fibrosis for hepatocellular carcinoma (HCC) prediction remains poorly known. Methods: Retrospective analysis of a NAFLD cohort from a single university hospital in Barcelona, Spain. Incidence rates and cumulative incidence for the overall cohort, as well as cirrhotic and non-cirrhotic patients were calculated. Logistic regression analyses were carried out to investigate risk factors of HCC. Results: From the entire cohort of 1040 patients, 996 patients (95.8%) were analyzed, in whom 35 cases of HCC were detected, of which 26 (72.4%) HCC incident cases were newly diagnosed during a median follow-up of 2.5 (1.9–3.6) years. Two-hundred and thirty-one (23.2%) were cirrhotic at baseline. With the exception of 2 (7.7%) cases of HCC, the rest were diagnosed in cirrhotic patients. Overall HCC cumulative incidence was 9.49 (95% CI 6.4–13.9) per 1000 person-years. The incidence rate for cirrhotic patients was 41.2 (95% CI 27.6–61.6) per 1000 person-years and 0.93 (95% CI 0.23–3.7) per 1000 person-years for patients without cirrhosis. Overall mortality was significantly higher amongst patients with HCC (4.4% vs. 30.8%, $p < 0.001$). In patients with available liver biopsy ($n = 249$, 25%), advanced fibrosis (F3–F4) was significantly associated with higher HCC incidence, but not steatosis, lobular inflammation, nor ballooning. In the overall cohort, FIB-4 ≥1.3 (HR 8.46, 95% CI 1.06–67.4, $p = 0.044$) and older age (HR 1.06, 95% CI 1.01–1.11, $p = 0.025$) were associated with increasing risk of HCC over time, whereas in cirrhotic patients predictors of HCC included decreasing values of albumin (HR 0.34, 95% CI 0.13–0.87, $p = 0.024$), platelets (HR 0.98, 95% CI 0.98–0.99, $p = 0.001$), and increasing values of liver stiffness (HR 1.03, 95% CI 1.00–1.06, $p = 0.016$). Conclusions: In a Spanish cohort of NAFLD patients, HCC was rare in non-cirrhotic patients. NITs might play a relevant role at predicting HCC.

Keywords: NAFLD; hepatocellular carcinoma; FIB-4; transient elastography

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1. Introduction

Liver cancer is considered the sixth-most common cancer and the second-most common cause of cancer-related death worldwide [1]. Hepatocellular carcinoma (HCC) represents 75–85% of all liver cancer and is associated with poor prognosis and higher need for liver transplant [1]. Importantly, non-alcoholic fatty liver disease (NAFLD) is nowadays the fastest increasing underlying cause of HCC globally [2–4].

Contrary to other causes of liver disease, there are increasing data suggesting that HCC may develop in the absence of cirrhosis in NAFLD patients [2,5]. Most data come from United States and United Kingdom reports [6,7]. However, these results have not been consistently reported across different geographical settings and, in particular, information in Southern European countries is limited. The increased risk of HCC in non-cirrhotic NAFLD patients implies a challenge for clinicians due to the absence of clear recommendations regardingt surveillance strategies in this scenario [2]. Sanyal et al., recently showed that patients with both fibrosis stages 3 and 4—due to NASH—had an increased risk of HCC development over time compared to those without advanced fibrosis [8].

In clinical practice, NAFLD patients are evaluated thorough non-invasive tests (NITs), such as serologic scores (i.e., FIB-4 index) or vibration-controlled transient elastography (VCTE) for disease staging and risk assessment, with liver biopsy usually being restricted for high-risk patients and consideration for inclusion in clinical trials at liver clinics. Meanwhile, whereas some studies suggest that liver stiffness measurements (LSM) by VCTE may accurately predict the development of HCC in patients with advanced liver disease [9–11], it remains unclear whether NITs can be used for HCC development prediction in NAFLD, particularly in the early assessment of patients with undiagnosed advanced liver disease [12]. Interestingly, recent studies found a significantly higher HCC cumulative incidence in NAFLD patients with a FIB-4 index $\geq$1.3 [13–15]. Yet, to date, the diagnosis of HCC risk in patients with chronic liver disease by NITs with LSM and FIB-4 index remains problematic due to its low area under the curve, which is not sufficient to enclose patients with high risk of HCC in actual clinical practice [16].

We aimed to analyze the incidence of HCC in an unicentric Spanish cohort of patients with NAFLD and investigate whether NITs are good predictors of HCC development in cirrhotic and non-cirrhotic patients.

2. Materials and Methods

2.1. Design and Setting

Single center, retrospective analysis of a prospectively collected cohort. Vall d'Hebron University Hospital is a 1200-bed hospital providing care to a reference population of approximately 650,000 people. From January 2016 onwards, a monographic NASH clinic was created and a prospective collection of data with patients diagnosed with NAFLD was initiated. Data were collected until 31 October 2021.

2.2. Patients

Diagnosis of NAFLD: either more than 5% of hepatocytes with steatosis in liver biopsy or controlled attenuation parameter (CAP) $\geq$ 275 dB/m by VCTE; diagnosis of HCC was based on imaging techniques (i.e., contrast-enhanced imaging such as multiphase computed tomography or magnetic resonance imaging showing hypervascularity in the arterial phase and a decreased signal compared with the rest of the liver in the venous and/or delayed phases of the study) plus pathology in non-cirrhotic patients or in patients in whom imaging findings were not conclusive [2]; diagnosis of cirrhosis was based in either histology (fibrosis 4 stage in liver biopsy), imaging volumetric alterations, or direct/indirect signs of portal hypertension. Metabolic syndrome was defined according to NCEP-ATPIII criteria [17].

Exclusion criteria: patients with other causes of liver disease and those with engaged in risk-inducing alcohol consumption (20 g daily for females and 30 g daily for males), patients with prior diagnosis of HCC before the inclusion in the prospective cohort, cirrhotic subjects who did not attend a follow-up, or those with a follow-up time <6 months.

2.3. Collected Variables

All patients: sex, age, alcohol consumption, smoking, metabolic risk factors (arterial hypertension, type 2 diabetes mellitus, dyslipidemia, overweight/obesity), blood tests (lipid profile, hemoglobin, INR, platelets, transaminases, bilirubin, albumin, glycated

hemoglobin, insulin, C peptide), NITs (steatosis, cirrhosis, and portal hypertension signs in liver ultrasound, LSM and CAP in VCTE, FIB-4 index), diagnosis of cirrhosis, time of follow-up, death, and cause of death.

Cirrhotic patients: hepatic venous portal gradient (HVPG), esophageal varices in upper endoscopy, Child–Pugh score, MELD score, hepatic decompensations (upper digestive bleeding, hepatic encephalopathy, ascites), liver transplant. Cirrhotic patients were systematically followed every 6 months with abdominal US and blood tests.

HCC-related: number of lesions, size, modified Barcelona Clinic Liver Cancer (BCLC) staging system score [18] at diagnosis, and type of treatment.

Liver-biopsy-related variables: where dichotomized (yes/no) in patients with available liver biopsy in order to calculate HCC incidence according to histologic findings as follows: steatosis (fat in $\geq$ 5%hepatocytes), lobular inflammation ($\geq$2 foci/200×), hepatocyte ballooning (grade $\geq$ 2), advanced fibrosis (F3–F4).

2.4. Outcomes

Primary: cumulative incidence of HCC. Only HCC diagnosed from the prospective collection of data in January 2016 were included in the analysis.

Secondary: mortality and hepatic decompensations.

2.5. Statistical Analysis

Continuous variables are expressed in mean (SD) or median (IQR) according to data distribution. Categorical variables are expressed in total number and percentages. Differences between baseline characteristics of HCC and non-HCC patients were tested using the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Comparisons between patients with and without HCC and with and without cirrhosis were performed through Chi^2 test or Kruskal–Wallis test when appropriate. Since not all patients in the cohort had liver biopsy results available, to calculate the area under the receiver operating characteristics curve (AUROC) of NITs (LSM and FIB-4) to predict HCC, we used the LSM cutoff values suggested by Eddowes et al., to classify the estimation of the fibrosis grade from the LSM results [19]. The cutoff values were defined as $\leq$8.1 kPa for F0–F1 (no or mild fibrosis), $\geq$8.2 kPa for F $\geq$ 2 (moderate fibrosis), $\geq$9.7 kPa for F $\geq$ 3 (severe fibrosis), and $\geq$13.6 kPa for F4 (cirrhosis). Kaplan–Meier survival curves analyzing the occurrence of HCC according to the presence of cirrhosis, FIB-4 adjusted to age, LSM, and fibrosis in liver biopsy were built, with categories compared through chi-square log-rank test. For the analysis of risk factors of HCC development, Cox regression analysis that included variables with $p < 0.10$ in the univariate analysis were used. A two-sided $p < 0.05$ was considered to be statistically significant. Missing values were kept as missing, and no specific statistical procedures were used for imputations. The statistical analysis was performed using SPSS for Windows, Version 21.0 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Sample

After excluding patients lacking enough clinical information or follow-up, and those still alive that had already been diagnosed with HCC before the beginning of the study period (n = 9), 996 patients were included in the analysis, of whom 26 (2.6%) were diagnosed with HCC during follow-up (Figure 1). Median follow-up time was 2.5 (1.9–3.6) years.

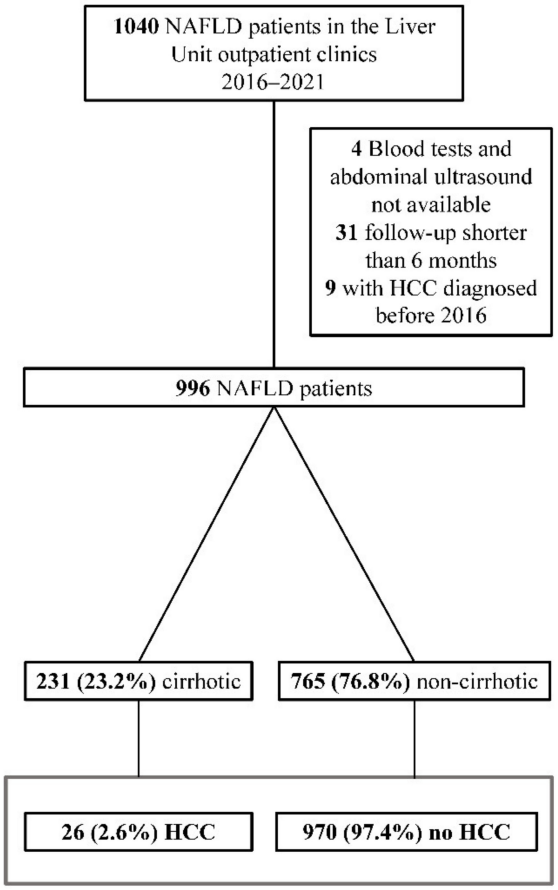

Figure 1. Flowchart of patients' disposition. HCC: hepatocellular carcinoma.

3.2. Baseline and Clinical Characteristics

The overall features of the NAFLD cohort, alongside the characteristics of patients with and without a new diagnosis of HCC during the study period are shown in Table 1. Female sex was present in 52.1% of patients without HCC and only in 13.2% of patients in the HCC group. Patients without HCC were significantly younger. Metabolic syndrome was present in 19.3% (n = 193) of all patients, and while there were no differences between the groups without and with HCC regarding mean BMI and rates of arterial hypertension, dyslipidemia was significantly more frequent in the former, whereas T2DM was significantly more frequent in the latter. Two-hundred forty-nine patients (25%) had available liver biopsy (24.5% of the no-HCC patients and 42.3% of HCC patients). Advanced fibrosis (F3–F4) was found in 53.7% of non-HCC and 90.9% of HCC patients.

Table 1. Characteristics and outcomes according to the diagnosis of hepatocellular carcinoma (entire cohort).

	No HCC n **= 970**	**HCC** n **= 26**	p
Female, n (%)	505 (52.1)	2 (7.6)	**<0.001**
Age, median (IQR)	60 (51–68)	69 (60–72)	**0.001**
Arterial hypertension, n (%)	530 (54.6)	10 (38.5)	0.102
Dyslipidemia, n (%)	595 (61.3)	8 (30.8)	**0.002**
Type 2 diabetes mellitus, n (%)	396 (40.8)	19 (73.1)	**0.001**
Body mass index, median kg/m^2 (IQR)	30.9 (27.7–34.7)	29.8 (27.5–35)	0.4

Table 1. *Cont.*

	No HCC ***n*** **= 970**	**HCC** ***n*** **= 26**	***p***
Metabolic syndrome, *n* (%)	187 (19.3)	6 (23)	0.6
Hb1Ac, median g/L (IQR)	5.8 (5.5–6.7)	5.8 (5–6.5)	0.2
Platelets, median $\times 10^9$/L (IQR)	228 (176–278)	102 (72–139)	**<0.001**
Bilirubin, median mg/dL (IQR)	0.61 (0.49–0.85)	1.1 (0.86–1.83)	**<0.001**
AST, median U/L (IQR)	32 (24–45)	48 (33–57)	**0.002**
ALT, median U/L (IQR)	36 (24–54)	40 (28–45)	0.9
Alkaline phosphatase, median U/L (IQR)	90 (72–115)	125 (96–189)	**<0.001**
GGT, median U/L (IQR)	63 (36–124)	187 (91–323)	**<0.001**
Albumin, median g/L (IQR)	4.3 (4.1–4.5)	3.8 (3.3–4.2)	**<0.001**
Total cholesterol, median mg/dL (IQR)	197 (173–228)	175 (145–200)	**<0.001**
c-HDL median mg/dL (IQR)	48 (42–58)	45 (41–59)	0.7
c-LDL median mg/dL (IQR)	117 (95–142)	100 (85–130)	0.09
Triglycerides median mg/dL (IQR)	137 (100–191)	91 (74–117)	**<0.001**
C Peptide, median U/L (IQR)	2.45 (1.81–3.22)	2.74 (2.23–2.83)	0.6
FIB-4, median (IQR)	1.3 (0.9–2.1)	5.5 (2.6–7.5)	**<0.001**
Liver stiffness, median kPa (IQR)	7.8 (5.4–12.6)	32 (17.8–58.2)	**0.001**
Control attenuation parameter, median dB/m (IQR)	319 (273–359)	297 (250–350)	0.3
Steatosis in abdominal US, *n* (%)	778 (82.8)	8 (30.8)	**<0.001**
Fibrosis in patients with available liver biopsy, *n* (%) F0 F1 F2 F3 F4	*n* = 238 29 (12.1) 47 (19.8) 34 (14.3) 43 (18.1) 85 (35.7)	*n* = 11 1 (9.1) 0 0 0 10 (90.9)	**0.009**
Death, *n* (%)	43 (4.4)	8 (30.8)	**<0.001**
Causes of death Liver Failure, *n* (%) HCC, *n* (%) Cardiovascular, *n* (%) Other neoplasms, *n* (%) Infectious, *n* (%) Other causes, *n* (%)	 12 (28%) 0 6 (14%) 8 (18%) 12 (27.9%) 5 (11.6)	 1 (12%) 2 (25%) 0 4 (50%) 1 (12.5%) 0	**0.005**
Follow-up, median years (IQR)	2.5 (1.9–3.6)	1.4 (0.85–2.4)	**<0.001**

HCC: hepatocellular carcinoma; IQR: interquartile range; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; Hb1Ac: glycosylated hemoglobin; HVPG: hepatic venous pressure gradient; US: ultrasound.

Out of the 996 subjects, 231 (23.2%) had cirrhosis at baseline. The characteristics of patients with cirrhosis according to the diagnosis of HCC are shown in Table 2. In the non-HCC group, 207 (21.3%) of patients had a diagnosis of cirrhosis at baseline, whereas 24 (92.3%) did in the HCC group. This was accompanied by significantly lower mean counts of platelets and albumin, and higher levels of bilirubin, as well as FIB-4 index and LSM in the latter group. Moreover, 90.9% and 62.5% of cirrhotic patients were Child–Pugh class A in the non-HCC group and the HCC group, respectively, whereas there were no significant differences between both groups regarding portal hypertension hallmarks.

Table 2. Characteristics and outcomes of cirrhotic patients according to the diagnosis of hepatocellular carcinoma.

	No HCC ***n* = 207**	**HCC** ***n* = 24**	***p***
% over total cohort	21.3	92.3	**<0.001**
Female, *n* (%)	98 (47.3)	2 (8.3)	**<0.001**
Median age, years (IQR)	65 (58–73)	69 (60–71)	0.3
Arterial hypertension, *n* (%)	145 (70)	10 (41.6)	**0.01**
Dyslipidemia, *n* (%)	122 (58.9)	8 (33.3)	**0.02**
T2DM, *n* (%)	148 (71.5)	17 (70.8)	0.9
HbA1c, %	6.2 (5.5–7.3)	5.8 (5–6.5)	0.07
BMI, kg/m^2	31.9 (28.5–35.2)	30 (27.9–35.1)	0.2
Metabolic syndrome, *n* (%)	70 (33.8)	6 (25)	0.3
Platelets, $\times 10^9$/L, median (IQR)	139 (94–208)	99.5 (71.5–126)	**0.01**
Bilirubin, median mg/dL (IQR)	0.8 (0.6–1.1)	1.2 (0.8–1.9)	**<0.001**
AST, median IU/L (IQR)	43 (30–57)	51 (33.5–58.5)	0.3
ALT, median IU/L (IQR)	36 (22–58)	40 (28.5–45)	0.7
Alkaline phosphatase, median IU/L (IQR)	103 (76–137)	129 (98–191)	**0.02**
GGT, median UI/L (IQR)	102 (55–223)	171 (87–326)	**0.01**
Albumin, median UI/L (IQR)	4.1 (3.8–4.4)	3.7 (3.2–4.1)	**0.001**
FIB-4, median (IQR)	3.3 (1.9–5.2)	6.09 (3.1–7.8)	0.054
Steatosis in US, *n* (%)	116 (56.5)	7 (29.1)	**0.01**
CAP, median dB/m (IQR)	310.5 (262–360)	308 (242.5–363.5)	0.8
Liver stiffness, median kPa (IQR)	27.3 (17.9)	38.3 (22.3)	**0.031**
Liver stiffness > 15 kPa, *n* (%)	137 (73.6%)	11 (84.6%)	0.3
Median HVPG, mmHg (IQR) *	10.5 (6.5–13)	8 (8–8)	0.5
Portal hypertension signs in abdominal US, *n* (%)	115 (56.1)	18 (78.2)	**0.04**
Varices, *n* (%) **	75 (45.1)	14 (60.8)	0.18
Child score, *n* (%) A B C	 157 (90.8) 15 (8.6) 1 (0.5)	 15 (62.5) 8 (33.3) 1 (4.1)	**0.001**
Child–Pugh score ≥6, *n* (%)	37 (17.8)	13 (54.1)	**<0.001**
MELD	7.7 (6.7–9.4)	9.4 (7.5–12)	**0.004**
Hepatic decompensation during follow-up, *n* (%)	59 (29.6)	15 (71.4)	**<0.001**
Ascites, *n* (%)	48 (31.1)	9 (37.5)	0.5
Hepatic encephalopathy, *n* (%)	26 (16.8)	10 (41.6)	**0.005**
Upper digestive bleeding, *n* (%)	22 (14.2)	5 (20.8)	0.4
Liver transplant, *n* (%)	1 (0.4)	9 (37.5)	**<0.001**
Death	37 (17.8)	8 (33.3)	0.09
Median follow-up, years (IQR)	2.2 (1.8–3.5)	1.5 (0.8–2.7)	**0.02**

* HVPG available in 41 patients; ** Gastroscopy available in 186 patients. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; Hb1Ac: glycosylated hemoglobin; HVPG: hepatic venous pressure gradient; US: ultrasound.

3.3. Incidence and Characteristics of HCC

Overall HCC incidence rate was 9.49 (95% CI 6.4–13.9) per 1000 person-years. When stratifying patients by cirrhosis status, the incidence rate for non-cirrhotic patients was 0.93 (95% CI 0.23–3.7) per 1000 person-years and 41.2 (95% CI 27.6–61.6) per 1000 person-years for patients with cirrhosis. As shown in Figure 2, the cumulative incidence of HCC was significantly higher in patients with cirrhosis, LSM (>8 kPa), and FIB-4 ($\geq$1.3 in patients < 65 years and $\geq$2 in patients > 65 years).

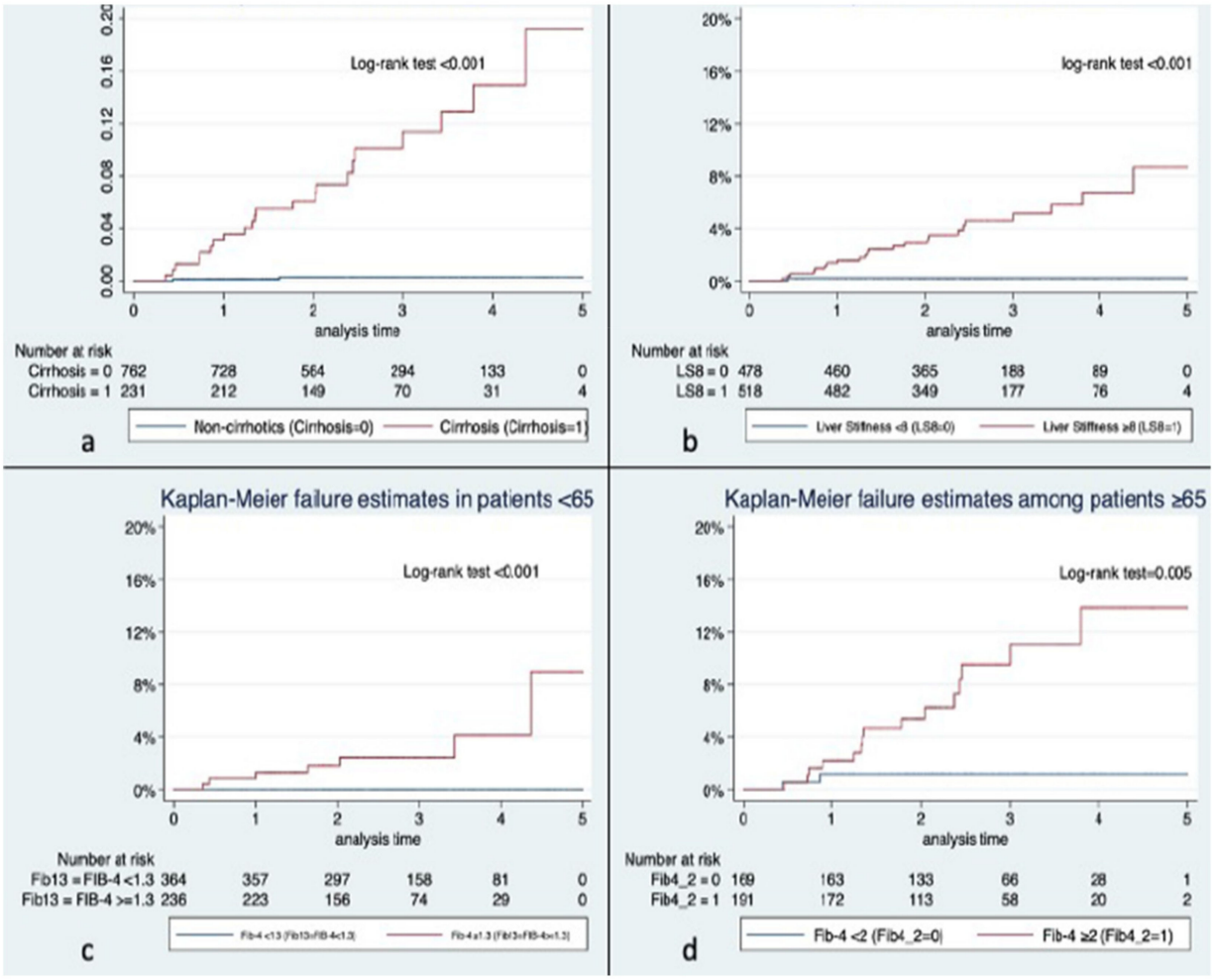

Figure 2. Kaplan–Meier survival curves for hepatocellular carcinoma incidence according to the presence of cirrhosis (**a**), liver stiffness measurement (**b**), and FIB-4 index adjusted to age (**c**,**d**).

Amongst the 249 patients with available liver biopsy, the incidence of HCC was not significantly different between patients with and without steatosis (6/207 (2.9%) vs. 4/40 (10%); $p = 0.06$). Similarly, there were no significant differences in the incidence of HCC when comparing patients with and without significant lobular inflammation (3/51 (5.8%) vs. 6/192 (3.1%); $p = 0.4$). Furthermore, the incidence of HCC was not significantly different between patients with and without prominent ballooning (1/90 (1.1%) vs. 8/153 (5.2%); $p = 0.1$). In contrast, patients with biopsy-proven grade 3–4 fibrosis had a significantly higher incidence of HCC when compared to patients with lower grades of fibrosis (10/138 (7.25%) vs. 1/111 (0.9%); $p = 0.02$). Figure 3 shows the Kaplan–Meier curve (log-rank $p = 0.02$).

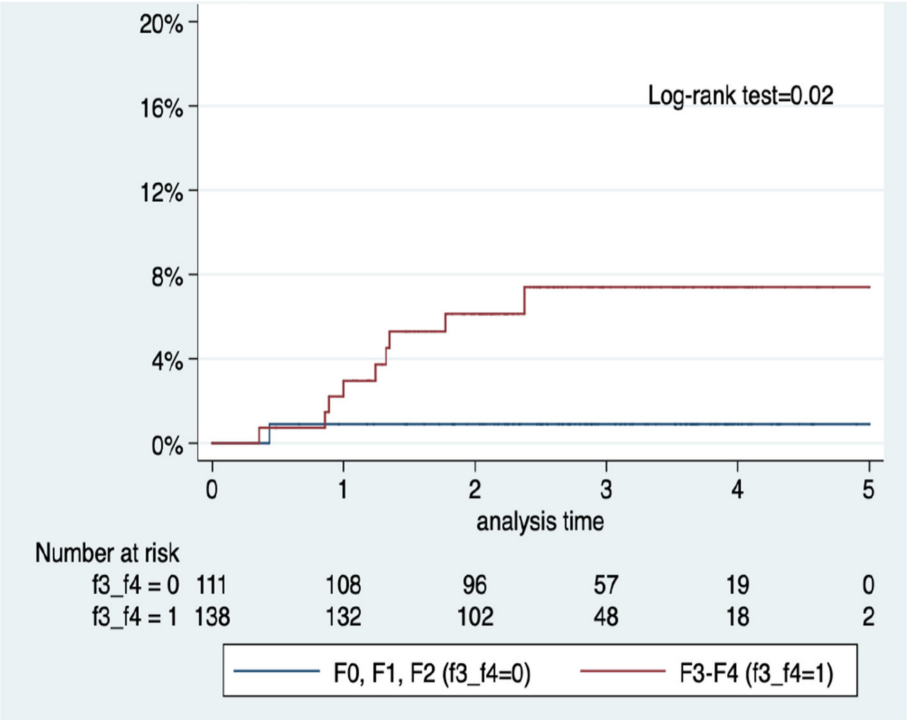

Figure 3. Kaplan–Meier survival curves for hepatocellular carcinoma incidence according to the presence of advanced fibrosis (F3–F4) in liver biopsy (n = 249).

Most patients (80.8%, n = 21) had a BCLC stage 0 or A at diagnosis and first-line therapy was aimed to be curative in 80.8% (n = 21). The median size was 30 (16–35) mm and the majority of patients had a single lesion (n = 19). Surgical treatment was applied in 23.1% (n = 6), radiofrequency ablation and trans-arterial chemoembolization (TACE) were performed in 10 (38.5%) and 7 (26.9%), respectively. Systemic therapy was used in two patients (Table 3).

Table 3. Characteristics of hepatocellular carcinoma cases included in the analysis.

Total Number of HCC	**26 (100)**
HCC BCLC stage, *n* (%)	0: 6 (23) A: 15 (57.6) B: 2 (7.6) C: 1 (3.8) D: 1 (3.8) Not classified 1 (3.8)
HCC size, median (IQR)	30 (16–35)
HCC type of treatment, *n* (%)	Curative: 21 (80.7) Surgical 6 (23) TACE: 7 (27) Radiofrequency Ablation: 10 (38.4) Systemic 2 (7.6)
HCC relapses, *n* (%)	8 (30.7)

3.4. Liver Events and Death

In the non-HCC group, 29.6% of patients with cirrhosis presented hepatic decompensation during follow-up, whereas 71.4% of cirrhotic patients in the HCC group decompensated ($p < 0.001$). The most frequent decompensation in the non-HCC group was ascites (31.1%). Meanwhile, hepatic encephalopathy was the most frequent type of liver event in

HCC patients, occurring in 41.6% of them. Liver transplant was significantly more frequent in HCC patients (0.4% vs. 37.5%, Table 2).

Overall mortality during follow-up was 5.1% (51 deaths), with liver failure (n = 13) and infections (n = 13) being the most common causes, while HCC was the direct cause of death in 2 (4%) patients. Mortality was significantly higher amongst HCC patients in the overall cohort (4.4% vs. 30.8%; $p < 0.001$, Table 1); however, this was not the case amongst cirrhotic patients (17.8% vs. 33.3%; $p = 0.09$, Table 2).

3.5. Predictors of HCC

In the overall cohort, multivariable analyses yielded two robust models when introducing NITs (FIB-4 index and LSM) (Table 4). In Model 1, older age and FIB-4 index ≥1.3 were associated with an increased risk adjusted odds of HCC, whereas T2DM and BMI, although entering the model, did not reach statistical significance. Model 2 included the following predictors: platelet count and albumin (the higher the value, the lower the risk), and liver stiffness (increasing values associated with increased risk of HCC over time).

Table 4. Summary of the multivariable analyses of factors associated with the incidence of hepatocellular carcinoma in NAFLD patients.

	HR	95% Confidence Interval		p
Model 1 (overall cohort)				
Type 2 diabetes mellitus	1.51	0.58	3.89	0.394
Body mass index (×1 kg/m^2)	0.99	0.91	1.09	0.930
Age (×1 year)	**1.06**	**1.01**	**1.11**	**0.025**
FIB-4 ≥ 1.3	**8.46**	**1.06**	**67.37**	**0.044**
Model 2 (cirrhotics)				
Platelets (x + 10 × 10^9/L)	**0.98**	**0.98**	**0.99**	**0.001**
Albumin (x + 1 IU/L)	**0.34**	**0.13**	**0.87**	**0.024**
Liver stiffness (x + 1 kPa)	**1.03**	**1.00**	**1.06**	**0.016**

The AUROC (95% CI) of FIB-4 and LSM for HCC diagnosis were 0.87 (0.78–0.96) and 0.85 (0.77–0.93), respectively. Regarding fibrosis stage, the AUROC (95% CI) of F0–F1, F2, F3, and F4 were 0.26 (0.22–0.30), 0.47 (0.43–0.51), 0.45 (0.41–0.48), and 0.80 (0.74–0.87), respectively. In patients ≥65 years, a 2.0 FIB-4 threshold had an AUROC of 0.65 (0.56–0.75). In younger patients (<65 years), a 1.3 FIB-4 threshold had an AUROC of 0.80 (0.78–0.82).

4. Discussion

4.1. Major Findings

In the present study, conducted in a large cohort of NAFLD patients followed for a median 2.5 years in a Southern European center, there are four major findings. First, the vast majority of HCC developed in patients with cirrhosis at baseline, and therefore the incidence of HCC in non-cirrhotic NAFLD was very low in our cohort. Second, HCC diagnosis is associated to high rates of overall mortality and hepatic decompensation in cirrhotic patients. Third, classical predictors of HCC such as advanced liver fibrosis, and platelets count and albumin in cirrhotic patients were found. Lastly, NITs as FIB-4 and LSM were found to have predictive ability for HCC.

4.2. Incidence of HCC

Although the total number of HCC diagnoses in our cohort is relatively small, incidence rates are not lower but rather higher than those of large and well-studied NAFLD cohorts. For instance, in their recent study, Sanyal et al., diagnosed nine cases of HCC amongst 1761 patients with biopsy-confirmed NAFLD, only one of them among F4 pa-

tients [8]. Cumulative probability of developing HCC was below 0.5/100 patients-year for F0–2, around 2/100 patients-year for F4, and close to 4/100 patients-year for F3 [8], the latter of which is similar to the overall cumulative incidence in our cohort.

Interestingly, we found that both F3 and F4 (advanced liver fibrosis) were associated with a significantly higher likelihood of developing HCC amongst patients with available liver biopsy, but not amongst the whole cohort. Oppositely to Sanyal et al.'s study and other American and English studies [1–8], the incidence of HCC in non-cirrhotic patients was very low in our cohort, only 7.7% (2/26) of total HCC new diagnoses. In a recent systematic review with metanalysis that analyzed 30 studies encompassing 13,371 subjects with NAFLD-associated HCC, Castellana et al., found that 37% (95% CI 28% to 46%) of patients developed HCC without cirrhosis [7]. In European studies, results were similar, with 36% (95% CI 13% to 58%) of HCC occurring in non-cirrhotic patients [7]. However, it is worth noting that data coming from Southern European countries are scarce. In a multicenter study encompassing Northern Italian hospitals, Piscaglia et al., analyzed 145 patients with NAFLD-associated HCC, and only 50% had cirrhosis [20]. Yet, average alcohol intake in Spain has been higher overall than in Italy and Anglo-Saxon countries during the last three decades [21], and although NAFLD patients in our study do not have either current or recent harmful alcohol consumption, prior exposure to alcohol might act as a contributing factor in carcinogenesis and fibrogenesis in our area, therefore reducing the proportion of non-cirrhotic NAFLD patients with HCC. Additionally, further studies assessing the impact of ethnicity, prevalence of PNPLA3 and TM6SF2 polymorphisms, as well as major epigenetic factors—such as Mediterranean diet and physical exercise—are needed to understand such wide variability.

4.3. Decompensations, Liver Transplant, and Mortality

All-cause mortality was 5.1% in our study. In the Sanyal et al., study, with 10-year follow-up, all-cause mortality was 2.7% [8]. In our cohort, 23.2% of patients were cirrhotic at baseline, whereas only 167 were F4 patients at baseline in the Sanyal study [8]. In the latter, there were 39 hepatic decompensations during follow-up (17 of which in F4 patients at baseline), thus 2.2% of the overall cohort and 35.9% of cirrhotics decompensated. Meanwhile, we recorded 74 decompensations amongst 996 patients and 231 cirrhotics (7.4% and 32%) in a much shorter period. Patients with HCC decompensated significantly more frequently in our cohort. Thus, potential explanations to the differing outcomes in Sanyal et al.'s study and our own might encompass, among other thing, the higher rate of cirrhosis at baseline and the higher incidence of HCC.

4.4. Predictors of HCC

Notably, T2DM was not found to be a predictor of HCC development amongst patients with NAFLD as widely described in the literature [1,2]. Meanwhile, LSM was found to be a predictive factor of HCC in cirrhotics. This is an interesting finding as VCTE is not recommended as a prediction tool for HCC development in guidelines [12]. Recently, Shearer and colleagues analyzed electronic health records data from 3028 patients in a multistate American study where the natural history of advanced chronic liver disease (LSM > 10 kPa) was assessed by means of VCTE [22]. Remarkably, the cumulative incidence of HCC before decompensation was low (1.3%) for NAFLD patients at 5 years after VCTE, whereas albumin bilirubin (ALBI) score was associated with the development of HCC from the compensated state [22]. Other prediction tools including LSM have been recently published [23,24]. In addition, we also found albumin and platelet count to be associated with an increased risk of HCC, which has already been described in NAFLD [25–27]. However, the finding of FIB-4 index $\geq$1.3 being associated to an increased risk of developing HCC over time has just recently been described, i.e., Loosen et al., found a much higher cumulative risk of HCC in NAFLD patients with FIB-4 $\geq$ 1.3 (0.47% vs. 0.04%; $p < 0.001$) [13]. Notably, Loosen et al., study was based on a large German outpatient population, whereas ours is based in a hospital-reference cohort with a large proportion of patients with advanced liver disease,

which adds to the potential use of NITs in non-population-based studies or in primary care practice but in the liver outpatient clinic or ward.

4.5. Limitations

Our study is constrained by several limitations. It is a single center study, and diagnosis of NAFLD was not confirmed via biopsy in all cases. Moreover, VCTE was not systematically performed. There might be a selection bias because only patients in the NAFLD monographic clinic were analyzed, whereas other potential HCC in non-cirrhotic NAFLD patients not followed in our clinic might have been diagnosed in our institution. The time elapsed since the onset of cirrhosis prior to the inclusion in the prospective cohort was unknown for some patients. In spite of these limitations, ours is the first large study providing data on NAFLD-HCC in a Southern European country, and findings—particularly those related to the potential utility of NITs in the prediction of HCC—might be relevant for public policy and to guide further studies in countries where data on NAFLD-HCC are lacking.

5. Conclusions

In our cohort, HCC was rare amongst non-cirrhotic patients. In spite of being largely diagnosed at early BCLC stages, HCC was associated with high rates of liver decompensation. Advanced liver fibrosis by biopsy and non-invasive tests (i.e., FIB-4 and LSM) were associated with the risk of developing HCC over time.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be available upon request.

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10.1.3. Estudio 3

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10.2. Anexo II: Otras publicaciones relacionadas con la tesis

10.2.1. Publicación anexa 1

Rivera-Esteban J, Armandi A, Augustin S, Bugianesi E. Outcomes and potential surrogate markers for future clinical trials of non-alcoholic steatohepatitis cirrhosis. Liver Int. 2021 Sep;41(9):1999-2008.

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REVIEW

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Outcomes and potential surrogate markers for future clinical trials of non-alcoholic steatohepatitis cirrhosis

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Abstract

Non-alcoholic steatohepatitis has emerged as a major public health problem, and the burden of non-alcoholic steatohepatitis cirrhosis is projected to increase by 64%-156% by 2030. The threat is aggravated by the fact that are currently no approved drugs for the treatment of non-alcoholic steatohepatitis. In this paper, we review the main challenges to drug development in patients with non-alcoholic steatohepatitis cirrhosis, and describe the opportunities brought by the advances in the understanding of the clinical and pathophysiological nuances of cirrhosis. The design of therapeutic regimens for non-alcoholic steatohepatitis cirrhosis will vary according to the specific cirrhosis substage (compensated vs decompensated), and the specific mechanistic basis of therapy, targeted either at improving aetiology-specific pathways and/or at more general aetiology-agnostic processes. The understanding of the probabilistic expectations for the whole range of potential outcomes, rooted at different mechanistic drivers at each specific substage, will be essential in order to choose adequate estimands and therapeutic strategies for clinical trials and individual patients with non-alcoholic steatohepatitis cirrhosis. Finally, we provide a summary of the main pitfalls and uncertainties in the design of clinical trials for non-alcoholic steatohepatitis cirrhosis and discuss potential biomarkers for use in trials and practice for these patients.

KEYWORDS

biomarkers, clinical trials, liver cirrhosis, metabolic associated fatty liver disease (MAFLD), portal hypertension, portal pressure

Abbreviations: ACLF, acute-on-chronic liver failure; CC, cryptogenic cirrhosis; CD, clinical decompensation; CSPH, clinically significant portal hypertension; CVD, cardiovascular disease; ELF, enhanced liver fibrosis; FDA, Food and Drug Administration; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; HVPG, hepatic venous pressure gradient; Hyperdynamic circ. Sd., hyperdynamic circulatory syndrome; IHVR, intrahepatic vascular resistance; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OLT, orthotopic liver transplantation; PBF, portal blood flow; PH, portal hypertension; PRO, patient reported outcome; RCTs, randomized controlled trials; T2DM, type 2 diabetes mellitus.

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1 | THE BURDEN OF CIRRHOSIS DUE TO NON-ALCOHOLIC STEATOHEPATITIS (NASH)

Non-alcoholic fatty liver disease (NAFLD) has emerged as a major public health threat, and the burden of end-stage liver disease due to NAFLD is projected to increase from 64% to 156% by 2030.[1] All longitudinal studies conducted on biopsy-proven NAFLD cohorts over the past 2 decades have highlighted the number of patients with cirrhosis. The prevalence of cirrhosis is currently estimated to range from 0.6% to 65%, with a median value of 9% (Table 1).[2-16] Overall mortality in patients with NAFLD is predicted to be approximately 20%, while liver-related mortality is approximately 4% across all stages of fibrosis, showing a stepwise progression from mild to severe fibrosis.[17-19]

By 2030, cirrhosis and end-stage liver disease related to NASH are projected to increase worldwide.[1] Models of future disease burden for incident decompensated cirrhosis have shown the highest percentage in France (164%) followed by the USA (150%).[1] In addition, projections from one Canadian study showed that cirrhosis due to NASH will increase in all birth cohorts by 2040, accounting for 73% of all new diagnoses of cirrhosis.[20]

As shown in Table 2, the median proportion of cirrhotic patients with liver decompensation in longitudinal studies of biopsy-proven NAFLD cohorts is 13.4%, ranging from 3% to 45% across the different cohorts.[4-6,8,10,12-16] Clinical events related to portal hypertension are the most common findings: ascites (median 8.2%, min–max: 0.4%-70%), variceal bleeding (median 8.6%, min–max: 0%-66.4%) and hepatic encephalopathy (median 4%, min–max: 0%-31.6%). Accordingly, the development of liver decompensation constitutes a healthcare burden that requires high resource utilisation, and is related to inpatient and short-term mortality.[21] Onset of hepatocellular carcinoma (HCC) represents another relevant clinical outcome, as well as occurrence of liver transplantation (median 0.5%, min–max: 0%-34.2%), that underlines the progressiveness of NAFLD. Otherwise, cardiovascular disease (CVD) involves a low rate of events in cirrhotic patients compared with liver-related outcomes and to the relative weight of morbimortality due to CVD in pre-cirrothic stages.[16]

One recent study in Wales[22] conducted in nearly 70 000 individuals affected by cirrhosis has shown that the incidence of NAFLD has increased 10-fold over the past 10 years and has become the predominant cause of liver damage. As compared with other aetiologies, the course of NASH-related liver disease appears to be milder, with a smaller proportion of decompensated patients (8% of patients with NASH cirrhosis, vs 25% of patients with alcohol-related cirrhosis). However, clinical outcomes extracted from current longitudinal studies need to be related to the rapidly increasing burden of NASH cirrhosis, which will eventually cause a considerable increase in liver-related events and mortality.

Key points

- Non-alcoholic steatohepatitis (NASH) represents a major public health problem, with the burden of NASH cirrhosis projected to increase 64%-156% by 2030.
- There are no Food and Drug Administration-approved drugs for NASH cirrhosis, and clinical outcomes are the only recommended endpoints for market approval.
- Therapeutic regimens will vary according to cirrhosis stage (compensated vs decompensated) and the mechanistic basis of therapy (aetiology specific vs symptomatic).
- A clear definition of NASH cirrhosis, understanding of liver disease biology and detailed patient risk stratification are required for future clinical trials.
- Non-invasive tests, eg enhanced liver fibrosis and liver stiffness, are promising biomarkers.

2 | CURRENTLY APPROVED ENDPOINTS IN NASH CIRRHOSIS TRIALS

There are currently no Food and Drug Administration (FDA)-approved drugs for compensated NASH cirrhosis. Patients with cirrhosis are the most likely to develop hard outcomes (eg death, HCC, liver transplantation). Current trials in patients with cirrhosis capture outcomes related to cirrhosis, such as hepatic encephalopathy, ascites and variceal haemorrhage, as well as the laboratory components of the Child–Pugh score. Improvement in histological fibrosis stage or hepatic collagen content have been also used as primary endpoints of outcome events in trials focused on NASH cirrhosis. However, the reversal of cirrhosis to lesser degrees of fibrosis is an ambitious goal, and the relationship between histological changes in cirrhosis and clinical outcomes has not been characterised. Improvements in fibrosis stage may reflect either true regression or sampling bias.

Currently, the only endpoints recommended by the FDA to support marketing approval in compensated NASH cirrhosis are clinical outcomes.[23] According to the FDA, Phase III trials in patients with compensated NASH cirrhosis should evaluate the effect of the investigational drug relative to placebo on the composite endpoint of time from randomisation to the first of any one of the following outcome events:

1. Complication of ascites including any of the following: spontaneous bacterial peritonitis, diuretic-resistant ascites (refractory ascites), hepato-pleural effusion.
2. Variceal haemorrhage.
3. Hepatic encephalopathy.
4. Worsening model for end-stage liver disease (MELD) score to greater than or equal to 15 (this endpoint approximates listing for liver transplant).
5. Liver transplantation.
6. Death from any cause.

TABLE 1 Prevalence of cirrhosis, and overall and liver-related mortality in longitudinal studies of biopsy-proven NAFLD cohorts[2-16]

Study	Year of publication	Number of subjects	Cirrhosis prevalence (%)	All-cause mortality (%)	Liver-related mortality (%)	Follow up (years)
Matteoni et al[2]	1999	132	20.4	48.9	9.1	8.3
Dam-Larsen et al[3]	2004	109	0.6	24.8	0.9	16.7
Adams et al[4]	2005	420	5[a]	12.6	1.7	7.6
Ekstedt et al[5]	2006	88	7.9	20.1	2.8	13.7
Sanyal et al[6]	2006	152	100[a]	19	4.6	10
Soderberg et al[7]	2010	118	9	40	6.7	28
Bhala et al[8]	2010	247	54	13.4	5.6	7.4
Younossi et al[9]	2011	210	10.5	26.6	8.5	12.1
Sebastiani et al[10]	2015	148	14.9	6	2	5
Ekstedt et al[11]	2015	224	1.8	42.4	4	26.4
Angulo et al[12]	2015	619	2.9	31.1	4.2	12.6
Seko et al[13]	2015	312	8	2.6	0.3	4.8
Leung et al[14]	2016	307	15.3	2.6	0.3	4.1
Hagström et al[15]	2017	646	3.1	33.1	7.9	20
Vilar-Gomez et al[16]	2018	458	65.2	8	6.7	5.5
Median values			9	20.1	4.2	

Abbreviation: NAFLD, non-alcoholic fatty liver disease.

[a]Diagnosed either by biopsy or clinical/imaging assessment.

TABLE 2 Proportions of cirrhotic patients with overt decompensation and most frequently reported liver-related events in longitudinal studies of biopsy-proven NAFLD cohorts

Study	Liver decompensation (%)	Ascites (%)	Variceal haemorrhage (%)	HCC (%)	HE (%)	OLT (%)
Adams et al[4]	3.1	2	1	0.5	2	0.2
Ekstedt et al 2006[5]	5.7	4.5	1.1	2.3	0	2.3
Sanyal et al[6]	45	42.8	66.4	1.2	31.6	34.2
Bhala et al[8]	19.4	7.7	10.5	2.4	7.7	NA
Sebastiani et al[10]	16.2	8.7	6.7	0.7	0	1.3
Angulo et al[12]	13.4	34.6	46	11.5	23.1	0.5
Seko et al[13]	NA	NA	NA	1.9	NA	0
Leung et al[14]	1.6	0.4	0	0.9	0.4	0
Hagström et al[15]	11.8	NA	NA	1.9	NA	0
Vilar Gomez et al[16]	19	70	24	9	6	8
Median values	13.4	8.2	8.6	1.9	4	0.5

Abbreviations: HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; NA, not applicable; OLT, orthotopic liver transplantation.

3 | WHICH PATIENTS WITH NASH CIRRHOSIS ARE ELIGIBLE FOR PHARMACOLOGICAL THERAPY AND HOW TO SELECT THEM: THE DIFFERENT STAGES OF NASH CIRRHOSIS

The structure and focus of therapeutic regimens for NASH cirrhosis will vary according to 2 main factors: (i) the specific substage of cirrhosis at which the therapeutic strategy is directed, and (ii) the specific mechanistic basis of therapy–either targeted at improving aetiology-specific pathways (such as the metabolic derangements typical of NASH) or at more general aetiology-agnostic processes (such as the intrahepatic or extrahepatic vascular dysregulation common to all forms of portal hypertension).[24-26]

The classical concept of cirrhosis is histological in nature, and has been traditionally regarded as a static, non-reversible last stage for all forms of progressive liver diseases.[24] However, both the success of antiviral therapies in cirrhosis, as well as the integration of clinical and haemodynamic knowledge generated in the past 2 decades, have helped to develop a more comprehensive, dynamic and

nuanced view of this last phase of chronic liver disease.[24] Nowadays, there are at least 4 distinct, well-differentiated substages of cirrhosis, classified according to the mechanisms driving progression and potential for regression of disease at each stage and the expected probabilities of a different range of outcomes.[24,26] Table 3 summarises a schematic view of these 4 substages and the potential implications for the treatment of patients with NASH cirrhosis.

From a clinical standpoint, the occurrence of a first clinical decompensation (CD) indicates a dramatic worsening of prognosis in patients with cirrhosis and enables division into 2 main stages: compensated and decompensated cirrhosis.[27] Indeed, the expected median survival of a patient with compensated cirrhosis is 10-12 years, compared with the 1.5-2 years that could be expected after a patient's first decompensating event[28,29] (of note, these rates are drawn from studies including patients with all aetiologies of cirrhosis–the impact on survival of the transition from compensated to decompensated stage has not been reported specifically for NASH-).

The compensated stage is the longest phase and can go undiagnosed given its asymptomatic nature. Nonetheless, early identification of patients at this stage would be of paramount importance, since this is precisely the phases at which therapies might be still able to prevent the transition to the decompensated stage, or even lead to a regression to pre-cirrhotic stages. Within the compensated stage, the presence and degree of portal hypertension is a key predictor of outcome. By integrating haemodynamic data, further distinction of prognostic relevance can be made into an early compensated stage and a late compensated stage.[24,26,27,30] The early compensated stage is characterised by a mild degree of portal hypertension (defined by an hepatic pressure venous gradient (HVPG) <10 mm Hg), and a low risk of CD (<10% at 4 years).[31,32] The late compensated stage is characterised by the presence of clinically significant portal hypertension (CSPH), defined by an HVPG ≥10 mm Hg, the threshold above which CD is up to 4 times more likely to occur.[31,32]

The distinction between the early compensated stage and CSPH is not merely prognostic but also carries pathophysiological and therapeutic implications. In the early compensated stage, the main drivers of progression and outcomes are aetiology-specific.[25,26] There seem to be intrahepatic vascular adaptations, both structural and functional–the latter being characterised by a moderate increase in intrahepatic vascular resistance (IHVR)–all of which translates into mild portal hypertension.[30,33] Thus, within this stage, therapeutic efforts should be directed towards arresting the driving mechanism of NASH. The underlying assumption (extrapolated from the lessons learnt from antiviral therapies[34-37]), is that the improvement of those aetiology-specific mechanisms will carry over indirectly into the arrest (or even regression) of fibrosis and the functional improvement of IHVR, leading to a decrease of portal pressure and the risk of CD. Within this clinical and pathophysiological framework, the choice of outcomes in this specific early stage should focus on histological improvement of NASH features and fibrosis as the main endpoint (as in current Phase III trials). Progression to CSPH and/or resolution of portal hypertension could be considered as secondary outcomes or for earlier Phase II trials.[25] In order to assess the impact of hard clinical endpoints in this early NASH cirrhosis population, large sample sizes and a longer duration of follow up are likely to be required, as seen in current Phase III/IV trials.[32,38]

Once the critical CSPH threshold is reached, the risk of CD increases considerably,[27,31,32] and therefore the prevention of CD should become the main therapeutic goal and the ideal endpoint for trials.[25] During this late compensated stage, structural intrahepatic changes are characterised by the presence of thicker fibrotic septa, thus making the regression of fibrosis unlikely.[24] Also, extrahepatic adaptations start to develop, with an increase in portal blood flow, the development of collateral circulation and systemic adaptations leading to the initial phases of a mild hyperdynamic circulatory state.[39,40] Once these adaptations appear, splanchnic vasoconstrictors start to become effective at halting the progression of portal hypertension and reducing the risk of decompensation.[40,41] Extrapolating from experience with antiviral therapies, it can be assumed that for NASH cirrhosis, there might also be a 'point of no return' somewhere after CSPH is established, for which the effects of aetiology-specific therapies are no longer able to reverse CSPH and eliminate the risk of CD without concomitant medication.[34-37] Under these pathophysiological assumptions, therapeutic efforts in this late compensated phase should aim to target the same aetiology-specific mechanistic pathways as well as both intrahepatic and extrahepatic vascular tone. As a matter of fact, in patients already harbouring oesophageal varices, the use of non-selective beta-blockers is the standard of care[25] and cannot be avoided as a comparator for trials.[26] The transition of HVPG values to <10 mm Hg could be considered as a potentially relevant surrogate endpoint in these patients,[25,26] but histological changes in NASH and fibrosis become less likely[32] and are thus not ideal endpoints.

Finally, the occurrence of CD signals that intrahepatic and extrahepatic structural and functional adaptations are insufficient and therefore that the patient has progressed to NASH cirrhosis.[24,30] The risk of further decompensation, progression to end-stage liver disease and death increases exponentially as decompensating events accumulate.[26,28] From a pathophysiological perspective, there is a progression of the hyperdynamic circulatory state, peaking in patients with refractory ascites, for whom there is a relative decrease in the cardiac index that leads to a further decompensated stage.[24,26] Mounting evidence also points to a deleterious hypercoagulable state in decompensated cirrhosis, especially affecting the micro-circulation.[42] All of these changes, along with the decrease in synthetic liver function, lead to an increase in gut bacterial translocation and systemic inflammation, which are key drivers of the decompensating inertia of this stage, eventually leading to a final high-mortality stage characterised by multiorgan failure.[24,27] The role of aetiology-specific therapies is likely secondary in these later stages and, for the specific case of NASH, might even be associated with deleterious effects, especially with weight loss-directed approaches, which could lead to worsening of patients' nutritional and performance status and an increased risk of complications and death.[43,44] Aetiology-agnostic approaches in this phase might be

TABLE 3 The different stages of NASH cirrhosis: Clinical features, mechanistic drivers and ideal therapeutic goals and endpoints

	Compensated NASH cirrhosis		Decompensated NASH cirrhosis	
	Early compensated	**Late compensated (compensated CSPH)**	**Early decompensated**	**Further decompensated**
Clinical features	Unspecific symptoms	Unspecific symptoms (± varices)	Few/mild decompensating events	• Recurrent/refractory decompensating events • ACLF (multiorgan failure)
Biological features				
Histology	Scar, X-linking (thin septa)	Acellular scar, nodules (thick septa)	Insoluble scar	Insoluble scar
Haemodynamic (HVPG)	5-10 mm Hg (mild PH)	>10 mm Hg (CSPH)	>12 mm Hg	>16 mm Hg
Mechanistic drivers				
Primary	NASH mechanisms (metabolic/ inflammation/ fibrosis)	• NASH mechanisms (fibrosis) • Increased IHVR	• Increased portal pressure (PBF+IHVR) • Hyperdynamic circ. Sd.	• Failed hyperdynamic compensation • Decreased liver function
Secondary	Increased IHVR	• Increased PBF • Collaterals development	• Decreased liver function • Increased inflammation • Increased bacterial translocation • Hypercoagulability	• Increased inflammation • Increased bacterial translocation • Hypercoagulability • Increased portal pressure
Therapeutic goals/ endpoints				
Primary	• Improvement in NASH histology • (NASH resolution and/or fibrosis regression?)	• Prevention of liver clinical events (CD)	• Transplant-free survival • Overall survival	• Overall survival • PROs
Secondary	• Prevention of liver +non-liver clinical events • Resolution of PH • Decreased progression to CSPH • New fibrosis biomarkers?	• Improvement in NASH histology • Prevention of non-liver clinical events • Decrease in HVPG (>20% and/or <10 mm Hg) • New fibrosis/liver function biomarkers?	• Recurrence/control of CD events • Mechanistic/proof-of-concept: specific to type of CD • Changes in MELD/Child-Pugh scores • PROs	• Recurrence/control of CD events • Mechanistic/proof-of-concept: specific to type of CD • Post-transplant outcomes

Abbreviations: ACLF, acute-on-chronic liver failure; CD, clinical decompensation; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; Hyperdynamic circ. Sd., hyperdynamic circulatory syndrome; IHVR, intrahepatic vascular resistance; MELD, model for end-stage liver disease; PBF, portal blood flow; PH, portal hypertension; PRO, patient reported outcome.

more generally directed to the modification of intra and extrahepatic vascular tone dysregulation or can be more specifically targeted to the specific complications (control of fluid retention for ascites, nitrogen metabolism for hepatic encephalopathy [HE], etc). Therapeutic efforts to control infection and systemic inflammation, and even to counterbalance the described hypercoagulable state, should also have a role in this stage.

Considering the poor prognosis in the decompensated stage, transplant-free survival is recommended as the primary endpoint for Phase III trials.[25,45] Either surrogate or secondary endpoints for Phase III trials (such as development of multiorgan involvement as in acute-on-chronic liver failure), or primary endpoints for Phase II trials focusing on specific decompensating events (recurrence of variceal haemorrhage, hepatic encephalopathy, control of ascites, etc), should be chosen according to the mechanism of action of the drug under investigation and the specific targeted patient subpopulation. Inclusion of patient reported outcomes should become standard practice in this symptomatic, late stage of NASH cirrhosis. Three excellent recent reviews provide a comprehensive view of the different tools and approaches for reporting quality of life in patients with advanced liver disease.[46-48]

In summary, the understanding of the probabilistic expectations for the whole range of potential outcomes, rooted at different mechanistic drivers at each specific substage of NASH cirrhosis, is essential in order to choose adequate estimands and therapeutic strategies for clinical trials, and tailor therapy to individual patients with NASH cirrhosis.

4 | PITFALLS AND UNCERTAINTIES IN CLINICAL TRIALS FOR NASH CIRRHOSIS

4.1 | Definition of NASH cirrhosis

An accurate case definition of NASH cirrhosis is critical for enrolling appropriate patients into clinical trials, but currently there are no published criteria for defining NASH cirrhosis. NASH is a histological diagnosis, but in clinical practice, the term NASH cirrhosis has been increasingly used by physicians to address the forms of end-stage liver diseases in the presence of known risk factors for metabolic derangement, mainly obesity and type 2 diabetes mellitus. However, the progression of NASH is associated with a loss of intrahepatic fat and diminished inflammatory activity.[49] Therefore, in the absence of a prior diagnosis of NASH, end-stage liver histology cannot be linked with a specific aetiology, and the term NASH cirrhosis remains hypothetical. In this line, the FDA requires fulfilment of full NASH histological criteria for enrolment in phase 3 trials in the F4 population. This certainly represents a significant hurdle for recruitment, since aproximatelhy, half of F4 patients do not show full NASH features,[49] which explain in part the high screen failure rate common in NASH trials.

The presence of a suggestive medical history and/or hallmarks of metabolic syndrome may help to characterise patients with true NASH cirrhosis, with respect to those with unexplained ('cryptogenic') cirrhosis (CC).[50] Data suggest that patients with NASH cirrhosis and CC form different parts of the same spectrum of chronic liver disease that originates in the setting of metabolic abnormalities, as these patients have similar metabolic and clinical features, but are not currently enrolled in clinical trials because a threshold of steatosis (>5%) is required as an inclusion criterion. In a recent study among CC patients with <5% fat at baseline biopsy who had another biopsy during follow-up, 40.4% had >5% fat on their subsequent biopsy, and, therefore, were no longer 'cryptogenic'. Furthermore, patients with CC seem to have a more aggressive disease than those with NASH cirrhosis, as indicated by greater hepatic collagen content and α-SMA expression on biopsy, higher serum fibrosis markers and MELD scores and a greater risk of liver-related clinical events during follow-up.[51]

The recent proposal to substitute the term NASH by Metabolic (dysfunction) Associated Fatty Liver Disease (MAFLD)[52] stems in part from these challenges with the disease definition. The new term brings the focus to the metabolic substrate at the mechanistic and clinical basis of the disease. For the particular of NASH cirrhosis, this recognition may help at clarifying in part the conundrum of CC described above, since most patients with CC share the same metabolic substrate of NASH and have overlapping clinical progression. The new term also provides an enabling framework from where to reinterpret the coexistence in real practice of metabolic and alcoholic liver disease, with the recognition that the two conditions are not mutually exclusive and can coexist together. Also, the removal of specific thresholds of alcohol intake from the definition, forces the revision of the assumption that slight to moderate alcohol intake have no deleterious hepatic effects, which is implicit in the classical definition of NASH. This is also relevant in patients with NASH cirrhosis, in whom the potential impact of alcohol in the progression of the disease becomes especially critical.

Nonetheless, adoption of the new term is still a matter of debate, and proof of it is that the whole MAFLD concept has not been applied in clinical trials yet, where precise and well-characterised criteria are still needed to avoid bias and confounders and for regulatory reassurance. In this regard, the Liver Forum recently developed a consensus case definition of NASH-related cirrhosis for inclusion in clinical trials that may be qualitatively categorised according to the degree of certainty of NASH as the cause of cirrhosis: definitive, probable and possible.[53] When histological evidence is absent, the presence of concomitant metabolic risk factors strengthens the likelihood that NASH is the cause of cirrhosis (Box 1).

4.2 | Biology of liver disease in cirrhosis

The drugs currently in development for NASH can be divided into 3 broad categories: metabolic, anti-inflammatory and antifibrotic. Favourable metabolic effects from a drug are desirable in patients with NASH, but to what extent the partial correction of these metabolic abnormalities leads to a more favourable outcome in cirrhosis

BOX 1 NASH Cirrhosis: Liver Forum consensus definitions for clinical trials[48]

1. Definite NASH cirrhosis	1a. Patients with current liver biopsy showing cirrhosis with steatohepatitis. 1b. Patients with a previous biopsy showing steatohepatitis, but now with evidence of cirrhosis, either by clinical history or current features, imaging, non-invasive tests or biopsy. 1c. Patients with a current biopsy showing cirrhosis[a] with steatosis (but no findings of active steatohepatitis) together with at least two coexisting or historical metabolic comorbidities including obesity and/or T2DM to corroborate a diagnosis of NASH as the cause of cirrhosis.
2. Probable NASH cirrhosis	2a. Patients with a previous biopsy with steatosis but not steatohepatitis, and current cirrhosis[a]. At least two coexisting or historical metabolic comorbidities including obesity and/or T2DM to support NASH as an underlying cause of cirrhosis. 2b. Patients with cirrhosis[a] with current or previous imaging showing evidence of steatosis. At least two coexisting or historical metabolic comorbidities including obesity and/or T2DM to support NASH as an underlying cause of cirrhosis. 2c. Patients with 'cryptogenic cirrhosis' without current or previous evidence of steatosis by imaging or steatosis/steatohepatitis by histology. At least two coexisting or historical metabolic comorbidities including obesity and/or T2DM to support NASH as an underlying cause of cirrhosis.
3. Possible NASH cirrhosis	3a. Patients with 'cryptogenic cirrhosis' without current or previous evidence of steatosis by imaging or steatosis/steatohepatitis by histology. At least one coexisting or historical metabolic comorbidity including obesity and/or T2DM.

[a] Either by a clinical history or current features, imaging, non-invasive tests or biopsy.

Abbreviations: NASH: non-alcoholic steatohepatitis; T2DM: type 2 diabetes mellitus.

is unknown. The biology of clinical decompensation in patients with previously compensated cirrhosis is not fully understood. For example, diabetes arising in liver cirrhosis (so-called hepatogenous diabetes) has a profound impact on the pathology and natural history of the liver disease.[54] Although peripheral insulin resistance and impairment of the hepatocellular function are two potential major causes, the beta-cell capacity plays a critical role. Recent evidence that the failing liver exerts an independent 'toxic' effect on beta-cells suggests that individuals with hepatogenous diabetes might benefit from interventions aimed at improving beta-cell function, such as thiazolidinediones and incretins.[55] On the other hand, cirrhosis is a condition of intense muscle protein wasting leading to sarcopenia even in individuals affected by obesity (so-called sarcopenic obesity).[43] Malnutrition and sarcopenia are associated with higher complication rates in patients with cirrhosis.[43] Moreover, they are associated with increased mortality in hospitalised patients with cirrhosis and those waiting for liver transplantation.[43] In this setting, drugs promoting weight loss may have a detrimental impact on the progression of cirrhosis. Therefore, it remains to be determined whether treatment of the underlying steatohepatitis as opposed to the fibrosis will prevent clinical decompensation. These possibilities are currently under active investigation.

4.3 | Risk stratification

As described above, cirrhosis represents a broader spectrum of disease compared with its usual histological classification (F4), and the main challenge is the stratification of NASH patients with compensated cirrhosis to identify strata for decompensation that represent a clinically meaningful outcome. The expected risk of each outcome changes markedly from stage to stage, and sample size and follow-up times should vary accordingly. This nuanced view of the different stages of cirrhosis is often overlooked in the design of large clinical trials in NASH cirrhosis. From an operational point of view, stratification by direct or indirect evidence of CSPH (HPVG>10 mm Hg, esophageal varices at endoscopy or, hopefully in the near future, non-invasive biomarkers of CSPH) and/or a decreased liver function (Child-Pugh score ≥6 points) could be used to fine-tune the assumptions around expected event rates informing sample size, trial duration, etc A more granular and detailed sub-stratification of patients with NASH cirrhosis and the corresponding choice of outcomes is needed to stimulate therapeutic development in this population.

4.4 | Placebo reponse and Hawthorne effect

Placebo response represents a major challenge in NASH clinical trials. Patients allocated to placebo arms in therapeutic trials show significant histologic, radiologic and biochemical responses[56,57] that must be taken into account as they can interfere with the overall trial design and an adequate interpretation of results. Hawthorne effect is particulary relevant in a lifestyle-driven disease such as NASH, as subjects may conciosuly or unconsciously change their behaviour after enrolment, directly affecting NASH biology and outcomes.[58] To control this effect adequately, trials should include a standardised approach to lifestyle interventions and an objective assessment of lifestyle, including physical activity, dietary and alcohol intakes questionnaires. In this regard, the Liver Forum has recently issued a comprehensive review and position paper on this important topic,

with specific recommendations.[59] Trial duration may also affect the impact of the Hawthorne effect. Long trail durations, as currently required for phase 3 trials in NASH cirrhosis, might mitigate the potential impact of a Hawthorne effect (since lifestyle modifications are harder to maintain in the long term), but this is yet to be proven. Other factors such as sample size or geographic location must be also considered when interpreting the results of placebo arms in RCTs.

5 | SUGGESTED SURROGATES AND NEW BIOMARKERS

5.1 | HVPG

As discussed above, the HVPG remains the most robust and accurate surrogate predictor of outcomes in patients with compensated and early decompensated cirrhosis of different aetiologies, including NASH.[25,31] In patients with cirrhosis of viral aetiology, improvement of HVPG after antiviral therapy translates into a clear improvement in outcomes.[34-37] In the case of NASH cirrhosis, observations from the simtuzumab programme suggest that reductions in HVPG (at least 20% and/or below 10 mm Hg) is associated with a significant decrease in the risk of clinical decompensation.[32,38] However, HVPG measurement is technically challenging, invasive and expensive, and is still regarded as logistically demanding for use in large clinical trials. Furthermore, in the case of earlier compensated stages (before CSPH), which are the focus of most current programmes in NASH cirrhosis, more studies are required to demonstrate that improvement in HVPG (due to reduced fibrotic remodelling of the liver or other intrahepatic structural or functional mechanisms) translates into improved clinical outcomes. On this basis, the most recent FDA guidance on clinical trials in NASH cirrhosis does not discuss HVPG as a valid endpoint for marketing approval in Phase III studies.[53] However, in the case of earlier development (Phases II and IIb), HVPG remains an attractive surrogate endpoint in compensated NASH cirrhosis, due to the large numbers of patients and long duration of follow up that would be required in trials with a primary endpoint of clinical outcomes, and the strong association of HVPG with clinical outcomes in this setting.

5.2 | Improved histological readings

In an attempt to mitigate the inter and intra-observer variability in NASH histology readings discussed in the first section, improved machine-driven methods to quantify basal amount of fibrosis and changes in time are being increasingly explored in the last few years. A recent paper evaluating a machine-learning (ML) based-approach with paired biopsies from 3 large NASH RCTs including patients with advanced fibrosis (STELLAR-3, STELLAR-4 and ATLAS)[60] showed that these artificial intelligence-driven techniques are sensitive and reliable, and represent promising approaches to correlate dynamic changes in fibrosis (even in the F4 stage) with clinical outcomes, although the number of events in those clinical trials was very small and further studies are required to increase the confidence in this novel and exciting techniques.

5.3 | New biomarkers in NASH cirrhosis

A major challenge in drug development is to identify and validate surrogate markers that predict a reduction in progression to hard outcomes. Different exploratory efficacy endpoints have been studied in clinical trials in patients with NASH cirrhosis, including changes in liver biochemistry tests; non-invasive tests (eg enhanced liver fibrosis [ELF], liver stiffness by transient elastography); markers of apoptosis and necrosis and other histological measures including hepatic collagen and fat content and α-SMA expression.[32,61] The demonstration that ELF score and liver stiffness had the ability to identify those most likely to progress from F3 to cirrhosis, in addition to predicting which patients with cirrhosis at baseline were most likely to have a liver-related event,[61] was of particular interest. The authors defined a change in ELF score of 0.5 points as the value that correlated with clinical liver events. This value also correlated with other non-invasive tests, liver biochemistry tests, glycaemic parameters, CK-18 values, serum bile acid values and body weight, but not with histological features. These findings give hope that in the future, reliance on histological endpoints will be a historical concept for NASH clinical trials. Validation from such biomarkers will hopefully be a reality as clinical events accumulate in ongoing Phase III trial efforts.

Functional testing is a novel approach to assess the severity of liver disease and changes in actual liver function in the context of treatment trials, especially in patients with cirrhosis. The HepQuant test simultaneously measure clearance from portal and systemic circulation as well as portal-systemic shunting.[62] Preliminary data indicate that this assessment of liver impairment correlates with hard endpoints, but more extensive validation is needed. Another test is the methacetin breath test, which is being evaluated in several ongoing clinical trials for NASH,[63,64] and longitudinal data that correlate this assessment with histological changes may be forthcoming. How the HepQuant, methacetin breath test and other tests of specific metabolic functions of the liver compare with clinical outcomes remains to be determined.

6 | CONCLUSIONS

NASH cirrhosis has emerged as a major healthcare problem and finding effective therapies for patients with NASH has become one of the main unmet clinical needs in hepatology. Drug development in this field will face important challenges, due to the time needed to gather hard outcomes and the unreliable nature of fibrosis as an endpoint. A deeper understanding of the clinical nuances of NASH cirrhosis, and the mechanistic processes driving disease progression

at each substage, will hopefully guide therapeutic efforts and appropriate patient selection, informative biomarkers and clinically meaningful endpoints.

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CONFLICTS OF INTEREST

Jesús Rivera-Esteban reports no conflicts of interest. Angelo Armandi reports no conflicts of interest. Salvador Augustin is now an employee of Boehringer Ingelheim but was not when the manuscript was written. Elisabetta Bugianesi reports advisory board activity for Boehringer Ingelheim, Bristol Myers Squibb, Gilead, Innova, Intercept, Novo Nordisk.

RESEARCH ETHICS AND PATIENT CONSENT

Not applicable.

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10.2.2. Publicación anexa 2

Rivera-Esteban J, Manzano-Nuñez R, Broquetas T, Serra-Matamala I, Bassegoda O, Soriano-Varela A, Espín G, Castillo J, Bañares J, Carrión JA, Ginès P, Graupera I, Pericàs JM. Impact of the COVID-19 pandemic on the care and outcomes of people with NAFLD-related cirrhosis. JHEP Rep. 2022 Nov;4(11):100574.

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Research article JHEP|Reports

Impact of the COVID-19 pandemic on the care and outcomes of people with NAFLD-related cirrhosis

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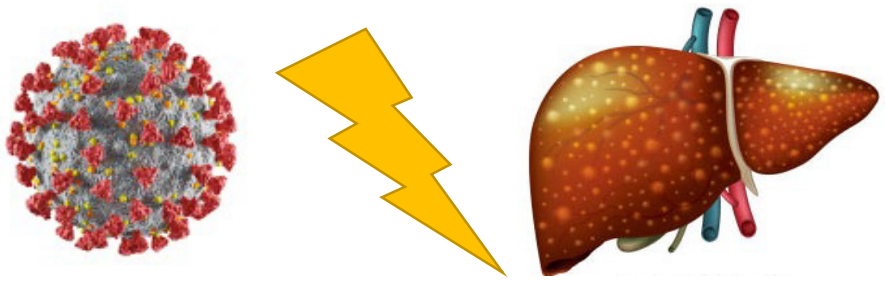
Graphical abstract



Outcomes amongst NAFLD cirrhotic patients without prior decompensations

	Pre-pandemic	Pandemic	*p* value
First liver-related event, n (%)	20/271 (7.4)	28/247 (11.3)	0.12
Cardiovascular events, n (%)	6/271 (2.2)	3/262 (1.1)	0.24
Mortality, n (%)	9/271 (3.3)	16/262 (6.1)	0.12
Worsening of metabolic status, n (%)	111/271 (40.9)	123/262 (46.9)	0.16

Highlights

- Patients with NAFLD cirrhosis did not present a higher rate of liver-related events during the COVID-19 pandemic.
- Usual predictors, such as diabetes, albumin and FIB-4 were associated with higher risk of a first liver event.
- Health system preparedness seems key to ensure patients with NAFLD cirrhosis receive appropriate care during health crises.

Lay summary

Mobility restrictions and social stress induced by the COVID-19 pandemic have led to increased alcohol drinking and worsened metabolic control (*e.g.*, weight gain, poor control of diabetes) in a large proportion of the population in many countries. We aimed to analyze whether people with cirrhosis due to non-alcoholic fatty liver disease, who are particularly vulnerable to such lifestyle modifications, were significantly impacted during the first year of the pandemic. We compared the clinical situation of 354 patients one year before the pandemic and one year after. We found that although metabolic control was indeed worse after the first year of the pandemic and patients presented worse clinical outcomes, the latter was mostly due to non-liver causes, namely COVID-19 itself. Moreover, the care provided to these patients did not worsen during the first year of the pandemic.

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Research article

JHEP|Reports

Impact of the COVID-19 pandemic on the care and outcomes of people with NAFLD-related cirrhosis

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Background & Aims: The COVID-19 pandemic has had a major negative impact on health systems and many chronic diseases globally. We aimed to evaluate the impact of the first year of the pandemic on the outcomes of people with NAFLD cirrhosis.
Methods: We conducted a before-after study in four University hospitals in Catalonia, Spain. Study subperiods were divided into Pre-pandemic (March/2019–February/2020) *vs.* Pandemic (March/2020–February/2021). The primary outcome was the rate of first liver-related event (LRE). Overall clinical outcomes (LREs plus cardiovascular plus all-cause mortality) were also assessed.
Results: A total of 354 patients were included, all of whom were compensated at the beginning of the study period; 83 individuals (23.5%) had a history of prior hepatic decompensation. Mean age was 67.3 years and 48.3% were female. Median BMI was 31.2 kg/m^2 and type 2 diabetes was present in 72.8% of patients. The rates of first LRE in the Pre-pandemic and Pandemic periods were 7.4% and 11.3% (p = 0.12), respectively. Whilst the rate of overall events was significantly higher in the Pandemic period (9.9% *vs.* 17.8%; p = 0.009), this was strongly associated with COVID-19-related deaths. The rate of worsened metabolic status was significantly higher in the Pandemic period (38.4% *vs.* 46.1%; p = 0.041), yet this was not associated with the risk of first LRE during the Pandemic period, whereas type 2 diabetes (odds ratio [OR] 3.77; 95% CI 1.15–12.32; p = 0.028), albumin <4 g/L (OR 4.43; 95% CI 1.76–11.17; p = 0.002) and Fibrosis-4 score >2.67 (OR 15.74; 95% CI 2.01–123.22; p = 0.009) were identified as risk factors in the multivariable analysis.
Conclusion: Overall, people with NAFLD cirrhosis did not present poorer liver-related outcomes during the first year of the pandemic. Health system preparedness seems key to ensure that people with NAFLD cirrhosis receive appropriate care during health crises.
Lay summary: Mobility restrictions and social stress induced by the COVID-19 pandemic have led to increased alcohol drinking and worsened metabolic control (*e.g.*, weight gain, poor control of diabetes) in a large proportion of the population in many countries. We aimed to analyze whether people with cirrhosis due to non-alcoholic fatty liver disease, who are particularly vulnerable to such lifestyle modifications, were significantly impacted during the first year of the pandemic. We compared the clinical situation of 354 patients one year before the pandemic and one year after. We found that although metabolic control was indeed worse after the first year of the pandemic and patients presented worse clinical outcomes, the latter was mostly due to non-liver causes, namely COVID-19 itself. Moreover, the care provided to these patients did not worsen during the first year of the pandemic.

Introduction

The COVID-19 pandemic has had a strong, overall negative impact on health systems globally in terms of patient suffering, healthcare overloads, and economic burden.[1] A specific impact was in the tertiary setting, particularly during the first wave, when healthcare resources were reassigned to COVID-19 and routine care was deferred for 'stable' patients to mitigate the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Hence, many other specialties (*i.e.*, hepatology

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units) suffered from a diversion and reduction of their resources that affected the delivery and quality of care.[2–6]

People with chronic liver disease, including non-alcoholic fatty liver disease (NAFLD), are at higher risk of severe COVID-19, disease progression and clinical decompensation.[7–16] Moreover, lockdown, economic hardship and the psychological impact of the pandemic all had a detrimental effect on people with liver disease, including poorer metabolic control in people with metabolic syndrome and fatty liver disease.[17,18] This likely had deleterious consequences on the liver and cardiovascular (CV) outcomes of people with NAFLD, particularly those with advanced liver disease. Mortality has been shown to increase in people with alcohol-associated liver disease,[19] and there have also been several reports on the impact of the COVID-19 pandemic on the diagnosis and management of hepatocellular carcinoma (HCC),[20–22] cirrhosis,[23–26] and patients requiring liver transplantation.[27]

However, the impact of the pandemic on people with cirrhosis due to NAFLD is poorly known. Therefore, in this study, we aimed to evaluate how the effect of COVID-19 on health systems during the first wave of the pandemic impacted outcomes in people with NAFLD cirrhosis.

Patients and methods

Design and setting

We conducted a multicentric before-after study based on NAFLD cohorts with retrospective data from four university hospitals in Barcelona (three) and Girona (one), Catalonia, Spain. The study period encompasses the period from March 2019 to March 2021 and has been divided into two subperiods: the year before the Spanish government declared the state of emergency (March 2019 – February 2020; Pre-pandemic period), and the year after that (March 2020 – February 2021; Pandemic period).

In the four participating hospitals, staff of hepatology units were assigned to COVID-19 clinical tasks at least during the first wave (March-May 2020) of the pandemic, and in some cases also in latter outbreaks. However, in all hospitals biannual visits were kept for people with cirrhosis; liver and metabolic changes were recorded; and blood tests and abdominal ultrasound schedules were maintained. During COVID-19 peaks, in-person visits were replaced by video calls or telephone calls. In decompensated patients, either admitted to the hospital or not, the frequency of follow-up calls was increased. In brief, the recommendations included in the EASL-ESCMID position paper on the care of people with liver disease during the COVID-19 pandemic[2] were followed.

Participants

People with a diagnosis of cirrhosis due to NAFLD before March 2019 under follow-up at liver clinics of the participating hospitals were included.

Definitions

NAFLD cirrhosis: one or more of the following criteria: liver biopsy with ≥5% steatosis and/or steatohepatitis by NASH clinical research network score[28] and fibrosis stage 4 or cryptogenic cirrhosis in a patient with known obesity, type 2 diabetes (T2D) or metabolic syndrome and no other detectable liver etiology; presence of steatosis on imaging and signs of ultrasonographic or endoscopic portal hypertension in a patient with compensated advanced chronic liver disease (cACLD) and obesity, T2D or metabolic syndrome in the absence of other etiologies of cACLD (signs of ultrasonographic portal hypertension were the presence of splenomegaly [>13 cm], portal-systemic collaterals, inversion of flow within the portal system, dilatation of portal vein [diameter >13 mm] or reduced portal vein velocity <10 cm/s); presence of steatosis on imaging and liver stiffness ≥18 kPa by vibration-controlled transient elastography (VCTE) in a patient with obesity, T2D or metabolic syndrome in the absence of other etiologies of cACLD. Of note, no other imaging technique different from VCTE was used for liver fibrosis estimation.

First liver event: first episode of ascites of any grade (stage 1 to 3), any grade of hepatic encephalopathy (HE) according to the West-Haven classification (stage 1 to 4), portal hypertension-related bleeding, or hepatocellular carcinoma in people with compensated cirrhosis.

Liver events: portal hypertension-related bleeding, any grade of HE, or ascites, spontaneous bacterial peritonitis (in people with refractory ascites), hepatocellular carcinoma, and liver transplant.

Cardiovascular events: acute coronary syndrome, acute stroke, others (*e.g.*, acute peripheral arterial syndrome).

Weight gain: any measured weight gain compared to one year earlier (under the assumption that people with NAFLD are supposed to lose weight or maintain it); *Significant body weight gain*: >5%.

Poor control of diabetes: new diagnosis of T2D and/or fasting glucose >140 and/or Hb1Ac >8%, and/or introduction of new drug to treat T2D.

Poor control of systemic hypertension: new diagnosis of high blood pressure and/or routine measurements of systolic arterial pressure >140 mmHg or diastolic arterial pressure >90 mmHg and/or episodes of hypertensive crisis-emergencies, and/or new drug added.

Poor control of dyslipidemia: new diagnosis of dyslipidemia (either due to hypercholesterolemia, hypertriglyceridemia or both) and/or total cholesterol >240 mg/dl and/or total triglycerides >200 mg/dl, and/or new drug added.

Worsening of metabolic status: Presence of at least one of the previous variables (significant weight gain and/or poor control of diabetes mellitus/arterial hypertension/dyslipidemia).

Delayed diagnosis of HCC: >2 months after an imaging test was performed suggesting HCC.

Delayed treatment of esophageal varices: >2 months after a gastroscopy showing new or advanced changes requiring new or additional treatment (either endoscopic or pharmacological).

Outcomes

The primary outcome was the development of clinical events during the study period, particularly a first liver-related event (LRE) amongst persons without prior decompensations. A first LRE was defined as the development of a clinical decompensation (ascites, hepatic encephalopathy, or upper gastrointestinal bleeding secondary to portal hypertension) or HCC.

As secondary outcomes, we investigated: the occurrence of overall clinical events (hepatic, also including spontaneous bacterial peritonitis, and CV), liver-related and all-cause mortality in the entire study cohort (including both persons with and without a prior decompensation at the beginning of the study period); worsening of metabolic status; and delay of management of cirrhosis complications (HCC diagnosis and endoscopic treatment of esophageal varices).

Ethics
Vall d'Hebron University Hospital Campus IRB approved the study protocol (code PR(AG)461/2021). All patients provided informed consent.

Statistical analyses
Continuous variables are presented as means (SD) or medians (IQR) as appropriate. Categorical variables are presented as frequencies and percentages.

Primary and secondary outcomes were compared for two periods: Pre-pandemic *vs.* Pandemic. Continuous variables were compared using paired *t* tests or the Wilcoxon matched-pairs signed-rank test, according to the normality of their distribution. On the other hand, categorical variables were compared by performing tests on the equality of proportions.

Incidence rate ratios were estimated for the Pandemic period and compared with the Pre-pandemic period using indicator variables. A logistic regression analysis was performed to identify risk factors associated with the development of a first LRE during the Pandemic period. We graphed Kaplan-Meier survival curves for the first LRE and a log-rank test was performed. All analyses were performed in Stata 13.1 Statistical Software (StataCorp, College Station, TX, USA).

Results
Sample
The study cohort comprised 354 persons with compensated NAFLD cirrhosis, 271/354 (76.5%) of whom had no history of decompensation, while 83 individuals (23.5%) had presented with a prior episode(s) of hepatic decompensation. Individuals with a Child-Pugh A score represented 86.9% of the sample, while those with Child-Pugh B and C represented 12.1% and 1%, respectively. The diagnosis of NAFLD cirrhosis was established by liver biopsy in 106 patients (35.6%), whereas 103 (29.1%) and 125 (35.3%) individuals were classified as having cirrhosis based on liver stiffness ≥18 kPa by VCTE or signs of portal hypertension, respectively. Of note, median follow-up time from the diagnosis of NAFLD cirrhosis was 2.54 years (IQR 1.23–5.13).

Baseline characteristics
Table 1 shows the main characteristics from the entire cohort. Mean age was 67.3 years (SD 9.6) and 48.3% were female. Seventy-six patients (21.5%) presented non-harmful alcohol consumption and 17.7% were active smokers. Median BMI was 31.2 kg/m^2 (IQR 27.6–35.1) and 57.8% were obese (BMI ≥30 kg/m^2). T2D was present in 72.9% of patients, while 70.9% and 51.1% had arterial hypertension and dyslipidemia, respectively. At baseline, 87% of patients were classified as Child-Pugh A.

Individuals with and without prior decompensations showed similar demographic and metabolic comorbidity rates, including overweight and obesity prevalence. Mean values of VCTE (liver stiffness and controlled attenuation parameter) were also comparable between groups.

Comparison before and after the pandemic outbreak
As shown in Table 2, platelet count, bilirubin, and renal function worsened during the Pandemic period in the overall cohort. Accordingly, Fibrosis-4 (FIB-4) and model for end-stage liver disease score showed higher values after the outbreak of the pandemic. Median time between blood analyses was 17.1 months (IQR 12.4–20.1). No changes were observed regarding transaminase levels, mean glucose or lipid profile. Paired individual before-after VCTE data were available in only 10.1% of the overall study cohort. No differences were found in liver stiffness and controlled attenuation parameter values between study periods.

Clinical outcomes in people with compensated NAFLD cirrhosis without prior decompensations
During the Pandemic period, 28 individuals (11.3%) presented a first hepatic event compared to 7.4% (20/271) before the pandemic outbreak (p = 0.12). The most frequent liver event was ascites in both periods (Table 3).

Table 1. Baseline characteristics of 354 people with compensated NAFLD cirrhosis included in the study.

	Overall n = 354	Without prior decompensations n = 271	With prior decompensations n = 83	*p* value
Age, mean years (SD)	67.3 (9.6)	66.9 (9.2)	68.8 (10.5)	0.11
Females, n (%)	171 (48.3)	132 (48.7)	39 (47.0)	0.78
Tobacco use, n (%)	38 (10.8)	27 (10.1)	11 (13.3)	0.41
Alcohol use, n (%)*	76 (21.5)	60 (22.1)	16 (19.3)	0.57
BMI, median kg/m^2 (IQR)	31.2 (27.6-35.1)	31.8 (27.8-35.3)	30.2 (26.9-32.9)	0.023
BMI ≥25 kg/m^2, n (%)	302 (92.9)	240 (94.1)	62 (88.6)	0.10
BMI ≥30 kg/m^2, n (%)	188 (57.8)	150 (58.8)	38 (54.3)	0.49
Arterial hypertension, n (%)	251 (70.9)	193 (71.2)	58 (69.9)	0.81
T2D, n (%)	258 (72.9)	196 (72.3)	62 (74.7)	0.67
Dyslipidaemia, n (%)	181 (51.1)	142 (52.4)	39 (47.0)	0.38
Previous stroke, n (%)	16 (4.5)	14 (5.2)	2 (2.4)	0.29
Previous ischemic heart disease, n (%)	35 (9.9)	26 (9.6)	9 (10.8)	0.73
Child-Pugh score A/B/C, n (%)	287 (86.9)/40 (12.1)/3 (1.0)			
Liver stiffness, mean kPa (SD)**	23.6 (14.8)	22.9 (13.8)	33.9 (25.4)	0.10
CAP, mean dB/m (SD)***	307.0 (58.0)	308.1 (57.6)	281.3 (77.6)	0.43

Hypertension: ≥140/90 mmHg or requiring treatment; type 2 diabetes: as a fasting plasma glucose ≥126 mg/dl or a non-fasting plasma glucose ≥180 mg/dl or requiring treatment.; dyslipidemia: serum triglycerides ≥150 mg/dl and/or total cholesterol >200 mg/dl, LDL >130 mg/dl, HDL<40 mg/dl in men and <50 mg/dl in women or requiring treatment.
Continuous variables were compared using *t* test or Wilcoxon rank-sum, depending on the normality of their distribution. Categorical variables were compared using the chi-square test. A p <0.05 was considered statistically significant.
CAP, controlled attenuation parameter; T2D, type 2 diabetes.
* Alcohol intake was defined as <20 g/day and <30 g/day for women and men, respectively.
** Data available in 83 individuals.
*** Data available in 75 individuals.

Table 2. Changes in biochemical and non-invasive tests before and after the outbreak of the COVID-19 pandemic.

	Overall, n = 354			Without prior decompensations, n = 271			With prior decompensations, n = 83		
	Pre-pandemic	Pandemic	*p* value	Pre-pandemic	Pandemic	*p* value	Pre-pandemic	Pandemic	*p* value
Platelets (10^9/L)	143 (68)	138 (67)	0.02	152 (70)	148 (68)	0.13	111 (50)	103 (50)	0.16
INR	1.15 (0.25)	1.17 (0.38)	0.33	1.14 (0.27)	1.14 (0.35)	0.94	1.19 (0.17)	1.27 (0.44)	0.07
Glucose (mg/dl)	141.1 (60.9)	146.7 (65.9)	0.25	143.9 (66.8)	148.3 (71.7)	0.48	132.5 (37.3)	142.1 (44.8)	0.19
Creatinine (mg/dl)	0.91 (0.47)	0.96 (0.57)	0.002	0.87 (0.35)	0.91 (0.48)	0.042	1.06 (0.73)	1.16 (0.79)	0.004
Bilirubin (mg/dl)	0.94 (0.55)	1.06 (1.21)	0.022	0.86 (0.48)	0.91 (0.53)	0.019	1.22 (0.69)	1.63 (1.34)	0.09
AST (U/L)	38.7 (17.8)	39.3 (22.2)	0.61	38.0 (17.5)	37.8 (18.9)	0.82	41.3 (18.7)	44.7 (31.0)	0.35
ALT (U/L)	34.7 (22.8)	33.4 (22.7)	0.32	35.2 (21.3)	33.4 (21.0)	0.14	33.2 (27.8)	33.3 (28.4)	0.98
Total CT (mg/dl)	166.3 (39.1)	168.1 (41.6)	0.3	168.0 (38.9)	171.4 (39.8)	0.054	159.9 (39.4)	155.8 (45.9)	0.41
HDL (mg/dl)	49.5 (17.2)	51.0 (17.9)	0.24	49.3 (17.4)	51.4 (17.9)	0.09	51.6 (15.5)	47.6 (18.1)	0.34
LDL (mg/dl)	92.8 (34.8)	92.9 (34.0)	0.95	92.9 (33.9)	93.3 (33.3)	0.82	92.4 (42.5)	90.0 (39.8)	0.84
Triglycerides (mg/dl)	141.5 (94.8)	142.1 (85.9)	0.88	147.3 (100.4)	150.7 (92.0)	0.47	120.1 (66.8)	110.3 (46.3)	0.13
Albumin (g/dl)	4.10 (0.52)	4.07 (0.58)	0.14	4.20 (0.48)	4.17 (0.53)	0.25	3.76 (0.53)	3.71 (0.64)	0.36
FIB-4 score	4.16 (2.93)	4.57 (3.82)	0.003	3.72 (2.51)	4.00 (3.29)	0.047	5.74 (3.71)	6.64 (4.79)	0.026
MELD score	7.67 (3.00)	8.16 (4.01)	0.001	7.36 (2.70)	7.55 (3.11)	0.12	8.77 (3.69)	10.35 (5.77)	0.001
Liver stiffness (kPa)*	22.3 (13.5)	22.8 (16.6)	0.84						
CAP (dB/m)**	315.0 (61.8)	293.8 (85.2)	0.14						

Data are presented as mean (SD). Continuous variables were compared using paired *t* tests or the Wilcoxon matched-pairs signed-rank test, according to the normality of their distribution. On the other hand, categorical variables were compared by performing tests on the equality of proportions. A p <0.05 was considered statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CT: cholesterol; FIB, Fibrosis-4; INR, international normalized ratio; MELD, model for end-stage liver disease.

* Data available in 35 individuals.

** Data available in 30 individuals.

No statistical differences were found when comparing the incidence of CV events between study subperiods (6/271 *vs.* 3/262; p = 0.24). The overall mortality rate before the pandemic outbreak was 3.3%, whereas it was 6.1% (16/262) during the Pandemic period (p = 0.12). Of note, during the latter 9/16 deaths were due to COVID-19.

Meanwhile, the incidence of overall events (LRE, CV event, and/or death from any cause) during the Pandemic period was significantly higher than that of the Pre-Pandemic period (17.8% *vs.* 9.9%, respectively; p = 0.009). The cumulative incidence of first LRE is shown in Fig. 1.

Secondary outcomes amongst the entire cohort of people with compensated NAFLD cirrhosis

As secondary outcomes, we assessed changes in the metabolic status, potential deferrals in the management of cirrhosis hallmarks (*i.e.*, esophageal varices or HCC) and the occurrence of clinical events (Table 4).

Although no significant differences were found in individual metabolic comorbidities between both subperiods, the rate of worse overall metabolic status was significantly higher in the Pandemic period (38.4% *vs.* 46.1% p = 0.041). No differences were found regarding HCC diagnostic delay and esophageal varices treatment.

No differences were found between periods when comparing the global number of patients that presented with any type of LRE nor by specific decompensating event. One patient underwent a liver transplant during the Pandemic period. The baseline characteristics of the entire study cohort according to Child-Pugh classification (A *vs.* B–C) are provided in the supplementary information.

Table 3. Clinical outcomes amongst people with NAFLD cirrhosis without prior decompensations.

Outcomes	Pre-pandemic	Pandemic	*p* value
First liver-related event, n (%)	20/271 (7.4)	28/247 (11.3)	0.12
Type of first LRE, n (% to all LRE)			
Ascites	10 (50.0)	17 (60.7)	
Hepatic encephalopathy	4 (20.0)	5 (17.8)	
Upper gastrointestinal bleeding	1 (5.0)	0 (0)	
HCC	5 (25.0)	6 (21.4)	
CV events, n (%)	6/271 (2.2)	3/262 (1.1)	0.24
Type of CV event, n (% to all CV)			
Cerebrovascular	1 (16.6)	1 (33.3)	
Ischemic heart disease	3 (50.0)	1 (33.3)	
Others	2 (33.3)	1 (33.3)	
Mortality, n (%)	9/271 (3.3)	16/262 (6.1)	0.12
Cause of death, n (% to all death)			
Liver-related	5 (55.5)	1 (6.2)	
CV	0	1 (6.2)	
Extrahepatic cancer	1 (11.1)	2 (12.5)	
COVID-19	0	9 (56.2)	
Other	3 (33.3)	3 (18.7)	
Composite endpoint (any clinical outcome), n (%)	27/271 (9.9)	44/247 (17.8)	0.009

All comparisons were performed using the test on the equality of proportions. A p <0.05 was considered statistically significant.
CV, cardiovascular; HCC, hepatocellular carcinoma; LRE, liver-related event.

Multivariable analysis of risk factors for LRE

Finally, we performed a logistic regression analysis to identify predictors of presenting a first LRE amongst people with cirrhosis without prior decompensations during the Pandemic period. As shown in Table 5, worsening of metabolic status during the Pandemic period was not associated with the development of a first LRE. However, T2D (odds ratio [OR] 3.77; 95% CI 1.15–12.32; p = 0.028), albumin <4 g/L (OR 4.43; 95% CI 1.76–11.17; p = 0.002) and FIB-4 score >2.67 (OR 15.74; 95% CI 2.01–123.22; p = 0.009) were identified as risk factors for a first LRE in the multivariable analysis.

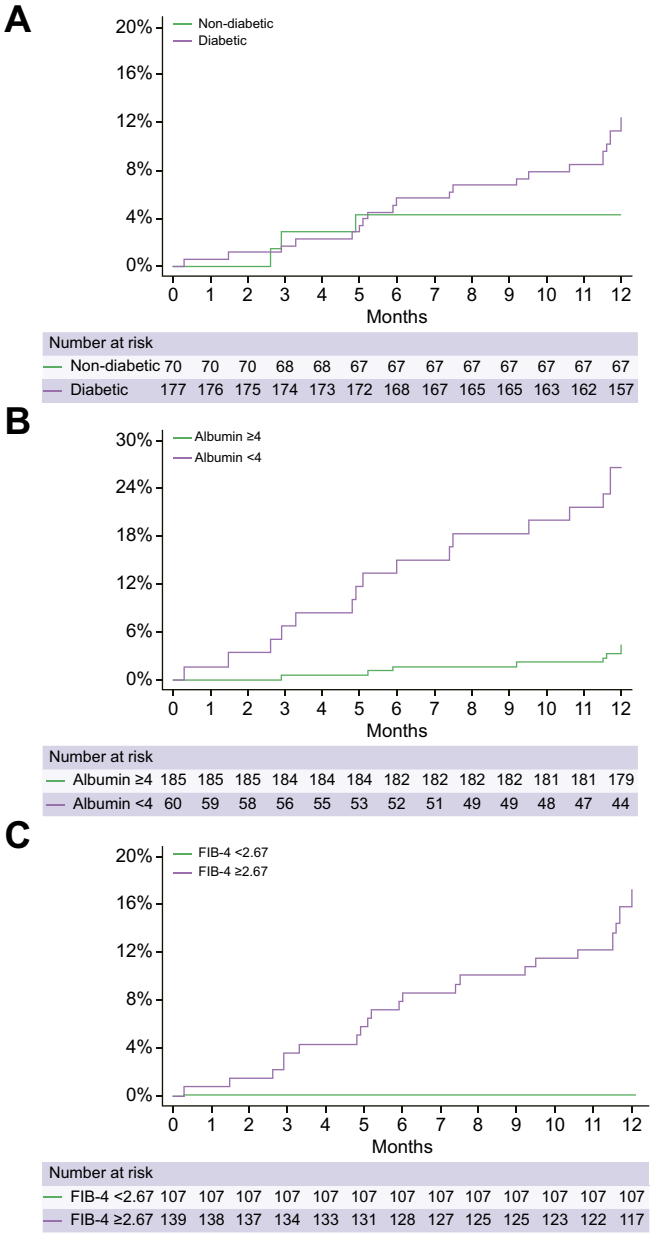

Fig. 1. Kaplan-Meier survival curves showing first liver-related events during the Pandemic period in people with compensated NAFLD cirrhosis without prior decompensations (n = 271). (A) By type 2 diabetes status (log-rank test = 0.08); (B) albumin serum levels ≥4 (log-rank test <0.001); and (C) FIB-4 score ≥2.67 (log-rank test <0.01). The equality of survivor functions was tested with the log-rank test and a p <0.05 was considered statistically significant. FIB-4, Fibrosis-4; NAFLD, non-alcoholic fatty liver disease.

Discussion

In the present study, we analysed a well-characterized multicentric cohort to investigate the impact of the COVID-19 pandemic on a particularly vulnerable population, namely people with cirrhosis due to NAFLD. Three hundred and fifty-four people with NAFLD cirrhosis were evaluated during two subperiods, from March 2019 to February 2020 (Pre-pandemic period) and between March 2020-February 2021 (Pandemic period). We observed that the proportion of people with compensated NAFLD cirrhosis presenting any clinical outcome (liver, CV event and/or death due to any cause) during the Pandemic period was higher than in the pre-Pandemic period, however this was due mostly to non-liver events and in particular to COVID-19 deaths. Moreover, worsening of metabolic status was not identified as a risk factor for a first cirrhosis decompensation.

The primary outcome we investigated was the incidence of a first LRE amongst people with compensated cirrhosis, since they comprise the bulk of NAFLD cirrhosis globally and therefore our findings could have informed strategies to prevent hepatic decompensation. In addition, we hypothesized that an overall lack of physical exercise, poor diet adherence, alcohol consumption, weight gain and psychological distress during the first year of the pandemic might have led to worsening of metabolic status in a significant proportion of patients, and this could be a major trigger of first LRE. However, we found that the incidence of LRE was similar between periods in compensated patients. No significant differences were found between periods when analyzing the incidence of LRE, CV events and mortality separately in the entire cohort (*i.e.*, also including patients previously decompensated at baseline). Conversely, a significantly higher proportion of the overall cohort presented impaired metabolic control during the pandemic. However, we did not find an independent association between a first LRE and metabolic worsening, which is in disagreement with prior reports.[17,18,29] We believe this could be partially explained by the relatively small number of events occurred during the study period and also because although metabolic status worsened overall none of its components separately worsened in a significant manner. Further prospective studies that systematically collect metabolic data on people with NAFLD and evaluate the longitudinal changes along the Pandemic period are needed.

On the other hand, when analyzing the occurrence of any clinical outcome together (LRE, CV and/or death) we observed that compensated patients were more likely to present an event during the pandemic with respect to the Pre-Pandemic period. This is in line with the observed worsening in liver function and renal parameters, which are well-known predictors of hepatic and extrahepatic events in cirrhosis, including NAFLD.[30] Yet, two observations prevent us from drawing clear conclusions. First, worsening liver and renal parameters mostly relied on previously decompensated patients, which is consistent with the natural history of the disease and might not be associated with the pandemic. Second, if it were not for the 9 deaths due to COVID-19, the rates of overall events would not have reached statistical significance and would actually have been similar. Therefore, we cannot conclude that the first year of the pandemic and its potentially associated factors had a strong impact on NAFLD outcomes other than the mortality induced by the viral infection itself, as previously described.[6,7] We believe that the enormous effort of all the healthcare professionals in these hospitals ensured that a high-quality clinical service was

Table 4. Metabolic and clinical outcomes before and after the outbreak of the COVID-19 pandemic.

	Overall, n = 354			Without prior decompensation, n = 271			With prior decompensation(s), n = 83		
Outcomes	**Pre-pandemic**	**Pandemic**	***p* value**	**Pre-pandemic**	**Pandemic**	***p* value**	**Pre-pandemic**	**Pandemic**	***p* value**
Metabolic status									
Significant weight gain, n (%)	40/331 (12.0)	37/247 (14.9)	0.31	32/259 (12.3)	30/197 (15.2)	0.37	8/72 (11.1)	7/50 (14.0)	0.63
Poor control of T2D, n (%)	49/353 (13.8)	44/286 (15.3)	0.59	39/271 (14.4)	38/224 (16.9)	0.43	10/82 (12.2)	6/62 (9.6)	0.63
Poor control of arterial hypertension, n (%)	13/354 (3.6)	20/301 (6.6)	0.08	11/271 (4.0)	15/251 (6.0)	0.31	2/83 (2.4)	5/70 (7.1)	0.06
Poor control of dyslipidaemia, n (%)	80/354 (22.6)	76/333 (22.8)	0.94	68/271 (25.1)	65/261 (24.9)	0.96	12/83 (14.4)	11/72 (15.2)	0.88
Overall worsening of metabolic status, n (%)	136/354 (38.4)	154/334 (46.1)	0.041	111/271 (40.9)	123/262 (46.9)	0.16	25/83 (30.1)	31/72 (43.0)	0.09
Delayed outcomes, n (%)	1/354 (0.3)	4/334 (1.2)	0.006	0	3/262 (1.15)	*	1/83 (1.2)	1/72 (1.4)	0.3
Type of delayed outcomes, n (% to all outcomes)									
Delayed HCC diagnosis, n (%)	0	4 (100)	*	0	3 (100)	*	0	1 (100)	*
Delayed varices treatment, n (%)	1 (100)	0	*	0	0	*	1 (100)	0	*
Type of LRE									
Ascites, n (%)	36/354 (10.1)	31/309 (10.0)	0.95	12/271 (4.4)	21/255 (8.2)	0.07	24/83 (28.0)	10/54 (18.5)	0.16
HE, n (%)	29/354 (8.1)	23/315 (7.3)	0.66	9/271 (3.3)	10/257 (3.8)	0.72	20/83 (24.1)	13/58 (22.4)	0.81
Upper gastrointestinal bleeding, n (%)	8/354 (2.2)	8/327 (2.4)	0.87	3/271 (1.1)	3/260 (1.1)	0.91	5/83 (6.0)	5/62 (7.4)	0.63
SBP, n (%)	4/354 (1.1)	7/333 (2.1)	0.21	2/271 (0.7)	3/262 (1.1)	0.43	2/83 (2.4)	4/71 (5.6)	0.09
HCC, n (%)	9/354 (2.5)	9/326 (2.7)	0.85	5/271 (1.8)	8/257 (3.1)	0.34	4/83 (4.8)	1/69 (1.4)	0.02
Liver transplant, n (%)	0	1 (100)	*	0	1 (100)	*	0	0	*
Total individuals with any LRE, n (%)	59/354 (16.7)	65/334 (19.4)	0.34	20/271 (7.3)	35/262 (13.3)	0.02	39/83 (46.9)	30/72 (41.6)	0.50
CV events, n (%)	9/354 (2.5)	6/334 (1.8)	0.5	6/271 (2.2)	3/262 (1.1)	0.24	3/83 (3.6)	3/72 (4.1)	0.8
Type of CV event, n (% to all CV)									
Stroke	1 (11.1)	1 (16.6)		1 (16.6)	1 (33.3)		0	0	
Ischemic heart disease	5 (55.5)	1 (16.6)		3 (50.0)	1 (33.3)		2 (66.6)	0	
Other	3 (33.3)	4 (66.6)		2 (33.3)	1 (33.3)		1 (33.3)	3 (100)	
Mortality, n (%)	20/354 (5.6)	28/334 (8.3)	0.15	9/271 (3.3)	16/262 (6.1)	0.12	11/83 (13.2)	12/72 (16.6)	0.55
Cause of death, n (% to all death)									
Liver-related	10 (50.0)	7 (25.0)		5 (55.5)	1 (6.2)		5 (45.4)	6 (50)	
CV	2 (10.0)	2 (7.1)		0	1 (6.2)		2 (18.1)	1 (8.3)	
Extrahepatic cancer	1 (5.0)	3 (10.7)		1 (11.1)	2 (12.5)		0	1 (8.3)	
COVID-19	0 (0)	9 (32.1)		0	9 (56.2)		0	0	
Other	7 (35.0)	7 (25.0)		3 (33.3)	3 (18.7)		4 (36.3)	4 (33.3)	
Composite endpoint (any clinical outcome), n (%)	71/354 (20.0)	82/334 (24.5)	0.15	29/271 (10.7)	49/262 (20.0)	0.009	42/83 (50.6)	33/72 (45.8)	0.55

All comparisons were performed using the test on the equality of proportions. A p <0.05 was considered statistically significant.
CV, cardiovascular; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; LRE, liver-related event; SBP, spontaneous bacterial peritonitis; T2D, type 2 diabetes.

maintained for people with cirrhosis. This is supported by the lack of differences in delayed diagnostic and therapeutic measures between the two periods.

We found that T2D, albumin levels and FIB-4 score were independently associated with the development of a first LRE in compensated patients during the first year of the COVID-19 pandemic. Our results are consistent and reproduce previous findings, where metabolic comorbidities, decline in serum albumin concentration and serologic non-invasive tests have proven to predict clinical events in people with cACLD due to NAFLD.[30–32]

Our results underscore the vulnerability of people with NAFLD cirrhosis and the importance of the healthcare system, from primary care to liver clinics, in their care. In order to avoid deleterious impacts of future healthcare crises, whatever the cause, healthcare providers and policymakers, alongside the patients and their communities, should advocate for health educational programs, community health interventions including screening and early diagnosis, e-health systems, and other measures that make people with cirrhosis less dependent on specialized care. Liver specialists should continue to play a key role in the follow-up and management of these patients, but

Table 5. Risk factors associated with the development of a first liver-related event during the pandemic period amongst people with NAFLD cirrhosis without prior decompensations.

	Univariate regression			Multivariable regression		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age	0.99	0.92-1.08	0.96			
Female sex	3.21	0.82-1.03	0.09	0.49	0.19-1.23	0.13
Arterial hypertension	1.03	0.28-3.74	0.95			
T2D	0.10	0.01-0.52	0.007	3.77	1.15-12.32	0.028
Dyslipidaemia	1.95	0.67-6.17	0.25			
Metabolic status worsening	2.65	0.78-8.88	0.11			
BMI	0.92	0.82-1.03	0.16			
Creatinine	1.88	0.12-28.77	0.64			
Albumin*	0.21	0.06-0.73	0.014	4.43	1.76-11.17	0.002
Bilirubin	0.62	0.13-2.86	0.54			
MELD score	0.87	0.61-1.24	0.46			
FIB-4 score**	1.49	1.18-1.89	0.001	15.74	2.01-123.22	0.009

Results from univariate and multivariable regression analysis. A p <0.05 was considered statistically significant.
MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; T2D, type 2 diabetes.
* Albumin cut-off <4.0 g/dl.
** FIB-4 score cut-off >2.67.

sustainable models for both the patients, the healthcare systems and the taxpayers that rely on transversal multidisciplinary teams are increasingly necessary to cope with the mounting complexity surrounding the care of people with cirrhosis. Meanwhile, contingency plans to face further pandemic waves, relying on a smooth coordination between the primary and the tertiary setting and on improved referral pathways, are essential.

Our study is constrained by several limitations. First, the low number of clinical events, likely determined by the short study period and the sample size analyzed. On the other hand, it is worth highlighting that the first and second COVID-19 waves (from March to December 2020, approximately) were particularly intense in Catalonia. Consequently, the overwhelmed healthcare system missed relevant information regarding non-fatal events or metabolic status during several months, thus likely leading to an underestimation of events. Moreover, information regarding non-invasive tests such as VCTE in the Pandemic period is limited due to restriction in routine tests until the end of 2020, hampering the utilization of liver stiffness data in the analyses of risk factors of first LRE.

In our study, people with cirrhosis due to NAFLD did not present a higher rate of LREs during the first year of the COVID-19 pandemic. Diabetes, lower albumin and higher FIB-4 were associated with a higher risk of a first LRE. Longitudinal studies with larger sample sizes are needed to assess the specific impact of the pandemic on people with NAFLD cirrhosis. Regardless of the epidemiological situation, it is fundamental to ensure a proper surveillance of people with cirrhosis and early management of complications.

Abbreviations

cACLD, compensated advanced chronic liver disease; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; T2D, type 2 diabetes; VCTE, vibration-controlled transient elastography.

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Conflicts of interest

TB has received educational and research support from Gilead and Abbvie. JMP reports having received consulting fees from Boehringer Ingelheim and Novo Nordisk. He has received speaking fees from Gilead, and travel expenses from Gilead, Rubió, Pfizer, Astellas, MSD, CUBICIN, and Novo Nordisk. He has received educational and research support from Gilead, Pfizer, Astellas, Accelerate, Novartis, Abbvie, ViiV, and MSD, and funds from European Commission/EFPIA IMI2 853966-2, IMI2 777377, H2020 847989, and ISCIII PI19/01898. Other authors: nothing to disclose. None of the authors have any personal conflict with regards to the present manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conceptualization and design: JRE, RM, JMP; Data collection: JRE, RM, TB, ISM, OB, ASV, GE, JC, JB; Drafting of first manuscript: JRE, JMP; Data analyses: JRE, RM; Data interpretation: JRE, RM, TB, JCar, PG, IG, JMP; Critical revision of the manuscript: RM, TB, ISM, OB, ASV, GE, JC, JB, PG, IG; Supervision: TB, ISM, PG, IG, JMP; Access and verification of data: JMP. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Data availability statement

De-identified data will be shared upon request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100574.

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10.2.3. Publicación anexa 3

Rojano-Toimil A, Rivera-Esteban J, Manzano-Nuñez R, Bañares J, Martinez Selva D, Gabriel-Medina P, Ferrer R, Pericàs JM, Ciudin A. When Sugar Reaches the Liver: Phenotypes of Patients with Diabetes and NAFLD. J Clin Med. 2022 Jun 8;11(12):3286.

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Review

When Sugar Reaches the Liver: Phenotypes of Patients with Diabetes and NAFLD

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Abstract: Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) have been traditionally linked to one another. Recent studies suggest that NAFLD may be increasingly common in other types of diabetes such as type 1 diabetes (T1DM) and less frequently ketone-prone and Maturity-onset Diabetes of the Young (MODY) diabetes. In this review, we address the relationship between hyperglycemia and insulin resistance and the onset and progression of NAFLD. In addition, despite the high rate of patients with T2DM and other diabetes phenotypes that can alter liver metabolism and consequently develop steatosis, fibrosis, and cirrhosis, NALFD screening is not still implemented in the daily care routine. Incorporating a clinical algorithm created around a simple, non-invasive, cost-effective model would identify high-risk patients. The principle behind managing these patients is to improve insulin resistance and hyperglycemia states with lifestyle changes, weight loss, and new drug therapies.

Keywords: type 2 diabetes; type 1 diabetes; ketone-prone diabetes; MODY diabetes; NAFLD; NASH; liver fibrosis

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in Western countries. Its incidence is expected to keep growing, parallel to the incidence of metabolic syndrome (MetS) and its determinants. It is estimated that NAFLD affects 25–35% of adults in the general population, and its prevalence increases to 60–80% in obese and diabetic patients [1]. The spectrum of the disease ranges from mild hepatic steatosis and liver inflammation (non-alcoholic steatohepatitis or NASH) to liver fibrosis and cirrhosis that lead to liver-related events and increased overall mortality [2].

Several studies demonstrate that insulin resistance (IR) plays a critical role in NAFLD pathophysiology and natural history of the disease [3]. The accelerated lipolysis associated with IR increases hepatic glucose production in NAFLD patients, which upregulates de

novo fat synthesis, accelerating NAFLD progression [4]. Other pathogenic pathways such as alterations in lipid metabolism, mitochondrial leakage, and pro-inflammatory cascades' activation are described in disease progression [5]. Although several drugs have demonstrated efficacy in NASH improvement in early-stage trials [6,7], at present, there are no US Food and Drug Administration (FDA) or European Medicines Agency (EMA) approved drugs for NAFLD.

Within the MetS spectrum, the bulk of research addresses the relationship between either type 2 diabetes (T2DM) or obesity with NAFLD [8–10]. However, a growing body of evidence shows that NAFLD is also prevalent in a variety of other forms of diabetes that typically have an earlier onset, such as T1DM, MODY and ketosis-prone diabetes. The lack of clinical awareness of the hepatic disease in these populations added to the scarce research in this area has resulted in the absence of protocols for screening and underdiagnosis of hepatic disease.

This review describes the relationship between DM and non-alcoholic fatty liver disease, the pathophysiological rationale behind this relationship, and its clinical relevance and outcomes. Figure 1 reflects the main mechanisms involved in the physiopathology of NAFLD in different types of diabetes.

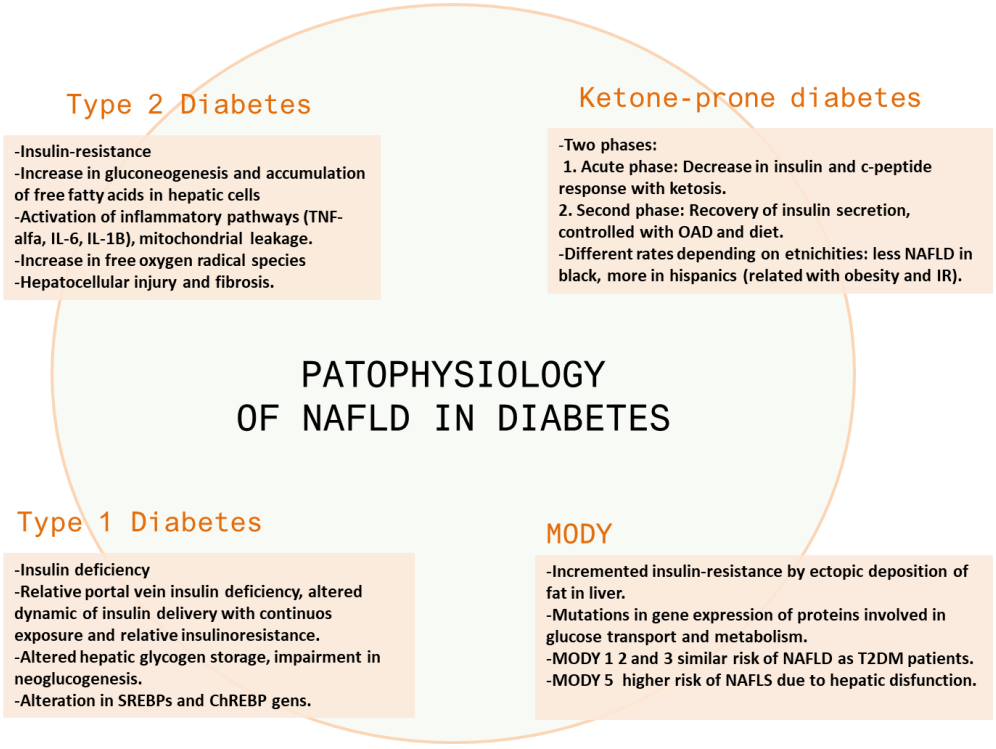

Figure 1. Main mechanisms involved in the physiopathology of NAFLD in different types of diabetes. OAD: oral antidiabetic drugs.

We furthermore propose a diagnostic algorithm to optimize diagnosis and contemporize treatment.

2. Methodology

A literature review was performed, including the information obtained from the search in the English language of 263 studies published in Ovid MEDLINE, PubMed, EMBASE, and Cochrane database. We defined as the primary outcome to compare between the different DM phenotypes related with NAFLD and the management and screening approaches. The search terms entered were 'T2DM', 'Obesity', 'ketone-prone diabetes', 'atypical diabetes', 'MODY', 'mature-onset of the young diabetes', 'type 2 diabetes', 'diabetes mellitus' 'T1DM', 'NAFLD', 'non-alcoholic fatty liver disease', 'NASH', 'non-alcoholic

steatohepatitis', and 'fatty liver'. Included studies were RCT, cross-sectional, longitudinal, or descriptive studies published in peer-reviewed journals between 1987 and January 2022. Inclusion criteria were as follows: published in English, peer-reviewed, addressed NAFLD and T2DM, T1DM, MODY, and ketone-prone DM. Exclusion criteria included abstract or non-peer reviewed articles or studies that were not in the English language or not related to the topic. A total of 197 articles out of 263 were included in the present review after adjusting by inclusion/exclusion criteria.

3. Diabetes Clinical Phenotypes

3.1. Type 2 Diabetes and Obesity—The Metabolic Syndrome Paradigm

3.1.1. Epidemiology

Type 2 diabetes is the most common form of diabetes. It has an estimated prevalence of 26.9 million people of all ages in the US (or 8.2% of the US population) and 536.6 million people worldwide [11,12]. In Spain, the incidence of DM obtained from the di@bet.es study cohort was estimated at 11.6 cases/1000 person-year (OR (95% CI) 11.1–12.1) [13]. T2DM is characterized by hyperglycemia, IR, and relative insulin deficiency [14]. The primary risk factors associated are those related to lifestyle behaviors such as poor physical inactivity and dietary habits, cigarette smoking, and alcohol consumption [15]. Moreover, overweightness and obesity contribute to approximately 89% of cases of T2DM [11].

T2DM has become an epidemic issue in occidental countries. Due to its asymptomatic onset, it is frequently diagnosed at later stages when micro and macrovascular complications are established [16]. Optimization of management of T2DM, screening in primary care, and advances in research have allowed a decrease in the associated complications [17]. In addition, obesity is directly linked to NAFLD due to IR and metabolic syndrome [18,19].

The 2020 Center for Disease Control and Prevention (CDC) report revealed 34.1 million patients diagnosed with diabetes among adults aged 18 years or older in the United States, with type 2 diabetes accounting for 90% to 95% of cases. Among the reported cases, 45.8% of individuals had obesity (body mass index (BMI) of 30.0 to 39.9 kg/m^2), and 15.5% presented extreme obesity (BMI of 40.0 kg/m^2 or higher) [11].

In global studies, the prevalence of T2DM is estimated at 10.5% [12], which has steadily increased during the last two decades due to changes in nutrition and physical exercise in Western countries. According to current predictions, the incidence of T2DM is expected to rise from 171 million in 2000 to 366 million individuals by 2030 [20]. One of the main drivers of such worrying forecasting is the increased prevalence of childhood obesity, with approximately 35.1% and 26.5% of children being overweight or obese in the US, respectively [21]. Social determinants of health such as gender, race/ethnicity, and socioeconomic status expose significant disparities amongst T2DM patients. For example, Black and Hispanic children have higher risk-adjusted obesity odds when compared to Asians or Caucasians [22]. Similar results are described for T2DM, with the highest prevalence among people of Hispanic origin (12.5%) and Blacks (11.7%) [11]. Low socioeconomic status has been found also associated with poorer outcomes [23]. Lifestyle behaviors, e.g., physical inactivity, smoking, or alcohol consumption, are also strong drivers of T2DM, independently of genetic predisposition [15,24].

3.1.2. Pathophysiology

Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) have been traditionally linked to one another. The main pathophysiological link between T2DM and NAFLD is IR [25], and hepatic steatosis and fibrosis are also related to the development of IR, which substantially increases the risk of subsequent T2DM [3]. On one hand, hyperglycemia was demonstrated to be a hazard in hepatic steatosis and fibrosis development: several in vitro models demonstrate the role of hyperglycemia in the process, e.g., Robin C et al. in 2017, demonstrated that hyperglycemic cell culturing conditions induced steatosis within a human hepatocyte cell line (HepG2 cells) by the accumulation of intracellular lipids [26]. Experimental models, such as in streptozotocin-induced diabetic models in

mice, show that progression in NAFLD is due to hyperglycemia-induced inflammation [27]. An increase in glucose substrate in the hepatocyte promotes the accumulation of free fatty acids in the cell and the stimulation of hepatic lipogenesis by upregulation of the Krebs cycle and an increase in the expression of ChREBPnad liver X receptor alfa. This cascade activates downstream fibrogenic pathways with oxidant stress and inflammasome activation that leads to cell apoptosis by cytokines such as IL-1B, IL-6, or TNF-alfa that finally result in inflammation, hepatocyte injury, and liver fibrosis [28–30]. On the other hand, the excess of triglyceride synthesis is another pathophysiological landmark of NAFLD. IR was proven to be responsible for an increase in adiposity in hepatic cells, as demonstrated in studies with the hyperinsulinemic-euglycemic clamp [31] and indirectly measured through the HOMA-IR index [32]. These changes result in a boost of gluconeogenesis cascades, an increase in free fatty acid (FFA) levels, and simultaneously impairment in hepatic glycogen synthesis. These metabolic changes lead to liver fat accumulation, resulting in a preferential shift from carbohydrate to FFA beta-oxidation [33]. FFA accumulation and IR activate several inflammatory pathways related to pro-inflammatory cytokines such as TNFα or IL-6 [34]. Other extrahepatic drivers of liver inflammation include IL-1B, or IL-6 liberated from adipose tissue in obese individuals [35]. These cytokines create a pro-inflammatory environment producing hepatotoxic free oxygen radical species that result in hepatocellular injury and fibrosis [36].

Despite of that, not all patients with NASH exhibit IR. This suggests that other factors may influence the progression to NASH. For example, genetic variants are strongly associated with histologic severity. Petersen et al. demonstrated that specific polymorphisms in the gene encoding for apolipoprotein C3 are markers of hepatic IR and inflammation [37], such as other research related IL-6 polymorphisms [38]. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a lipid droplet-associated protein that is increased in obese patients. Higher levels are associated with augmented liver fat content [39]. Finally, hepatic lipid metabolism disruptions, inflammation in adipose tissue, and ectopic sites of fat deposition interfere with normal hepatic function and give way to excess storage of lipid droplets in hepatocytes [40]. In fact, de novo lipogenesis is threefold higher in patients with NAFLD [41]. In addition, hepatocellular FFA accumulation is sustained by impaired synthesis and secretion of very-low-density lipoproteins and excessive importation of FFA from adipose tissue [42]. The activation of these cascades eventually leads to impaired antioxidant capacity with increased oxidative stress and mitochondrial function defects, leading to hepatic fibrosis and IR [36].

3.1.3. Clinical Manifestations

T2DM and obesity are among the most important risk factors of NASH, liver-related events, hepatocellular carcinoma (HCC), and mortality in patients with NAFLD [43–46]. The prevalence of NAFLD in T2DM patients is over 50%, and when both T2DM and obesity coexist, the prevalence of NAFLD ranges between 60% and 80% [47–50]. A global meta-analysis showed that T2DM was present in 22.51% of patients with radiologically defined NAFLD and 43.36% among patients with NASH determined by biopsy. Of note, 56% of the individuals with histologically proven NASH had normal liver enzymes [2,43]. Data recollected from the Edinburgh Type 2 Diabetes Study (ET2DS) registered a prevalence of 42.6% of NAFLD in patients with T2DM. Independent predictors were BMI, duration of diabetes, HbA1c, triglycerides, and metformin use [51]. These studies also showed that some factors play a critical role in the relationship between NAFLD and diabetes. For example, compared to T2DM only, patients with T2DM and NAFLD were more likely to present hypertension, dyslipidemia, and cardiovascular diseases [52] which means that the presence of NAFLD determines a higher risk of poor cardiovascular and metabolic outcomes, a fact that was proven in previous studies. Indeed, a meta-analysis including 16 studies representing more than 34,000 patients demonstrated that NAFLD was associated with a higher cardiovascular risk and conferred a more elevated risk adjusted-odds of cardiovascular events, including myocardial infarction and stroke [53].

Emerging data suggest that up to 20% of patients diagnosed with NASH will develop cirrhosis in the disease course [54]. The fibrosis stage is the main predictor of liver-related illness, liver transplantation, and liver-related mortality [2,43]. Both T2DM and overweight/obesity are predictors of advanced fibrosis and comorbidities in patients with NASH [45,55] with a >10-fold risk of HCC [56]. Remarkably, and contrary to other causes of chronic liver disease, HCC can develop in non-cirrhotic livers in the setting of NAFLD. In addition, patients with obesity have an increased incidence of HCC without cirrhosis compared with non-obese patients [57,58]. Numerous studies linked pro-inflammatory cytokines derived from IR and hyperinsulinemia to activation of carcinogenic pathways [59]. Yet, cardiovascular events such as stroke or myocardial infarction are the leading cause of death in patients with NAFLD [60]. NAFLD also is shown to worsen the prognosis of T2DM, e.g., to worsen glycemic control and microvascular complications such as nephropathy and retinopathy [61,62]. To remark, another factor with a close relationship with metabolic syndrome is the presence of hepatitis virus C infection (HCV). Recent evidence from prospective studies suggests that the treatment of HCV in NAFLD patients with and without fibrosis, would reduce the risk of T2DM by 81% by restoring normal glucose metabolism. The same effect was reported for cardiovascular events such as acute coronary syndrome, myocardial infarction, stroke, or transient ischemic attack, with a decrease between 56 and 75 cases per year for 10,000 HCV patients with long-term remission, independently of other risk factors such as smoking, dyslipidemia, or hypertension [63–65].

3.1.4. Management: Diagnosis and Interventions

All patients with NAFLD should be screened for T2DM, and anthropometric data must be recorded. Most T2DM patients are asymptomatic; thereby, early screening will help these patients prevent complications. The American Diabetes Association (ADA) criteria for the diagnosis of T2DM focus on the following tests [66]:

- Glycated hemoglobin (HbA1c) measures the average blood sugar levels over the prior 90 days. Hb1Ac 6.5% or higher is diagnostic of T2DM [67].
- A fasting plasma glucose of more than 126 mg/dL is a very specific parameter, but its sensitivity has been reported to be unsatisfactory due to false negatives among individuals with impaired glucose tolerance [68].
- The oral glucose tolerance test measures the blood sugar level before, and two hours after, 100 g glucose overload; the diagnosis is established when blood sugar is equal to or greater than 200 mg/dL [69].

In patients with symptoms suggestive of diabetes, a random plasma glucose test equal or greater to 200 mg/dL is sufficient for T2DM diagnosis. A three-year control at age 45 should be carried out according to the ADA, especially in people who are overweight or obese. These screenings should be conducted annually if risk factors are present: family background of DM, prediabetes, personal history of gestational diabetes, dyslipidemia, or age greater than 65 years old [66].

Although the concomitant occurrence of T2DM and hepatic disease is becoming increasingly common, both the ADA and European Association of Diabetes (EASD) recommendations for screening NALFD are still not implemented in the daily care routine. Incorporating a simple clinical diagnostic algorithm formed by a simple, non-invasive, and cost-effective test would allow for identifying patients who need a liver biopsy. Relying on a cohort of 1249 patients with histology-proven NASH, Bazick et al. developed a score to identify T2DM patients at risk of NASH, showing a specificity of 90.0% and a sensitivity of 56.8%. This score includes BMI, waist circumference, HbA1c, HOMA-IR, and serum levels of transaminases, albumin, and ferritin [70]. Other scoring systems implemented for the general population, such as FIB-4, with a sensitivity of 69% and specificity of 77% [71] and NAFLD fibrosis score [72], were demonstrated to be valuable tools for evaluating the risk of hepatic fibrosis [73,74] as first-line non-invasive tests and support decision-making regarding referral to a liver specialist and performing a second-line test such as transient elastography.

Abdominal ultrasonography is the first-line imaging technique recommended to identify steatosis. It is considered the most cost-effective non-invasive tool for large-scale fatty liver screening [75–77]. Even though non-invasive methods are currently available, the definite diagnosis of NAFLD/NASH still relies on liver biopsy and histologic examination [78].

Treatment should not be deferred in patients with NAFLD, especially if significant fibrosis is present. Some general measures for these patients include abstinence from alcohol [79], vaccination for hepatitis virus A and B [80], and modification of risk factors for cardiovascular disease [81]. Several studies examined the role of weight loss on NAFLD progression. A weight loss of at least 5 percent of body weight demonstrated an improvement in hepatic steatosis, and a weight loss $\geq$7% also improved fibrosis and inflammation [82,83]. Another prospective study recruited 293 patients with histologically proven NASH and were endorsed in a one-year program with restriction calorie intake to reduce their weight; although only 30% of patients achieved the goal, a 5% weight loss showed histological improvement, and patients who lost $\geq$10% of their weight had a more dramatic regression in fibrosis [84]. The first line therapy for patients with T2DM and obesity includes an integrated diet and exercise plan. A randomized, cross-over 6-week dietary intervention study of patients with biopsy-proven NAFLD showed that the Mediterranean diet induced a reduction in liver steatosis and insulin resistance, with subsequent beneficial effects on inflammation and fibrosis [85]. Additionally, physical activity is linked to better outcomes in NAFLD. A longitudinal study made by the US National Health and Nutrition Examination Survey from 2003–2006 to 2015 demonstrated that exercise was associated with lower risk of all-cause mortality and lower risk of cardio-vascular-disease-related mortality [86]. For patients with NASH who do not meet their weight loss goals, additional treatments such as bariatric surgery are proposed. At present, there is a lack of randomized clinical trials that demonstrate the effectiveness of bariatric surgery in NAFLD patients. Although some studies showed improvement in inflammation and fibrosis following bariatric surgery in patients with NASH, the methodology is heterogeneous [87,88] Furthermore, recent studies showed worsening of NAFLD/NASH features in some patients following bariatric surgery. Because of that, it is mandatory to monitor liver enzymes and semiology in all patients postoperatively [89].

Some antidiabetic therapies are efficacious in treating NASH. For example, pioglitazone improved histologic features of NASH in biopsy-proven NASH patients. In a meta-analysis of 4 trials that compared thiazolidinediones with placebo in 334 patients with NASH, patients in treatment with thiazolidinediones had a reduction in alanine aminotransferase levels ($-$10.9 vs. $-$36.2 u/L; $p = 0.009$), gamma-glutamyltransferase levels ($-$9.4 vs. $-$41.2 u/L; $p = 0.002$), hepatocellular injury ($p = 0.005$), and fibrosis ($p = 0.05$) [90]. However, liver specialists seldom prescribe pioglitazone to treat NAFLD/NASH. Glucagon-like peptide-1 (GLP-1) receptor agonism seems to be a promising pharmacological approach, with preliminary data from Phase 2 RCT for both liraglutide and semaglutide [9,10]. Metformine is the antidiabetic agent most commonly used in T2DM patients. Although these biguanides demonstrated an effective reduction in hepatic and peripheral insulin resistance [91], a difference in reduction in hepatic fat content or a decrease in inflammatory markers was not observed in several studies [92–94]. Other groups of diabetic agents that demonstrated lack of clinical efficacy are SGLT-2 inhibitors, although few RCT are registered regarding its hepatic effects. At present, only empaglifozin induced a significant reduction in liver fat [95,96], but not a statistical difference in fibrosis compared with control groups. Other new agents are being tested, such as dual PPARα and PPARδ agonist elafibranor (or GFT505) that showed a resolution of steatohepatitis without worsening fibrosis, with a favorable safety profile [97].

3.2. Type 1 Diabetes (T1DM)

Although it appears that the most common liver disease in patients with T1DM is NAFLD [98], the links between both disorders remain largely unelucidated. T1DM is an autoimmune disorder that typically presents in children and younger adults. Until

recently, there was not a traditional association between obesity, metabolic syndrome, and T1DM [14], yet with the increasing prevalence of obesity in the general population, a parallel rise has been observed in patients with T1DM and overweight or obesity [99]. Moreover, a pathogenic role of obesity in T1DM was recently described, related to β-cell stress, ectopic adipose tissue, and an increase in autoimmune disorders [100–102].

3.2.1. Epidemiology

A global increase in T1DM incidence has been observed, with a 2–3% increase per year [103–105]. The higher incidence is found among children younger than five years, and variations occur according to environmental and behavioral factors such as diet, obesity, vitamin D sufficiency, gut-microbiome changes, or exposure to certain viruses [100,105]. As in the case of T2DM, socioeconomic factors play a paramount role in the differences in the prevalence among genetically similar patients [106]. Although T1DM can also develop in adulthood, the higher incidence of T2DM among adults and the lack of strong diagnostic criteria make such late diagnosis rare and challenging [107].

3.2.2. Pathophysiology

Although T1DM and T2DM share hyperglycemia as their landmark, various underlying mechanisms primarily differentiate both entities. While an absolute insulin deficiency characterizes T1DM, the natural history of T2DM is marked by IR in peripheral tissues. Yet, T2DM can also lead to insulin deficiency when insulin production is depleted due to exhaustion of pancreatic synthesis [14,108]. As in T2DM, the state of hyperinsulinemia found in T1DM affects glucose and lipid liver metabolism and triggers pro-inflammatory cascades that produce liver fibrosis and cirrhosis [3,29,109,110].

A proposed differential mechanism of liver damage in T1DM relates to the increase in visceral adiposity secondary to frequent snacking in the hypoglycemia context, which increases caloric and fructose intake [111]. Other mechanisms that may explain the development of hepatic complications in patients with T1DM are the relative portal vein insulin deficiency that leads to altered hepatic glycogen storage, absence of inhibition of hepatic gluconeogenesis, and overall metabolic disturbance with a shunt to lipogenic pathways [14,112,113]. The lack of endogenous insulin also alters the dynamic of insulin delivery with more minor variation in the plasma range, ending in downregulation of insulin receptors on hepatic cells due to continuous exposure [114]. This altered pharmacokinetics implies the development of a relative IR with the subsequent hepatic damage [109].

As mentioned above, not all patients with NAFLD are associated with obesity and MetS. This statement acquires relevance in T1DM due to its genetic footprint. Several genetic risk alleles associated with fatty liver disease are described in the literature, including those containing the genes PNPLA3 and transmembrane six superfamily member 2 (TM6SF2) [115]. Other potential genes that are related to T1DM and insulin-dependent T2DM are those that encode sterol regulatory element-binding protein (SREBPs) and carbohydrate-responsive element-binding protein (ChREBP). Alterations in these proteins stimulate lipogenic and glycolytic pathways that contribute to NAFLD [116,117].

3.2.3. Clinical Manifestations

Despite T1DM being at an increased risk of developing NAFLD, likely higher than T2DM patients (both because of the intensity of metabolic dysfunction and the earlier onset of the disease), a limited number of studies are addressing the prevalence of NAFLD in T1DM. A meta-analysis by Vries et al. reported an overall prevalence of 19.3% in T1DM, which increased to 22% in adults [98]. A recent study showed a NAFLD prevalence of 16–21% in T1DM patients by share wave elastography at liver ultrasound [118].

Evidence on the outcomes of patients with NAFLD and T1DM is also preliminary. In a cohort of 4641 patients with T1DM, Harman et al. found that overall, patients with T1DM were at increased risk of developing liver cirrhosis compared to the general population [119]. Targher et al. demonstrated that NAFLD increases the risk of both microvascular and

macrovascular complications in T1DM, i.e., chronic kidney disease (37.8% vs. 9.9% in patients without NAFLD), retinopathy (53.2% vs. 19.8%), coronary artery disease (10.8% vs. 1.1%), cerebrovascular (37.3% vs. 5.5%), and peripheral vascular disease (24.5% vs. 2.5%, $p < 0.001$ for all comparisons) [53,120]. Further prospective studies are needed to evaluate whether NAFLD is an independent factor for hepatic and cardiometabolic complications that might contribute to the excess mortality in T1DM cohorts [121,122].

3.2.4. Management: Diagnosis and Interventions

T1DM is characterized by autoimmune β-cell dysfunction and loss compared to patients with T2DM [14]. Typically, these patients present hyperglycemic symptoms at onset as polyuria, polydipsia, and body weight loss. An estimated 26.3% to 31.7% of patients debut with a life-threatening diabetic ketoacidosis due to the total absence of insulin production [123,124]. The definite diagnosis is confirmed through positive autoimmunity (including insulin, glutamate decarboxylase, islet antigen 2, zinc transporter eight, and tetraspanin-7 autoantibodies) [110], yet in around 10% of patients who do not express such antibodies, low C-peptide measurements also confirm the diagnosis [125].

The cornerstone of drug therapy in T1DM is exogenous insulin administered through either subcutaneous injections or an insulin pump. The objective is to mimic physiologic insulin release with a basal dose that controls glycemia overnight and between meals, as well as bolus doses that cover carbohydrate rations when feeding [126]. These patients require close monitoring with dose adjustment for physical activity, illness, or stress. Management of T1DM rapidly changed over recent years, with continuous glucose monitoring, intermittently viewed devices, and closed-loop systems, and an artificial pancreas leading to substantial improvements in glycemic control and quality of life [127–130].

There is a current increase in obesity prevalence, IR, and MetS among T1DM patients, yet NAFLD screening is not systematically recommended in patients with T1DM in the recent ADA 2022 guidelines [131]. However, it is reasonable to recommend yearly NALFD screening in this group, as proposed for patients with T2DM. In addition, the use of antidiabetic drugs in patients with T1DM and NAFLD is controversial. Previous studies demonstrated that endogenous GLP-1 levels are low in T1DM patients [132]. The levels of GLP-1 correlate with the presence of metabolic syndrome [133,134]. Despite favorable results in trials with liraglutide [135], GLP-1 analogs are not approved in T1DM. In NAFLD cohorts, GLP-1 agonists demonstrated a reduction in liver steatosis and IR [136]. The potential effect on hepatic fibrosis and reduction in liver fat content in this cohort of patients remain unexplored, and the current standard of care for NAFLD in T1DM relies on ensuring optimal glycemic control besides diet/weight loss and physical exercise. Interestingly, HbA1c appears not to be a good predictor of NAFLD development in T1DM [82]; hence, new parameters obtained with glycemic control devices such as time in range and coefficient of glycemic variation that are strongly associated with diabetic complications might be explored [137–139].

3.3. MODY Diabetes

MODY is a mild, largely asymptomatic form of diabetes that occurs in nonobese children and young adults with a dominant inheritance pattern [140]. This form of diabetes is commonly misdiagnosed as T1DM or T2DM and is often inappropriately managed with insulin, whereas the adequate treatment consists of a sulfonylurea [141,142].

3.3.1. Epidemiology

MODY represents a clinically heterogeneous form of β-cell dysfunction caused by genetic mutations with an autosomal dominant form of inheritance. Because of the diverse patterns of presentation and the need for costly molecular diagnosis, it is frequently misdiagnosed as other types of diabetes [142,143]. HNF-1α/MODY3 and GCK/MODY 2 are the most common mutations [144,145]. One of the first prevalence studies estimated 35.2 cases per million with a confirmed genetic test in the UK [146]. In a study carried out

on Norwegian diabetes registers, MODY accounted for 0.4% of patients [147]. In the US, the estimated prevalence is 2.1 per 100,000 individuals younger than 20 years [148]. In these groups, we will include a subtype of inherited diabetes associated with lipodystrophies that are associated with severe insulin resistance, premature diabetes, hypertriglyceridemia, and hepatic steatosis due to defects on adipocyte development, differentiation, and apoptosis [149,150]. Although acquired lipodystrophies related with chronic corticosteroids therapy or human immunodeficiency virus (HIV) infection are more common in the general population [151], this group is characterized by a translocation of subcutaneous adipose to ectopic parts of the body, including the liver, which subsequently drives to hepatic inflammation and fibrosis. Therefore, NAFLD has been described in these patients, as confirmed in histologically confirmed cohorts, with a prevalence around 82–90% [152–154]. Clinicians must be aware that some of these patients are characterized by low weight and BMI; therefore, recent guidelines recommend the screening of lipodystrophy in lean individuals with a diagnosis of NASH [155]. The severity of NAFLD will depend on the type of lipodystrophy, being more severe in generalized lipodystrophy, but also on specific mutations, such as LMNA mutations [156].

3.3.2. Pathophysiology

MODY was recognized as a disease in 1964 at the Fifth Congress of the International Diabetes Federation in Toronto, and since then, significant progress has been made in understanding its pathophysiology [157]. In 1974, Tattersall et al. reported a new form of diabetes with an autosomal dominant pattern of inheritance that typically presented in young patients who could discontinue insulin therapy over the course of the disease [158]. Since 1975, various mutated genes were identified and associated with different subtypes of MODY with a wide diversity of clinical features, severity of hyperglycemia, and age onset [159]. GCK (MODY 2) and HNF1A (MODY 3) mutations account for almost 70% of all cases of MODY, followed by HNF4A (MODY 1). HNF1A and HNF4A genes encode the transcription factors hepatocyte nuclear factor-1 alpha and factor-4 alpha [160] and coordinate gene expression of proteins involved in glucose transport and glucose metabolism, and β-cell apoptosis, which lead to an increase in cellular apoptosis and defects in insulin secretion [161]. The Glucokinase gene is responsible for detecting bloodstream glucose by its transformation to glucose-6-phosphate by the glucose transporter 2 (GLUT2). Heterozygous mutations imply less function of this enzyme, and affected β-cells are less sensitive to glucose variations that result in elevated fasting and postprandial blood sugar.

3.3.3. Clinical Manifestations

Patients diagnosed with the most common forms (MODY 1, 2, and 3) have a similar risk for complications as those with T1DM and T2DM. Therefore, an optimal glycemic control is inversely related to poor micro- and macrovascular outcomes [161]. Patients carrying heterozygous mutations rarely develop micro- or macrovascular complications [162]. Concerning the association of MODY and NAFLD, again, the body of evidence is overall poor. Multisystemic forms that imply alterations in the transcription factor hepatocyte nuclear factor 1β (HNF1B) (MODY 5) with clinical features that include early-onset diabetes mellitus, pancreatic hypoplasia, genital tract, kidney hypoplasia, cognitive impairment, and abnormal liver function might be the subgroup at higher risk of NAFLD. Hepatic dysfunction is presented in 65% of patients, and the classical phenotype is characterized by elevated serum transaminases, steatosis, and periportal fibrosis. Yearly abdominal ultrasonography and biannual laboratory monitoring are proposed for patients with MODY 5 [163–165].

3.3.4. Management: Diagnosis and Interventions

Molecular diagnosis of MODY should be performed in patients with phenotypical features: young nonobese patients with absence of pancreatic autoimmunity with low or null insulin requirements and strong familiar association [166]. Some experts proposed assessing endogenous C-peptide levels on serum or urine samples in patients with T1DM

to identify those who may benefit from MODY genetic testing [167]. GCK-MODY onset typically occurs in pregnant women. These patients usually have an altered fasting glycemia with an increase in oral glucose tolerance (OGTT) less than 4.6 mmol/L [168]. HNF1A and HNF4A MODY present during adolescence or young adulthood, and large glucose increases are observed on OGTT, but fasting blood glucose levels are usually normal. These diabetes subsets present an excellent response to sulfonylureas [169]. Finally, the MODY 5 form has an earlier onset with absolute pancreatic deficiency and the need for insulin therapy. In addition, extra-pancreatic alterations are present such as liver disturbances and genital tract and urinary malformations [170].

3.4. Ketone-Prone Diabetes (KPD)

In 1987, Winter et al. reported a new form of diabetes called 'atypical diabetes' by then [171]. The discovery led to a rise in recognizing this uncommon clinical presentation, with an abrupt onset—typically with ketoacidosis—and transient insulin requirements that usually occurred in African-American or Hispanic patients and were associated with obesity and a strong family history of type 2 diabetes [172–175]. Some evidence suggests that a primary glucose desensitization acts as a trigger to β-cell exhaustion and dysfunction that lead to acute metabolic failure. Initial aggressive treatment with insulin therapy and antidiabetic drugs such as metformin predict better outcomes and a delay in the recurrence of hyperglycemia [176,177].

3.4.1. Epidemiology

These patients are often Afro-American and Hispanic, obese, middle-aged men, with a family history of type 2 diabetes. Most prevalence studies on KPD made are US-based, with an estimate of 20% and 50%, respectively, in African American and Hispanic patients with new diagnoses of diabetic ketoacidosis [171,173,178,179]. Obesity is present in 56% of newly diagnosed patients, and more than 80% of patients have a family history of T2DM [180,181]. More recent studies estimated an average incidence of 60% among patients attending emergency rooms due to ketoacidosis [182]. The prevalence of this diabetes seems to be lower in Asian and White Americans, representing less than 10% of cases of diabetic ketoacidosis [183,184]. Patients with KPD present with acute IR, elevated glucose, Hb1Ac, and ketone levels, and unlike T1DM, they do not exhibit β-cell antibodies.

3.4.2. Pathophysiology

The natural history of KPD has two phases. These individuals initially present an acute form presentation with severe hyperglycemia and ketosis due to a lack of response and stimulus of β-cell insulin secretion [178]. The causes of these acute onsets in patients affected with ketosis-prone diabetes remain unknown. Patients present a diminished insulin and c-peptide response to an oral glucose load in the second phase. In studies with a euglycemic insulin clamp, there was no difference in baseline glucagon levels and glucagon suppression between KPD patients and the normal controls. In short term follow-up, the insulin secretion increased after a few weeks of exogenous insulin treatment, and after months, there was no difference in the beta-cell response and the insulin secretion compared to controls [185]. IR is not systematically found and appears to be associated with ethnicity and geographic variability [186,187].

3.4.3. Clinical Manifestations

The typical presentation is a new clinical onset with severe hyperglycemia with high ketones or diabetic ketoacidosis, negative GAD, and islet cell autoantibodies. The type and rate of complications are similar to T2DM [162,164–166]. Provided that IR characterizes KPD and related metabolic abnormalities, pathogenic liver pathways that contribute to NAFLD are certainly stimulated; yet, there are no specific studies of NAFLD in KPD. It is worth underscoring that African Americans with T2DM are at lower risk for hepatic steatosis than White Americans, which is not explained by ethnic differences in BMI,

HOMA-IR, or toxic or drug ingestion [55,180]. In contrast, Hispanics are at higher risk of steatohepatitis, seemingly due to the higher prevalence of obesity and IR [188,189].

3.4.4. Management: Diagnosis and Interventions

Accurate clinical history and physical examination are essential to distinguish KPD from T2DM. Central obesity and acanthosis nigricans in skin folds related to IR are more prevalent in KPD [190]. The diagnosis of asymptomatic forms could be made with the HbA1c test, OGTT, and two basal altered glycemia as recommended for T2DM [57]. Intensive insulin and fluid replacement may be necessary at onset [165,170]. A significant percentage of patients will need exogenous insulin after several weeks since they can be managed with oral agents. Sulfonylurea agents have been proved efficacious and safe in the longstanding treatment of KPD [180,182]. There is a lack of studies on SGLT2 inhibitors, and because of its direct link with euglycemic ketoacidosis, further data are needed before being recommended to patients with KPD.

4. Algorithm of Diagnosis and Treatment

Numerous guidelines such as those of the ADA [131], the European Association for the Study of Diabetes, the European Association for the Study of the Liver [191], or the American Association for the study of Liver Disease [192] recommend yearly screening of NAFLD in diabetic patients. However, there are relevant controversies on fundamental aspects of screening, such as the non-invasive test to be used, whether it should be carried out systematically or on a case-finding basis, or whether the approach might or might not be the same at the primary care level and the diabetes clinic regarding when to refer to the liver specialist, etc. [193,194]. Hence, widespread systematic NAFLD screening is not still implemented in many countries. Although a FIB-4 cut-off < 1.3 is considered to rule out advanced fibrosis in the general population accurately, and thus it is recommended by the latest EASL guidelines on the use of non-invasive tests [195], Boursier et al. recently found that a substantial proportion of diabetic patients with NAFLD and FIB-4 < 1.3 who underwent liver biopsy had F3-F4 fibrosis [196]. Therefore, in diabetic patients with FIB-4 < 1.3 and concomitant factors that might lead to the suspicion of significant or advanced fibrosis (e.g., age, obesity, poor control of DM, elevated transaminases, or other altered non-invasive serologic tests for fibrosis such as ELF), it might be reasonable to perform liver elastography to assess liver stiffness or directly refer the patient to the hepatologist clinic. At this moment (Figure 2), we propose an algorithm that will indeed be refined in the following years as further studies shed light on critical points of NAFLD screening and referral pathways.

The scope of the proposed algorithm is limited as there is still much uncertainty regarding the most accurate non-invasive method to detect NAFLD. On the other hand, studies show that biopsy is a reliable method to detect and stage the degree of fibrosis once NASH is established and the only one to detect active inflammation or steatohepatitis [197], which is known as a prevalent feature of liver damage among diabetic patients and in some cases the only one. Additionally, a diagnostic approach based on liver biopsy was demonstrated to add a survival benefit compared to a non-invasive-based approach. However, liver biopsy is not without perils, and we believe that biopsies should be reserved for patients with higher values of liver stiffness on transient elastography. The decision to perform a liver biopsy should be individualized and taken by an expert hepatologist.

Currently, there are no NAFLD-specific pharmacologic therapies approved for widespread use, and the ones that do show beneficial effects are available in randomized clinical trials. Some of these drugs were tested in diabetic patients and are commercially available. For example, GLP-1 agonists, a part of the routinely pharmacological armamentarium for diabetic patients, show promising results in phase-2 randomized clinical trials. In a randomized clinical trial of semaglutide in patients with non-alcoholic steatohepatitis, Newsome et al. [7], showed that once-daily semaglutide resulted in a higher proportion of patients with NASH resolution without worsening of fibrosis and higher loss of body

weight after 72 weeks of treatment. Liraglutide demonstrated similar properties in a randomized clinical trial where 39% of patients treated with the drug had NASH resolution compared to 9% of patients treated with a placebo [6].

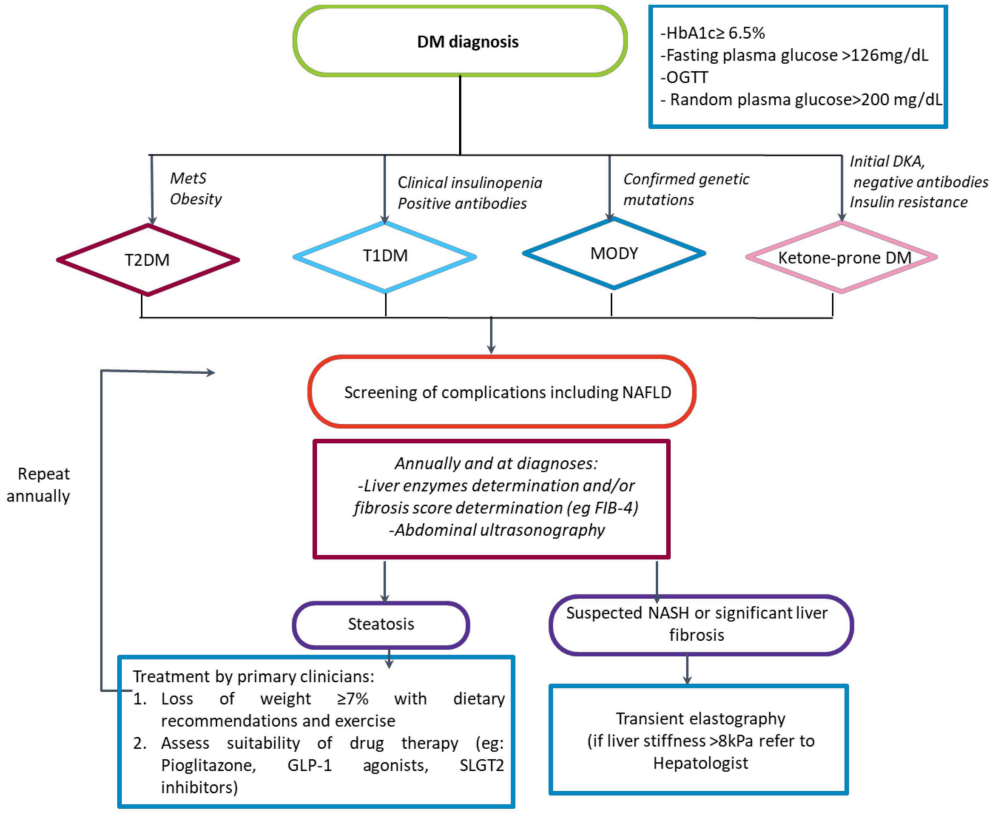

Figure 2. Algorithm proposed for the management of NAFLD in different typed of diabetes. DM: Diabetes mellitus; HbA1c: glycated hemoglobin; OGTT: Oral glucose tolerance test; MetS: Metabolic syndrome; T2DM: Type 2 diabetes mellitus; T1DM: Type 1 diabetes mellitus; MODY: Maturity-onset diabetes of the young; DKA: Diabetic ketoacidosis; OADs: Oral antidiabetic drugs; NAFLD: Non-alcoholic fatty liver disease; GLP1a: GLP-1 receptor agonists.

5. Concluding Remarks

In recent years, in parallel to the increase of its prevalence worldwide, the knowledge of NAFLD diagnosis, pathophysiology, and treatment has grown exponentially. The relevance of NAFLD as a global public health threat is reinforced by its parallel evolution alongside DM, obesity, and MetS. This review aimed to underscore that different DM phenotypes have various types of liver involvement that need personalized management. Despite the lack of approved drugs by the FDA and EMA to treat NAFLD, several pharmacological treatments are used to treat DM, obesity, or dyslipidemia that are shown to be efficacious in treating NAFLD; yet, the evidence is still preliminary. Unfortunately, in the diabetes field, there is no consensus on how to screen for NAFLD. In the hepatology field, there are gaps in the recommendations for liver fibrosis screening in diabetic patients. The gold-standard method to evaluate the presence of NASH is the liver biopsy, as we could observe in some prospective studies that demonstrated a prevalence of 58.52% among patients with metabolic syndrome and 96.82% in T2DM patients [197]; other studies recorded lower prevalence such as that registered in a global systematic review performed in 2017 with a value of 54% [44]. Transient elastography is the screening test more implemented in the daily care routine, although it has a moderate sensitivity compared with the results of biopsy. In a meta-analysis published in 2013, this test reported a high diagnostic accuracy, with an Area under the curve (AUC) in the range of 0.84–0.87 for fibrosis stage $\geq$ F2, 0.89–0.91 for fibrosis stage $\geq$ F3, and 0.93–0.96 for fibrosis stage F4 [197], which

was also recommended for NAFLD because of its cost-effectiveness and the lack of adverse events. We proposed a simple algorithm for early intervention in patients with DM and suspected NASH. Further studies are needed to improve referral pathways, work-up, and the standard of care for diabetic patients with NAFLD.

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10.2.4. Publicación anexa 4

Manzano-Nuñez R, Rivera-Esteban J, Comas M, Ángel M, Flores V, Bañares J, Ciudin A, Vilallonga R, Pericàs JM. Outcomes of Patients with Severe Obesity and Cirrhosis with Portal Hypertension Undergoing Bariatric Surgery: a Systematic Review. Obes Surg. 2022 Nov 24.

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Risk of infections in patients with NAFLD and Type 2 Diabetes under treatment with SGLT2 inhibitors and relationship with liver outcomes: A retrospective case-control study

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease in developed countries, with its incidence growing parallel to the epidemics of obesity and type 2 diabetes mellitus (T2DM). Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are becoming a cornerstone in the management of cardiovascular health and some studies suggest the potential role in NAFLD. However, patients under treatment with SGLT2i are at risk of developing genitourinary fungal infections (GFIs). Moreover, both NAFLD and SGLT2i have a strong influence on the immune system, and therefore the risk of infections other than GFIs could be increased in NAFLD patients treated with SGLT2i. We aimed to examine the possible association of SGLT2i with infections and hepatic outcomes in NAFLD patients.

Methods: We conducted a case-control study including NAFLD patients with T2DM visited at the Liver Unit outpatient clinic from 2016 to 2021 with a minimum follow-up of 6 months by selecting 65 patients receiving SGLT2i and 130 matched patients with other types of antidiabetic treatment.

Results: During follow-up, GFIs were significantly higher in the SGLT2i group (15.4% vs. 3.8%; p=0.008), whereas there were no differences in the occurrence of overall infections (41.5% vs. 30%; p=0.1) nor in other types of specific

infections. In the multivariable analysis, treatment with SGLT2i was not independently associated with higher odds of overall infection. On the other hand, SGLT2i patients showed a significantly lower incidence of hepatic events (1.5% vs. 10.7%; p=0.02). There were no significant different in all-cause mortality between cases and controls.

Conclusions: NAFLD patients with T2DM receiving SGLT2i more frequently presented GFIs, whereas the incidence of other types of infections was not found to be higher than in other patients with NAFLD and T2DM treated with other drugs. Moreover, SGLT2i-treated patients had a lower occurrence of hepatic events. Further studies are warranted to validate our data.

KEYWORDS

NAFLD, type 2 diabetes mellitus, sodium-glucose co-transporter-2 inhibitors, infections, hepatic outcomes

Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a progressive clinical spectrum: from simple steatosis, through inflammation [i.e., non-alcoholic steatohepatitis (NASH)] and fibrosis, to liver cirrhosis (1, 2). From an epidemiological standpoint, parallel to the growing epidemic of obesity and type 2 diabetes mellitus (T2DM), NAFLD has emerged as the most prevalent liver disease in the US and probably will become the leading cause of liver transplantation in the upcoming years (3). It is estimated that 25% of general population in Western countries to have NAFLD, but this prevalence increases up to 60-80% in patients with obesity or T2DM and can reach 80-100% when both risk factors are present (2, 3). In addition, it is estimated that 20-30% of patients with NAFLD will progress to liver inflammation and fibrosis (2–4).

NAFLD is considered the liver manifestation of metabolic syndrome, both sharing multiple pathophysiological mechanisms such as insulin resistance (1–3). Moreover, improvements in metabolic factors such as weight loss, are associated with amelioration in inflammation and liver fibrosis (1). Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are now solidly established amongst the armamentarium to improve metabolic status and cardiovascular health in T2DM, chronic kidney disease and cardiovascular diseases (5, 6). Emerging data suggest that SGLT2i may play a role in treating NAFLD being associated with an impact in the metabolic status, including reduction in liver fat content and even histologic improvement in liver steatosis and fibrosis (7–10). Such effects could have a clinically relevant impact on the outcomes of patients with T2DM and NAFLD, making SGLT2i an attractive therapeutic alternative. Even though there are currently several clinical trials underway assessing their efficacy to treat NAFLD (11, 12), there is insufficient information regarding an impact in clinical practice.

SGLT2i inhibit glucose reabsorption in the kidney *via* inhibition of the SGLT channels primarily located in the proximal tubules, promoting glycosuria, which has been associated with a higher incidence of urinary and genital infections, mainly caused by fungi, with odds ratios ranging approximately from 3 to 5 (13–17). Moreover, patients with T2DM have increased susceptibility to a wide array of infections due to variable degrees of baseline immunosuppression caused by complex mechanisms that are tightly intertwined with pathways leading to the enhanced systemic inflammation and immune system dysfunction characteristic of advanced liver disease, while obesity and NAFLD may also increase infection susceptibility (1, 18, 19). However, no studies have investigated whether T2DM patients with NAFLD treated with SGLT2i present increased rates of infections and particularly genitourinary fungal infections (GFIs).

The present study aimed to examine the impact of SGLT2i treatment in the incidence of infections in patients with NAFLD and T2DM. In addition, we aimed to investigate whether NAFLD patients treated with SGLT2i presented significant differences in liver outcomes compared to those not receiving SGLT2i.

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; SGLT2i, Sodium-glucose co-transporter-2 inhibitors; GFIs, genitourinary fungal infections; NASH, non-alcoholic steatohepatitis; VHUH, Vall d'Hebron University Hospital; HE, hepatic encephalopathy; COVID-19, Coronavirus disease 2019; IQR, interquartile range; BMI, body mass index; OR, odds ratio; CI, confidence of interval; MRI, magnetic resonance imaging.

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Methods

Design and setting

Case-control study conducted at the Vall d'Hebron University Hospital (VHUH), a tertiary care setting with 1,300 beds in Barcelona, Spain.

Participants

An ongoing prospective cohort study on NAFLD was used to identify patients considered eligible to be included in the present case-control study. The ongoing prospective cohort study includes consecutive patients from the VHUH NAFLD outpatient clinics diagnosed with NAFLD and T2DM from 2016 to 2021. Patients with a diagnosis of both NAFLD and T2DM were considered eligible for inclusion. Patients with NAFLD and T2DM receiving treatment with SGLT2i and with a minimum of 6 months of follow-up were identified and defined as cases. NAFLD and T2DM patients receiving other antidiabetic drug different than SGLT2i were considered as potential controls (see flowchart in Figure 1). Matching variables were sex and age (+/- 3 years).

We excluded patients with Child B and C cirrhosis from the analysis to avoid bias in the allocation of SGLT2i treatment, as we considered that patients with more severe liver disease were less likely to receive treatment with SGLT2i because these are not widely recommended in patients with moderate-severe hepatic insufficiency, and also because patients with advanced chronic liver disease are at higher risk of infections and these might trigger hepatic events.

Outcomes

The occurrence of any type of infection was considered as the primary outcome of interest. Secondary outcomes included the occurrence of each type of infection separately, the occurrence of hepatic events (only the first episode accounted for each patient), and all-cause mortality.

Definitions

The diagnostic criteria for NAFLD and T2DM are described elsewhere (1, 20). Briefly, NAFLD was diagnosed either based on steatosis in abdominal ultrasound or transient elastography and the presence of at least one feature of metabolic syndrome or by liver biopsy. T2DM was diagnosed following current clinical practice recommendations from the American Diabetes Association (20). Cirrhosis was defined by histological analysis when available and/or by a combination of clinical, analytical, liver stiffness measurement, endoscopic assessment and/or radiological evidence of advanced chronic liver disease. Hepatic events were defined as the presence of ascites, hepatic encephalopathy, and upper gastrointestinal bleeding. Infections were defined as urogenital fungal infections, urogenital bacterial infections, bacteremia, upper and lower respiratory tract infections, COVID-19, intra-abdominal, skin and soft tissues infections, and spontaneous bacterial peritonitis. Infections were diagnosed in a real clinical setting by means of clinical presentation and, when needed, radiological, laboratory and microbiological analysis.

Ethics

The study protocol was approved by the VHUH Ethics Committee for Clinical Research (protocol code: PR(AG)222/2021) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Statistical analyses

Data were collected into a pre-specified and de-identified database in electronic format. Categorical variables were presented as frequencies and percentages. Continuous variables were expressed as means with standard deviation or medians with interquartile ranges as appropriate according to data distribution. Categorical variables were compared with Chi square test or Fisher exact test where appropriate. Continuous variables were compared using T test or nonparametric tests when necessary. A multivariate logistic regression analysis adjusted by relevant covariates was used to examine the association between SGLT2i use and the occurrence of infections. Findings were presented as odds ratios (OR) with 95% confidence intervals. Kaplan-Meier survival curves and long-rank test were calculated for the occurrence of overall infections and hepatic events along time. All analyses were performed in STATA statistical software.

Results

Patients

The flowchart of patients' disposition is shown in Figure 1.

After applying the inclusion and exclusion criteria, sixty-five patients with NAFLD and T2DM received SGLT2i and were considered as cases, whereas 130 diabetic patients with NAFLD not

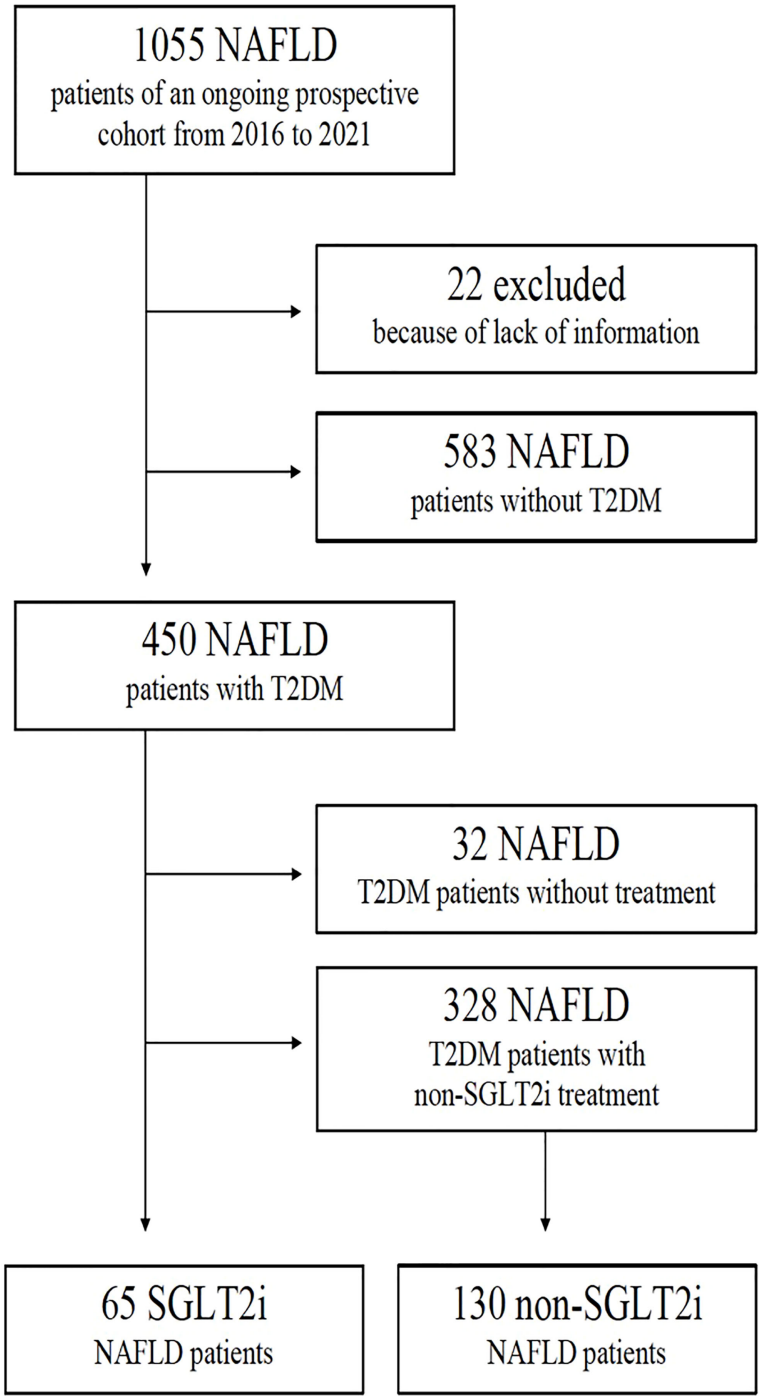

FIGURE 1
Patients' disposition flowchart.

receiving SGLT2i were matched as controls. Characteristics of patients are shown in Table 1. Overall median follow-up was 34.4 months (IQR 17-48.3), with no significant differences found between cases and controls. There were neither differences regarding age, sex (matching variables), the prevalence of comorbidities, or the values of glycosylated hemoglobin, albumin, and liver enzymes between groups. Patients receiving SGLT2i had significantly higher BMI values and were more likely to present obesity.

Infections

Outcomes' information is outlined in Table 2. There were no significant differences in the occurrence of overall infections between groups (41.5% vs. 30%; p=0.1). With respect to the infection's etiology, the proportion of patients presenting genital mycotic infections was significantly higher in patients receiving SGLT2i compared to those not receiving SGLT2i (15.4% vs. 3.8%; p=0.008), with incidence rates of 5.12 and 1.28 cases per 100 person-year in SGLT2i and non-SGLT2i groups, respectively (p<0.001). There were no significant differences in the proportion of the other types of infections between the two group of patients, including urinary tract infections of bacterial etiology (10.7% vs. 7.7%, p=0.4).

As shown in Table 3, multivariate logistic regression analysis showed no significant risk-adjusted odds of infections with the use of SGLT2i (OR 1.68, 95% CI 0.93-3.05; p=0.084).

Kaplan-Meier survival curve on the occurrence of overall infections is shown in Figure 2A (log-rank test= 0.02).

Hepatic events

Overall, patient who received SGLT2i showed a significantly lower incidence of hepatic events (1.5% vs. 10.7%; p=0.02) although the proportion of cirrhosis was similar between groups. Each individual decompensating event was less frequent in SGLT2i treated patients (Table 2) although the difference did not reach statistical significance. Figure 2B shows the Kaplan-Meier curve for hepatic events (log-rank test= 0.1).

Mortality

No significant differences in overall mortality [1.5% vs 6.9%; p=0.1] were observed between groups. Out-of-hospital cardiac arrest was the cause of death of the only patient who died in the SGLT2i group. In the non-SGLT2i group 5 patients died because of an infection (two of them of COVID-19) and one as a direct complication of hepatocellular carcinoma. The other three died of cardiac arrest, extrahepatic cancer and a motor vehicle collision.

Discussion

In this study we aimed at examining the frequency of infections and hepatic events in patients with NAFLD and T2DM treated with SGLT2i as compared to patients treated with other antidiabetic agents. Although we did not find a significantly higher rate of overall infections in patients treated with SGLT2i, we described for the first time that genitourinary fungal infections are also more frequent in T2DM patients with NAFLD treated with SGLT2i. Moreover, we found that SGLT2i-treated patients had lower rates of hepatic events during the follow-up time included in the study.

Despite there were no significant differences in the occurrence of overall infectious episodes among SGLT2i patients, these were more likely to present genitourinary fungal infections (GFIs). In the general population, the incidence of GFIs has been estimated to be approximately 1-2% (13). As previously reported across a large body of literature, through their glycosuric effect there is a higher risk of these types on infections in SGLT2i users, with rates between 3 to 8% (13, 15, 16). Several randomized controlled trials revealed a 3- to 4-fold increase for SGLT2i compared with placebo (14, 17), which led manufacturers to include GFIs as common adverse reactions in the prescribing information of SGLT2i. Furthermore, observational real-world studies analyzing claims data revealed similar results. For example, a large population-based observational study, analyzing data of more than 40,000 patients, found a two-fold increase in the risk of GFIs with the use of SGLT2i in older diabetic patients (16). Our study is the first to corroborate these findings in a NAFLD cohort. Furthermore, the 15% rate of GFIs in NAFLD patients found in our cohort is higher than those previously reported in SGLT2i-treated diabetic patients without NAFLD (17), which could point to a synergistic effect of NAFLD-related underlying immune dysfunction (1) added to the SGLT2i intrinsic increased risk of these infections. NAFLD indeed has been linked to immune system malfunctioning (18), including microcirculation disarrangements leading to diminished microbial clearance, undermined function of neutrophils and natural killer cells, and deficiency of vitamin-D levels, which in turn further impair innate immunity (19).

Notwithstanding the increased risk of GFIs infection, treatment with SGLT2i was not associated with and overall increase of infections. In contrast, HbA1c levels were independently associated with the risk of infections. This has been previously described in patients with T2DM (21–23). To our knowledge, this is the first study that corroborates this finding in NAFLD patients. As infections can precipitate acute decompensation and acute-on-chronic liver failure in cirrhotic patients (24), improving glycemic control could reduce the risk of infection. Further studies are required to better understand the pathologic pathways and clinical implications of these findings.

We found that patients with T2DM and comorbid NAFLD receiving treatment with SGLT2i were less likely to present hepatic events. Though preliminary, these data suggest the potential beneficial effects of SGLT2i in avoiding liver disease-related complications amongst patients with T2DM and

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TABLE 1 Demographics and clinical characteristics of cases (SGLT2i users) and controls (non-SGLT2i users) in a cohort of patients with NAFLD and type 2 diabetes.

	Total (n = 195)	SGLT2i users (n = 65)	Non-SGLT2i users (n = 130)	p-value
Follow-up, median months (IQR)	34.4 (17-48.3)	34.4 (16.5-48.2)	34.4 (17.1-49)	0.5
Age, median (IQR)	62 (57-68)	61 (57-66)	63 (57-68)	0.3
≥70 years, n (%)	40 (20.5)	10 (15.4)	30 (23)	0.2
Male sex, n (%)	104 (53.3)	35 (53.8)	69 (53.1)	0.9
Comorbidities				
Body Mass Index, median (IQR)	31.5 (28-34)	33.5 (29.7-36.2)	30.4 (27.3-34.2)	0.001
Obesity, n (%)	116 (61.4)	47 (74.6)	69 (54.7)	0.008*
Hypertension, n (%)	145 (74.3)	51 (78.5)	94 (72.3)	0.3
Dyslipidemia, n (%)	152 (78.0)	55 (84.6)	97 (74.6)	0.1
Ischemic Heart Disease, n (%)	18 (9.2%)	5 (7.8%)	13 (10)	0.7
Heart Failure, n (%)	17 (8.7)	7 (10.7)	10 (7.7)	0.4
COPD, n (%)	24 (12.3)	12 (18.4)	12 (9.2)	0.06
CKD, n (%)	20 (10.2)	6 (9.2)	14 (10.7)	0.7
Child A cirrhosis, n (%)	65 (33.7)	21 (33.3)	44 (33.8)	0.9
SGLT2i				
Canagliflozin, n (%)	–	9 (13.9)	–	
Dapagliflozin, n (%)	–	31 (47.7)	–	
Empagliflozin, n (%)	–	35 (35.4)	–	
Other/No data, n (%)	–	2 (3.1)	–	
Concomitant T2DM treatment				
Insulin, n (%)	76 (39.0)	32 (49.2)	44 (33.9)	0.03
Metformin, n (%)	161 (82.6)	53 (81.5)	108 (83.1)	0.7
DDP-4 inhibitors, n (%)	53 (27.2)	18 (27.7)	35 (26.9)	0.9
GLP-1 receptor agonists, n (%)	50 (25.6)	26 (40.0)	24 (18.5)	0.001
Pioglitazone, n (%)	35 (18.0)	12 (18.5)	23 (17.7)	0.9
Sulfonylureas, n (%)	4 (2.1)	2 (3.1)	2 (1.5)	0.5
Metaglinides, n (%)	5 (2.6)	2 (3.1)	3 (2.3)	0.8
Number of antidiabetic drugs				
Monotherapy	49 (25.1)	0 (0.0)	49 (37.7)	<0.001
2-drugs regime	68 (34.9)	13 (20.0)	55 (42.3)	0.002
3-drugs regime	52 (26.7)	30 (46.2)	22 (16.9)	<0.001
4-drugs regime	19 (9.7)	16 (24.6)	3 (2.3)	<0.001
5-drugs regime	7 (3.6)	6 (9.2)	1 (0.8)	0.002
Lipid-lowering agents				
Statins†	110 (56.4)	43 (66.2)	67 (51.5)	0.05
Fibrates†	35 (17.9)	18 (27.7)	17 (13.1)	0.1
Ezetimibe†	12 (6.2)	2 (3.1)	10 (7.7)	0.2
Other/No data	4 (2.1)	0 (0.0)	4 (3.1)	0.146
No treatment	58 (29.7)	13 (20.0)	45 (34.6)	0.03
Baseline Blood Tests				
HbA1c, median (QIR)	7.25 (6.7-8)	7.5 (6.9-8.5)	7 (6.5-8)	0.01
Glucose, median (IQR)	137 (110-167)	141 (114-167)	133 (108-165)	0.1
Platelets, median (IQR)	224 (161-273)	232 (183-271)	215 (152-274)	0.1
Creatinine, median (IQR)	0.79 (0.63-0.93)	0.8 (0.63-0.95)	0.77 (0.62-0.92)	0.5
Albumin, median (IQR)	4.3 (4.1-4.5)	4.3 (4.1-4.5)	4.3 (4.1-4.5)	0.5
INR, median (IQR)	0.97 (0.92-1.04)	0.96 (0.93-1.02)	0.98 (0.92-1.04)	0.6
AST (GOT), median (IQR)	36 (24-52)	35 (24-52)	36 (24-51)	0.8
ALT (GPT), median (IQR)	40 (26-56)	39 (25-56)	40 (27-60)	0.6

(Continued)

TABLE 1 Continued

	Total (n = 195)	SGLT2i users (n = 65)	Non-SGLT2i users (n = 130)	p-value
GGT, median (IQR)	72 (37-137)	67 (34-132)	72 (38-147)	0.2
Alkaline phosphatase, median (IQR)	85 (69-114)	81 (62-100)	89 (72-123)	0.01*
FIB-4, median (IQR)	1.66 (1.16-2.9)	1.56 (1.16-2.6)	1.68 (1.16-3.17)	0.3
LSM, median kPa (IQR)				
Overall	10 (6.8-18.3)	10.1 (7.7-16.6)	10 (6.4-18.6)	0.6
Cirrhotics	19.8 (15.6-34.9)	19.8 (15-27)	20.4 (17-43.2)	0.4

[†]Lipid-lowering agents alone or in combination with other drug classes.
ALT, alanine transferase; AST, aspartate transferase; GGT, gamma glutamyl transferase; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; HbA1c, glycosylated hemoglobin; LSM, liver stiffness measurement; SGLT2i, sodium-glucose co-transporter-2 inhibitors. * means statistical significance at, $p < 0.05$.

NAFLD. Translational research in animal models and clinical studies has shown that SLGT2i use is related to improved hepatic metabolism (9). Its use has been associated with reductions in hepatic steatosis measured by controlled attenuation parameter, MRI-proton density fat fraction or magnetic resonance spectroscopy (7), as well as liver stiffness ameliorations in patients with previous significant fibrosis (8). In a single-arm pilot study with paired biopsies demonstrated that SGLT2i might induce histological improvements in NASH features without a worsening of fibrosis (10, 25). Moreover, a small randomized controlled trial found that SGLT2i treatment resulted in reduced liver fat content by MRI-derived proton density fat fraction and improvements in liver enzymes, compared to placebo (12). Several ongoing clinical trials are currently assessing the effects of SGLT2i in patients with NAFLD and NASH, which may provide more definitive answers. Parallel to their well-known beneficial cardiovascular and renal effects (5), SLGT2i may exert additional hepatic protection, which could be a cornerstone in the treatment of T2DM patients with NAFLD. Moreover, while SLGT2i undoubtedly increase GFIs, by improving the metabolic control the overall risk of infection could decrease. This balancing situation presents a trade-off for physicians and patients between metabolic control and adverse effects. Cirrhotic patients, especially those that have already presented decompensations, are of special interest regarding the role of SGLT2i because their combined glycosuric and natriuric effects might be beneficial in patients with hypervolemia and renal dysfunction such as those with hepatorrenal syndrome (26). Nonetheless, SGLT2i are seldom prescribed in patients with advanced liver disease. Preliminary findings by Saffo, Garcia-Tsao and Tadei in a cohort of seventy-eight patients with cirrhosis treated with SGLT2i, including 39 (50%) with non-alcoholic steatohepatitis and 63 (81%) with compensated disease, rates of hepatic decompensation and mortality were not increased compared to most published cohorts of cirrhotics, and SGLT2i use was not identified as a cause of decompensation (27). Whether administering SGLT2i before first decompensation or as earlier as possible in both diabetic and non-diabetic patients might be not only safe

TABLE 2 Outcomes in cases (SGLT2i users) and controls (non- SGLT2i users) in a cohort of patients with NAFLD and type 2 diabetes.

	Total (n = 195)	SGLT2i users (n = 65)	Non-SGLT2i (n = 130)	p-value
Overall infection (total), n (%)	66 (39)	27 (41.5)	39 (30)	0.1
Respiratory tract infection, n (%)	16 (8.2)	5 (7.7)	11 (8.4)	0.9
Abdominal infection, n (%)	4 (2)	2 (3.1)	2 (1.5)	0.6
Bacterial UT infection, n (%)	17 (8.7)	7 (10.7)	10 (7.7)	0.4
Mycotic UT/genital infection, n (%)	15 (7.7)	10 (15.4)	5 (3.8)	0.008
Skin and Soft tissue infection, n (%)	4 (2)	1 (1.5)	3 (2.3)	1
Bacteremia, n (%)	4 (2)	1 (1.5)	3 (2.3)	1
COVID-19, n (%)	2 (1)	1 (1.5)	1 (1)	1
SBP, n (%)	1 (1.9)	0 (0)	1 (2.5)	1
Hepatic Events, n (%)	15 (7.7)	1 (1.5)	14 (10.7)	0.02
Ascites, n (%)	10 (5.1)	1 (1.5)	9 (6.2)	0.1
HE, n (%)	6 (3.1)	0 (0)	6 (4.6)	0.1
UGB, n (%)	7 (13.2)	0 (0)	5 (17.5)	0.1
Mortality, n (%)	10 (5.1)	1 (1.5)	9 (6.9)	0.1

SGLT2i, Sodium-glucose co-transporter-2 inhibitors; UT, urinary tract; COVID-19, Coronavirus disease 2019; SBP, Spontaneous bacterial peritonitis; HE, hepatic encephalopathy UGB, upper gastrointestinal tract bleeding.

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TABLE 3 Multivariate logistic regression analysis for risk of infection.

Variable	Adjusted HR (95% CI)	p-value
SGLT2i	1.68 (0.93-3.05)	0.084
HbA1c	1.09 (0.87-1.36)	0.43
Obesity†	1.35 (0.70-2.59)	0.36
Platelets	1.0 (0.99-1.004)	0.39
Age	1.02 (0.98-1.07)	0.17
Sex (males)	0.95 (0.52.1.74)	0.89

HbA1c, glycosylated hemoglobin; SGLT2i, Sodium-glucose co-transporter-2 inhibitors †(BMI >30 kg/m^2).

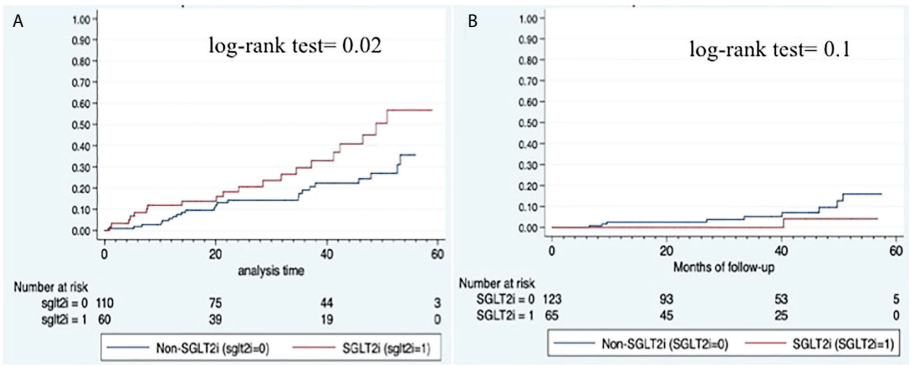

FIGURE 2
Kaplan-Meier survival curves. **(A)** Overall infections; **(B)** Hepatic events.

but beneficial as suggested by our findings warrants further investigation.

Limitations

This study is not without limitations and results should be interpreted in the context of the study design. First, we performed a case-control study, which is prone to selection bias thereby limiting the validity of the results presented. In the case of our study, cases had higher rates of obesity, were using more drugs for T2DM, had more insulin use and also statin, and had also higher glycated hemoglobin, which might contribute to both infections and hepatic events. Second, the collection of relevant data was retrospective. Third, the relatively small sample size constrains the robustness of multivariate analyses. Fourth, the potential effects of medications other than SGLT2i (e.g., GLP1 receptor agonists) were not addressed in our analysis. Despite our limitations, we provide data about infectious and metabolic outcomes that are relevant to clinical practice. Nevertheless, these results should be interpreted in the context of hypothesis generating research and should be tested in subsequent, larger, studies.

Conclusion

When compared with patients with NAFLD and T2DM without SGLT2i treatment, patients receiving SGLT2i were more likely to present genitourinary fungal infections but not other types of infections. In addition, patients receiving SGLT2i had a lower occurrence of hepatic events. Further studies are required to confirm these results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Vall d'Hebron University Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization and design: JB, JP; Data collection: JB, AP, JR-E, LC-R, AV, LR-O, MS; Data analysis: RM-N, MP; Interpretation: JR-E, AC, MS, VV, JG, JP; First manuscript drafting: JB, RM-N; Revision and acceptance of last version: All authors; Supervision: JP. All authors contributed to the article and approved the submitted version.

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Conflict of interest

JP reports having received consulting fees from Boehringer Ingelheim and Novo Nordisk. He has received speaking fees from Gilead, and travel expenses from Gilead, Rubió, Pfizer, Astellas, MSD, CUBICIN, and Novo Nordisk. He has received educational and research support from Gilead, Pfizer, Astellas, Accelerate, Novartis, Abbvie, ViiV, and MSD. Funds from European Commission/EFPIA IMI2 853966-2, IMI2 777377, H2020 847989, and ISCIII PI19/01898.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.945626/full#supplementary-material

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