UNIVERSITAT DE BARCELONA

Factors de risc de trombosi portal no tumoral en la cirrosi hepàtica

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Programa de Doctorat de Medicina i Recerca Translacional

Facultat de Medicina i Ciències de la Salut

FACTORS DE RISC DE TROMBOSI PORTAL NO TUMORAL EN LA CIRROSI HEPÀTICA

Memòria de tesi doctoral presentada per **Fanny Turon Masferrer** per optar al grau de Doctora per la Universitat de Barcelona

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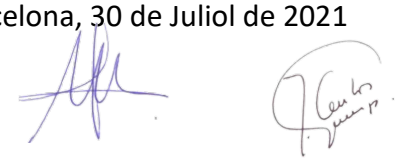
La tesi "*Factors de risc de trombosi portal no tumoral en la cirrosi hepàtica*" presentada per Fanny Turon Masferrer ha estat realitzada sota la seva direcció i reuneix les condicions necessàries per a la seva lectura i defensa pública per optar al títol de Doctor per la Universitat de Barcelona en el marc del Programa de Doctorat de Medicina i Recerca Translacional.

A Barcelona, 30 de Juliol de 2021



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AGRAÏMENTS

Als directors de la tesi, Joan Carles i Virginia i a tot l'equip d'hemodinàmica hepàtica. Als meus pares, germana i amics i sobretot a en Ferran, a la personeta que ha arribat fa menys temps al meu costat, la Carla, i a la que vindrà. Gràcies a tots.

ÍNDEX

1. ABREVIACIONS

AAD	Antivirals d'acció directa
BBNC	Beta-bloquejants no cardioselectius
FVIII	Factor VIII
FVL	Factor V Leiden
FVW	Factor Von Willebrand
GPVH	Gradient de pressió venós hepàtic
IL-6	Interleucina-6
NETs	Trampes extracel·lulars de neutròfils
PAI	Activador del plasminogen
RVS	Resposta viral sostinguda
TAFI	Inhibidor de la fibrinòlisi activable per trombina
TNF-α	Factor necrosi tumoral α
TP	Trombosi portal
t-PA	Activador del plasminògen dels teixits
VHC	Virus hepatitis C

2. ENUMERACIÓ D'ARTICLES QUE COMPONEN LA TESI

Tesi en format compendi d'articles. La tesi consta de 5 objectius i de 2 articles:

Article 1:

Fanny Turon, Ellen G. Driever, Anna Baiges, Eira Cerda, Ángeles García-Criado, Rosa Gilabert et al. Predicting portal thrombosis in cirrhosis: A prospective study of clinical, ultrasonographic and hemostatic factors. Journal of Hepatology 2021. doi: 10.1016/j.jhep.2021.07.020. Factor d'impacte 25,083, primer quartil, àrea gastroenterologia i hepatologia.

Article 2:

Mattias Mandorfer*, **Fanny Turon***, Sabela Lens, Anna Baiges, Ángeles García-Criado, Anna Darnell et al. Risk of non-tumoural portal vein thrombosis in patients with HCV-induced cirrhosis after sustained virological response. Liver International 2021. doi: 10.1111/liv.15009. *Primera co-autoria compartida. Factor d'impacte 5,828, segon quartil, àrea gastroenterologia i hepatologia.

3. INTRODUCCIÓ

3.1. Aspectes generals

La cirrosi hepàtica és l'estadi final de diferents malalties hepàtiques i s'associa a una alta morbi-mortalitat. Globalment, suposa 1.32 milions de morts anuals al món, essent així la onzena causa de mort a nivell mundial en l'actualitat (1,2). Les principals causes de cirrosi hepàtica inclouen les hepatitis víriques per virus de l'hepatitis C (VHC) o virus de l'hepatitis B, el consum d'alcohol o la malaltia per fetge gras entre d'altres.

En la cirrosi hepàtica es produeix una alteració a nivell de l'arquitectura hepàtica com a conseqüència d'un dany crònic i mantingut sobre el fetge provocat per diferents factors etiològics (alcohol, virus, autoimmunitat, ...) que promou la síntesis i dipòsit de col·lagen formant ponts entre els espais porta i venes centrals delimitant nòduls de regeneració (3). Aquesta distorsió de l'estructura normal del fetge juntament amb un increment del to vascular hepàtic fa que augmentin les resistències vasculars intrahepàtiques, provocant el desenvolupament d'hipertensió portal (4). La hipertensió portal es defineix com l'increment del gradient de pressió portal (GPP) que és la diferencia entre la pressió a la vena porta i la pressió a la vena cava inferior. En condicions normals, la pressió dels dos territoris és similar i el GPP és menor de 5 mmHg.

Anatòmicament, el fetge té la particularitat de rebre una doble aportació vascular, la que prové de la vena porta i la que prové de l'artèria hepàtica. Aquesta doble aportació de sang es barreja a nivell del sinusoid hepàtic i flueix cap a la circulació sistèmica a través de les venes hepàtiques que desemboquen a la vena cava inferior. El flux arterial té la capacitat de modificar-se quan varia el flux sanguini portal i això fa que l'aportació sanguínia hepàtica es mantingui relativament constant en individus sans. El GPP, com en qualsevol sistema hepodinàmic ve definit per la Llei de Ohm (Pressió = Q x R), on la pressió es directament proporcional al flux sanguini portal (Q) i la resistència (R) que s'oposa al flux tant a la vena porta com a la circulació intrahepàtica o a la vena cava inferior. Així doncs, donat que la circulació

hepàtica te una gran elasticitat i és capaç d'acomodar grans augments de flux, la principal causa responsable de l'elevació del GPP es l'augment de la resistència vascular. Al territori esplàncnic, en estadis més avançats de la malaltia també té lloc una vasodilatació esplàncnica progressiva secundària a l'hiperproducció de substàncies vasodilatadores (fonamentalment òxid nítric) que incrementa el flux sanguini portal que agreuja i perpetua encara més la síndrome d'hipertensió portal (4).

Durant l'evolució de la cirrosi, el desenvolupament d'hipertensió portal es un punt clau en l'historia natural de la malaltia ja que es el factor de risc més important per l'aparició de descompensacions i complicacions de la cirrosi tenint així un gran valor pronòstic. Una de les complicacions observades de forma relativament freqüent en els pacients amb cirrosi hepàtica es el desenvolupament d'una trombosi portal no tumoral (TP).

Aquesta tesi pretén aprofundir en l'estudi de la trombosi portal no tumoral en la cirrosi hepàtica des d'un punt de vista etiològic, estudiant els factors de risc més rellevants implicats en el seu desenvolupament.

3.2. Trombosi portal no tumoral en la cirrosi hepàtica (TP)

La trombosi portal (TP) no tumoral es caracteritza per la presencia d'un trombus d'origen no tumoral a la vena porta. Pot afectar el tronc venós portal i/o les branques portals intrahepàtiques, i es pot estendre a la vena mesentèrica superior, vena esplènica o ambdues.

Tradicionalment, la cirrosi s'havia considerat una malaltia amb un estat hipocoagulant subjacent i amb un elevat risc hemorràgic. No obstant, durant els últims anys, aquest paradigma ha canviat i ja no es considera una afecció associada a un risc baix de desenvolupar esdeveniments trombòtics. De fet, diversos estudis han demostrat que els pacients amb cirrosi tenen un major risc de patir trombosi venosa, i molt especialment en el territori esplàncnic (5,6).

El desenvolupament d'una trombosi venosa portal no tumoral, contràriament all que passa a la població general, és un esdeveniment relativament freqüent en pacients amb cirrosi, amb una incidència anual estimada que va del 4,6 al 26%, essent la incidència major en estadis més avançats de la malaltia quan aquesta es troba en fase descompensada i en pacients en llista d'espera per a transplantament hepàtic (6–10).

L'impacte de la TP en la història natural de la cirrosi hepàtica no està ben establert i no hi ha prou evidència per definir si la TP és una causa d'un major deteriorament hepàtic o si la TP es una conseqüència més de la cirrosi hepàtica en estadis més avançats (7,11,12). El que si que està més clar, es que la TP en context del trasplantament hepàtic s'associa a una major morbi-mortalitat en el post trasplantament, sobretot quan la trombosi és extensa afectant la vena mesentèrica superior i impedeix realitzar una anastomosi portal anatòmica (13–17). Així mateix, en algunes ocasions la TP pot contraindicar el trasplantament hepàtic limitant així les opcions terapèutiques d'aquests pacients i conseqüentment la seva supervivència.

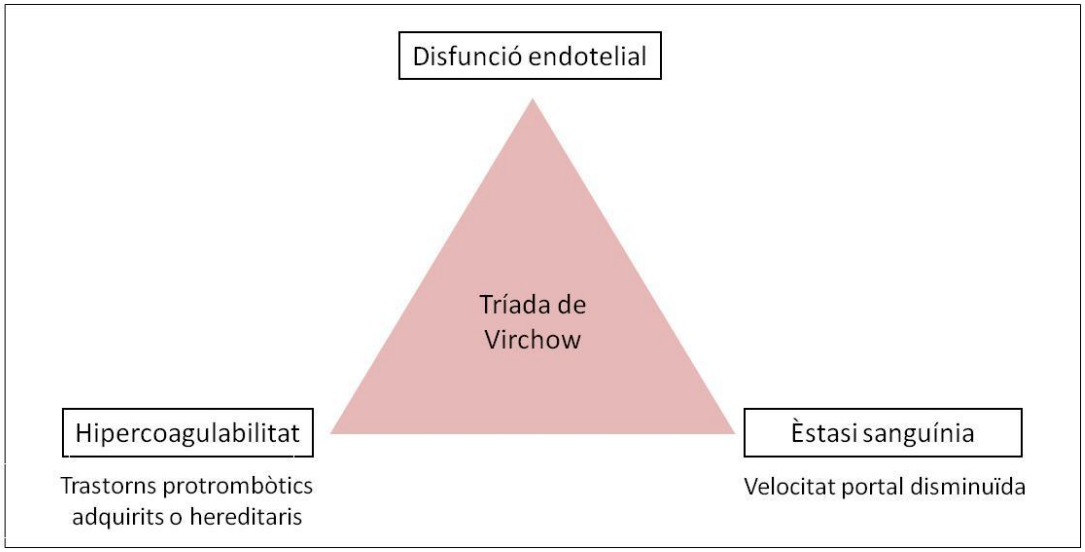
3.3.Fisiopatologia i factors de risc de trombosi portal en la cirrosi hepàtica

En la patogènesi de la trombosi en qualsevol territori, s'han implicat diferents factors que s'han agrupat en tres pilars principals que contribueixen a la formació del trombus i que s'anomena "Tríada de Virchow" (Figura 1): 1) Disminució de la velocitat del flux sanguini o estasi sanguínia (obstrucció vascular, immobilitat…), 2) la hipercoagulabilitat (trombofílies adquirides o hereditàries, fàrmacs, malalties autoimmunes…) i 3) Lesió o disfunció endotelial (p.ex. traumatisme, cirurgia, sèpsia, aterosclerosis…).

No obstant, la contribució exacta de cadascun d'aquests mecanismes en el desenvolupament de la trombosi portal no tumoral en la cirrosi hepàtica és desconeguda. Hi ha alguns estudis que han avaluat el possible paper de diferents d'aquests factors sobre el risc de desenvolupar TP en la cirrosi hepàtica però la

majoria son estudis retrospectius i transversals i pocs estudis ho han avaluat de forma prospectiva (7,8,10,18,19).

Figura 1. Factors que contribueixen a la formació d'una trombosi: Tríada de Virchow.



3.3.1 Disminució de la velocitat del flux sanguini o estasi sanguínia

El sistema venós portal representa un territori vascular únic, degut a que en condicions normals, és un sistema de baixa pressió, alt volum i amb una alta elasticitat ja que el vas es pot distendre fàcilment per compensar els canvis en el flux portal. Això fa que en condicions normals la trombosi en aquest nivell sigui un event poc freqüent. No obstant, en la cirrosi, el territori esplàncnic és el territori on es més freqüent que es produeixi una trombosi, suggerint que els canvis que es produeixen en la cirrosi en aquesta àrea puguin jugar un paper rellevant en la seva patogènesi.

En la cirrosi es produeix un increment en les resistències vasculars intrahepàtiques al flux portal que provoca el desenvolupament de la hipertensió portal i en estadis més avançats, es produeix una vasodilatació del territori arterial esplàncnic que fa que s'incrementi el flux sanguini portal i que agreuja encara més la hipertensió portal (4). Així mateix, l'increment de la pressió portal promou la formació de circulació colaterals que ocasiona la derivació de part del flux portal cap a la circulació sistèmica com un intent de descomprimir el sistema portal però que paradoxalment

es produeix un augment compensatori de l'aportació de sang que prové de la circulació esplàncnica i afavoreix la disminució de la velocitat portal.

Tenint en compte les particularitats hemodinàmiques del territori portal, estudis previs han avaluat el rol de la velocitat portal en el desenvolupament de TP i s'ha descrit la velocitat portal disminuïda, especialment quan es troba per sota de 15 cm/seg com un dels principals factors de risc independents per a desenvolupar TP (8,20), suggerint que els canvis hemodinàmics locals al sistema portal son factors rellevants en la patogènesi de la TP en la cirrosi.

3.3.2 Hipercoagulabilitat

El fetge juga un paper primordial en la coagulació ja que sintetitza gran part dels factors de coagulació o de les proteïnes involucrades en la fibrinòlisi. A més a més, el fetge també produeix la trombopoetina, que és responsable de la producció de plaquetes per part dels megacariòcits. I addicionalment, s'associa esplenomegàlia amb hiperesplenisme secundaris a la hipertensió portal que provoca plaquetopènia. Per tant, conseqüentment, les malalties hepàtiques tenen un impacte rellevant en el sistema hemostàtic (21).

Durant molts anys hi ha hagut la creença generalitzada que en les malalties hepàtiques cròniques, sobretot en estadis avançats o cirrosi, hi ha un estat d'hipocoagulabilitat o anticoagulant subjacent i que existeix una tendència hemorràgica. Aquest fet està recolzat perquè aquests pacients presenten alteracions en les proves de coagulació rutinàries com un temps de protrombina allargat i plaquetes baixes i perquè freqüentment tenen complicacions hemorràgiques. No obstant això, els darrers anys hi ha hagut un canvi en aquest paradigma, ja que s'ha evidenciat que els pacients amb hepatopaties cròniques avançades, en concret en estadi de cirrosi, tenen un equilibri hemostàtic molt complex que resulta en el descens concomitant tant dels factors procoagulants com dels anticoagulants (22,23). Així doncs, a pesar de què els tests de laboratori habituals mostren un temps de protrombina allargat i un descens en el recompte de plaquetes suggerint

l'existència d'un estat d'hipocoagulabilitat, els tests de coagulació més sofisticats han evidenciat alteracions que afavoreixen un estat d'hipercoagulabilitat. Per això, es considera que aquests pacients tenen una hemostàsia balancejada o equilibrada (Figura 2) (22,23).

No obstant, es tracta d'un equilibri fràgil i menys estable que l'equilibri hemostàtic en persones sanes (23). Això es podria explicar perquè els nivells de la majoria de factors es troben substancialment disminuïts en ambdós sentits (tant anti com procoagulants) i perquè aquest balanç hemostàtic es pot veure fàcilment alterat per complicacions que freqüentment tenen lloc en els pacients amb cirrosi com les infeccions o la insuficiència renal. Així doncs, es freqüent que en els pacients amb cirrosi hepàtica es produeixin complicacions tant hemorràgiques com trombòtiques durant la seva evolució.

El perfil hemostàtic d'un pacient amb cirrosi típicament presenta les següents alteracions:

a) Alteracions en l'hemostàsia primària

L'hemostàsia primària es refereix als processos mitjançant els quals es produeix el tap plaquetari a través de l'adhesió, activació i agregació plaquetària. Les alteracions de l'hemostàsia primària en els pacients amb cirrosi inclouen la trombopènia i alteracions en la funció plaquetària, nivells elevats de FVW i disminució de nivells d'ADAMTS-13 (A desintegrin and metalloproteinase with thrombospondin type 1, member 13). En condicions normals, les plaquetes s'adhereixen a les parets dels vasos danyats a través d'una interacció amb el FVW, promovent així l'agregació i, finalment, la formació del tap primari hemostàtic. Així doncs, la trombopènia, característica típica en la cirrosi (24), es una característica a favor de l'estat anticoagulant. No obstant això, els nivells alts de FVW, troballa també freqüent en aquests pacients, poden restablir l'adhesió plaquetària (25). A més, els nivells d'ADAMTS-13, una metaloproteasa plasmàtica que s'encarrega de degradar els multímers de FVW disminuint la seva activitat, es troben reduïts en els pacients amb

cirrosi i això pot contribuir encara més a la restauració de la funció plaquetària (26) a pesar de trobar les plaquetes en nivells disminuïts.

b) Alteracions en l'hemostàsia secundària

L'hemostàsia secundària involucra l'activació del sistema enzimàtic de coagulació amb l'objectiu principal de la formació de trombina i fibrina per a l'estabilització del coàgul (Figura 3 a).

L'alteració característica en l'hemostàsia secundària dels pacients amb cirrosi consisteix en la disminució concomitant dels nivells dels factors anticoagulants (antitrombina III, proteïna C i proteïna S) i dels factors procoagulants (factor II, V, VII, IX, X, XI) excepte el Factor VIII que està incrementat i és un dels impulsors més potents de la generació de trombina. El fibrinogen, proteïna precursora de la fibrina, també es troba disminuït en la cirrosi.

c) Fibrinòlisi

La fibrinòlisi és el mecanisme mitjançant el qual, després de que s'hagi dipositat fibrina dins del sistema vascular, es converteix el proenzim plasminògen en l'enzim actiu plasmina, que al seu torn degrada la fibrina. En condicions normals, aquesta conversió del plasminògen a plasmina està regulada per activadors (profibrinolítics) com l'activador del plasminògen dels teixits (t-PA), l'activador del plasminògen de la urocinasa i el factor XII activat. Aquests activadors alhora estan regulats per els antiactivadors (antifibrinolítics) com els inhibidors de t-PA (principalment l'inhibidor de l'activador del plasminogen (PAI)), l'inhibidor de la plasmina i l'inhibidor de la fibrinòlisi activable per trombina (TAFI). Aquest és un procés altament regulat i qualsevol pertorbació d'aquest equilibri pot provocar hiperfibrinòlisi augmentant el risc d'hemorràgia o pel contrari, hipofibrinòlisi, augmentant el risc de trombosi (Figura 3 b).

En la cirrosi s'han descrit canvis en els dos sentits, trobant tant alteracions que afavoreixen l'hiperfibrinòlisi com l'augment dels nivells de t-PA i nivells disminuïts de l'inhibidor de plasmina i de TAFI però també alteracions que afavoreixen

l'hipofibrinòlisi com la reducció dels nivells de plasminògen i l'increment dels nivells de PAI. No obstant, no està del tot establert el rol patogènic de la fibrinòlisi en l'hemorràgia ni en la trombosi sobretot degut en què no es disposa de proves de laboratori adequades per a la seva valoració, ja que la majoria es basen en la mesura dels components del sistema de forma individual i no d'una forma global. Probablement el balanç en el sistema de la fibrinòlisi es trobi compensat degut als canvis paral·lels en els factors pro i antifibrinolítics (27,28) .

Figura 2. Alteracions de la coagulació (canvis anti i pro coagulants) en la cirrosi hepàtica que condueixen a una hemostàsia rebalancejada.

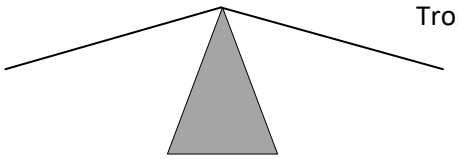
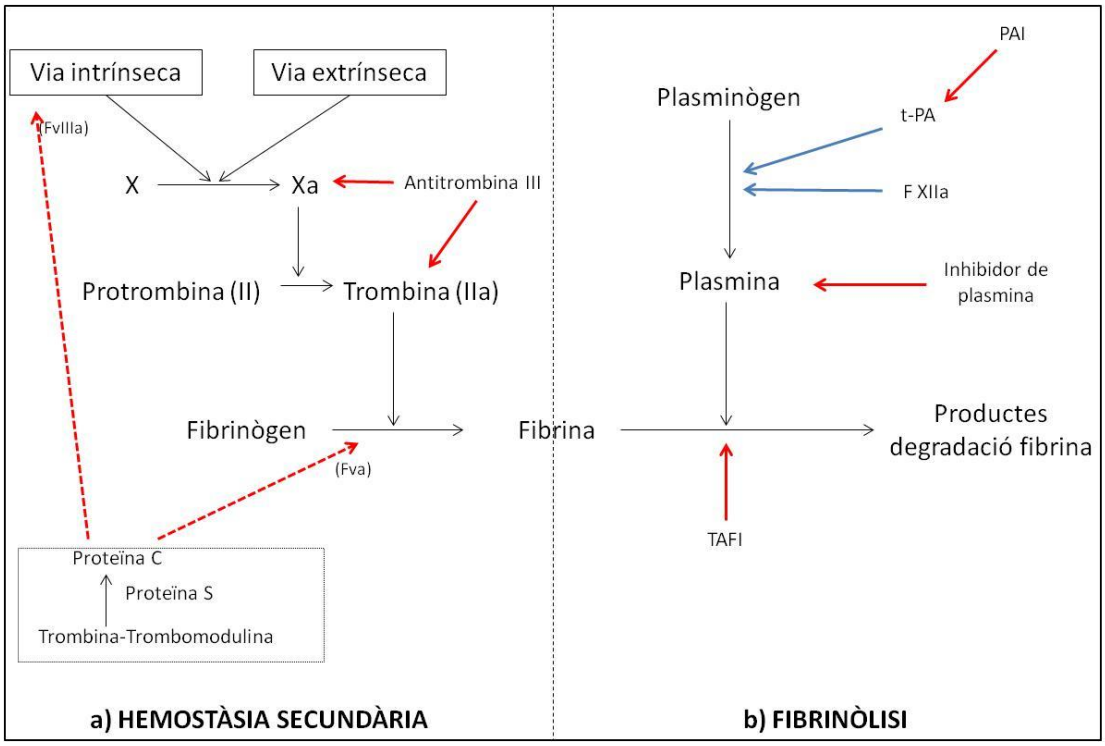
	HEMOSTÀSIA REBALANCEJADA	
	Hemorràgia  Trombosis	
	Canvis anticoagulants	**Canvis procoagulants**
Hemostàsia primària	↓Plaquetes	↑FVW ↓ADAMTS-13
Hemostàsia secundària	↓ factors de coagulació II, V, VII; IX, X , XI i fibrinogen	↑ Factor VIII ↓proteïna C, S, antitrombina
Fibrinòlisi	↓ TAFI ↓Inhibidor plasmina ↑ t-PA	↓Plasminògen ↑PAI

Figura 3. Esquema simplificat del sistema de la coagulació: a) Hemostàsia secundària; b) Fibrinòlisi.



Avaluació de l'hemostàsia

Com a problema addicional, els tests de laboratori convencionals que s'utilitzen de rutina a la pràctica clínica, com el temps de protrombina o el temps de tromboplastina parcial activada, no son adequats per valorar l'estat real de coagulació en la cirrosi ja que només reflecteixen una part del problema i no son sensibles a l'acció dels anticoagulants que es troben disminuïts en la cirrosi.

Durant els últims anys, s'han avaluat altres mètodes per estudiar l'estat global de coagulació en els pacients amb cirrosi. El test de generació de trombina es un bon exemple dirigit a explorar la funció de coagulació tenint en compte els factors pro i anticoagulants. La trombina és l'enzim final de la coagulació que actua sobre el fibrinogen per formar fibrina. Els estudis realitzats mostren que la generació de

trombina (mesurada sense trombomodulina, que es el principal activador de la via anticoagulant de la proteïna C) està reduïda en la cirrosi, igual que el factor II (protrombina) que també es troba en nivells disminuïts. No obstant això, quan el test es modifica i s'afegeix trombomodulina, aquesta permet l'activació completa de la proteïna C (principal anticoagulant que regula a la baixa la generació de trombina) i els pacients amb cirrosi generen gairebé la mateixa quantitat de trombina que les persones sanes (29). Això suggereix que en la cirrosi, la reducció del factor II (procoagulant) es troba equilibrat per la reducció de proteïna C (anticoagulant). De fet, la ratio entre el Factor II i els nivells de proteïna C, considerat un índex aproximat del balanç entre l'estat pro i anticoagulant responsable de la generació de trombina, es troba augmentat en els pacients amb cirrosi respecte els controls sans indicant que la proteïna C es redueix de forma més significativa que el factor II (29). Així mateix, estudis posteriors han mostrat que la ratio de generació de trombina (amb/sense trombomodulina) es major en pacients cirròtics que en controls sans, indicant això que a més d'una disminució de factors anti i procoagulants també existeix una marcada resistència a l'acció de la trombomodulina, contribuint així a un major estat procoagulant (30,31) Aquesta resistència es major en pacients Child-Pugh C que en Child-Pugh A o B suggerint que existeix una major hipercoagulabilitat del plasma en pacients amb cirrosi més avançada (Child-Pugh C) (30).

Factors de de coagulació implicats en el desenvolupament de de TP

En quant a l'entorn hemostàtic en la cirrosi, s'han proposat alguns factors procoagulants com a factors de risc per a la TP com la ratio Factor VIII/Proteïna C incrementada (32), un potencial endogen de generació de trombina augmentat (33), nivells plasmàtics elevats de Factor VIII (34), nivells baixos d'ADAMTS-13 (35) o nivells plasmàtics elevats de VWF (32) suggerint el potencial paper de l'hipercoagulabilitat en el desenvolupament de trombosi portal. No obstant aquests factors han estat avaluats de forma aïllada en els diferents estudis sense tenir en compte els altres potencials factors confusors implicats pel que s'han d'interpretar amb precaució.

Addicionalment, també s'han avaluat factors hereditaris de coagulació, i principalment s'ha suggerit que el factor V Leiden (FVL) i la mutació del gen de la protrombina G20210A es troben més freqüentment de forma significativa en els pacients amb cirrosi i TP que en aquells sense TP (36), però existeixen dades discrepants i no ha estat confirmat en altres estudis (7,37).

3.3.3 Disfunció endotelial

L'evidència actual suggereix que diferents insults com la inflamació o les forces mecàniques podrien activar les cèl·lules venoses endotelials i que aquestes cèl·lules activades poden canviar el seu fenotip i l'expressió molecular promovent un estat més protrombòtic (38). No obstant, aquestes dades provenen d'estudis que han avaluat el sistema venós sistèmic i no es coneix el potencial rol fisiopatològic de les alteracions endotelials del territori portal en el desenvolupament de la TP.

3.3.4 Paper de la inflamació associada a la cirrosi

A més a més de les alteracions hemodinàmiques i de la coagulació, dades recents suggereixen que l'existència d'una reacció inflamatòria sistèmica en la cirrosi probablement també jugui un paper en la progressió de la malaltia i el desenvolupament de les seves complicacions clíniques. Els pacients amb cirrosi i ascitis presenten translocació bacteriana (pas de bactèries o productes bacterians des de l'intestí als ganglis limfàtics mesentèrics), a conseqüència principalment de l'augment de la permeabilitat intestinal. Mitjançant aquest mecanisme s'estimulen els monòcits i limfòtics que alliberen citoquines pro-inflamatòries com el factor de necrosis tumoral α (TNF- α) o la Interleucina 6 (IL-6) condicionant així una reacció inflamatòria. Aquesta reacció inflamatòria sistèmica i activació del sistema immune agreujarien encara més la vasodilatació i l'estat hiperdinàmic d'aquests pacients (39).

Existeix una estreta relació entre el sistema de coagulació i la inflamació. Els neutròfils han estat descrits recentment com a promotors de la trombosi mitjançant el mecanisme pel qual alliberen NETs (Trampes extracel·lulars de neutròfils o "neutrophil extracelular traps"). Els NETs són expulsats per els neutròfils activats i estan compostos per una matriu d'histones, filaments de ADN i enzims dels neutròfils com la mieloperoxidasa (40,41). Els NETs originalment van ser descrits com a un mecanisme de defensa contra patògens (42) però posteriorment s'ha demostrat que es poden generar en absència de patògens i que son activadors potents de la coagulació. Han estat implicats en diferents malalties trombòtiques com la trombosi venosa profunda o l'infart de miocardi (40,41,43–46) suggerint que puguin ser factors de risc pel seu desenvolupament. Els principals mecanismes involucrats en el seu risc trombogènic inclouen l'activació i agregació plaquetària (desencadenada per la unió dels NETs a les plaquetes) i l'activació de la cascada de la coagulació promovent la formació de fibrina (41,44,47,48).

Durant els últims anys, la generació de NETs ha estat avaluada en diferents escenaris en relació a les malalties hepàtiques. S'ha descrit un augment de generació de NETs durant el trasplantament hepàtic (49) i en context d'insuficiència hepàtica aguda sobre crònica (ACLF-Acute-on-chronic liver failure) (50). Respecte el possible rol de la inflamació sistèmica en el desenvolupament de la TP en la cirrosi, s'ha descrit l'Interleucina-6 (IL-6) com a factor predictiu independent de TP en un estudi recent (51). El possible rol d'altres marcadors inflamatoris i dels NETs en el risc de TP no ha estat avaluat prèviament.

3.3.5 Altres factors de risc

S'han descrit altres factors de risc relacionats principalment amb una major severitat de la malaltia hepàtica i de la hipertensió portal: recompte plaquetari baix (8,10,52), nivells plasmàtics d'albúmina baixos (53), varius esofàgiques grans (7,18,54), tractament previ de les varius amb escleroteràpia (55), descompensacions hepàtiques prèvies (10) o presència de col·laterals portosistèmiques grans (9).

Estudis més recents han suggerit que els beta-bloquejants no cardioselectius (BBNC), emprats en la profilaxi de l'hemorràgia variceal, podrien tenir un paper afavorint el desenvolupament de TP (18,54,56). No obstant això, el disseny d'aquests estudis no permet descartar un efecte confusor amb altres factors de risc de TP i tampoc no s'han tingut en compte canvis en el tractament previs a la aparició de la TP, pel que aquest fet no ha estat adequadament avaluat ni confirmat.

3.4. Trombosi portal post curació del VHC

Es coneix poc sobre la història natural de la TP no tumoral en la cirrosi i sobre el potencial impacte en el pronòstic de la malaltia i per això hi ha un gran debat sobre la necessitat de tractar aquestes trombosis amb anticoagulants o no, sobretot quan la trombosi és poc extensa. De fet, existeix controvèrsia sobre si la TP és una causa o una conseqüència d'un major deteriorament hepàtic (7,11,12) i inclús s'ha suggerit que pot existir una transitorietat del trombus que pot desaparèixer espontàniament en fins el 70% dels casos (7). El que si que està clar, com s'ha mencionat anteriorment, es l'impacte de la TP en el trasplantament hepàtic que condiciona una major morbi-mortalitat associada.

Existeix una situació molt concreta en la que s'ha descrit una potencial regressió de la cirrosi al desaparèixer l'agent etiològic (finalització consum alcohol, erradicació VHC...) però es disposen d'escasses dades sobre l'impacte de la regressió sobre la hipertensió portal i les complicacions de la cirrosi incloent la trombosi portal. Això és especialment important en la cirrosi per VHC ja que durant l'última dècada, han aparegut nous tractaments antivirals pel tractament de l'hepatitis crònica C, en concret règims sense interferó basats en antivirals d'acció directa (AAD), que son molt efectius inclús en pacients amb cirrosi hepàtica i hipertensió portal, una població que fins ara era de molt difícil tractament (57,58).

Diferents estudis s'han centrat en avaluar l'impacte del tractament antiviral en l'evolució de la malaltia hepàtica després d'assolir la resposta viral sostinguda (RVS) (59–61), evidenciant un clar efecte beneficiós sobre la hipertensió portal (62–67)

reduint així el risc de presentar descompensacions hepàtiques (66), millorant la funció hepàtica i amb un clar impacte en la supervivència (60).

Estudis recents han avaluat l'impacte de la RVS després del tractament antiviral amb AAD sobre les complexes alteracions de la coagulació presents en els pacients amb cirrosi (61,68–70). S'ha evidenciat que després d'assolir RVS després del tractament antiviral en els pacients amb cirrosi es produeix una milloria en la síntesi hepàtica dels factors pro i anticoagulants conduint a una milloria de la hipercoagulabilitat (69,70). També s'ha demostrat una disminució significativa dels nivells de FVIII i un increment en els nivells plasmàtics de proteïna C (69), una disminució dels nivells de FVW (70) i canvis significatius en la generació de trombina després del tractament, indicant així una reversió de la coagulopatia del cirròtic (69). Aquesta correcció de la coagulopatia es produeix particularment en els pacients Child-Pugh A, mentre que en els pacients Child-Pugh B no es tant evident, suggerint així que la possibilitat de millorar la coagulació després del tractament es inversament proporcional a la gravetat de la cirrosi (69).

No obstant, l'impacte clínic en el desenvolupament de trombosi portal no ha estat del tot avaluat. Només dos estudis han reportat la taxa de TP després del tractament antiviral en la cirrosi per VHC i s'ha suggerit que a pesar de que hi ha un clar impacte beneficiós sobre la funció hepàtica, la hipertensió portal i en la supervivència, la incidència de TP podria estar incrementada. En l'estudi de Russo et al. dos dels 58 pacients avaluats van desenvolupar TP durant el seguiment post RVS. Tots dos eren Child-Pugh B abans de rebre el tractament (al mes i als tres mesos de la RVS) i en cap dels dos casos no hi va haver milloria en els paràmetres de generació de trombina, indicant que en aquests pacients, la RVS no s'associava a una milloria dels paràmetres de coagulació (69). Un altre estudi ha descrit un potencial augment de la incidència de TP després del tractament antiviral, reportant 7 pacients amb cirrosi compensada que van desenvolupar una TP a la setmana 12 post RVS (representant una incidència del 3% a la setmana 12) suggerint així un risc incrementat de TP post RVS (71).

4. JUSTIFICACIÓ I HIPÒTESI

4.1. Justificació

La trombosi portal no tumoral és una complicació relativament freqüent durant la història natural de la cirrosi hepàtica, sobretot en estadis més avançats de la malaltia. Actualment no existeix forma de predir quins pacients desenvoluparan o no una trombosi portal. Els estudis previs que han avaluat diferents factors de risc que podrien estar implicats en el desenvolupament de la TP tenen limitacions, ja que molts d'ells son estudis retrospectius o transversals , que inclouen pocs pacients, i on els potencials factors de risc estudiats no han estat avaluats de forma global i conjunta i no han tingut en compte tots els factors que poden estar implicats en la patogènesi de la TP (característiques clíniques i bioquímiques, característiques ecogràfiques com la velocitat portal i paràmetres de coagulació i inflamació). Per això, els factors de risc per desenvolupar trombosis portal, i en concret el potencial paper de les alteracions de la coagulació en la cirrosi, no han estat determinats.

Addicionalment, en el cas concret de la cirrosi per VHC, durant els últims anys han aparegut els antivirals d'acció directa que han permès la curació del VHC en pacients amb cirrosi en els que fins fa pocs anys no existia aquesta possibilitat d'eliminació del VHC. La curació del VHC s'ha associat a una millora de la funció hepàtica, millora de la hipertensió portal i a un augment de la supervivència. No obstant, es desconeix el potencial impacte de la curació del VHC sobre el risc de desenvolupar trombosi portal i dades recents suggereixen que aquest risc podria estar incrementat.

4.2. Hipòtesi

- L'avaluació prospectiva incloent els diferents factors potencialment implicats en el desenvolupament de trombosi portal no tumoral en la cirrosi (variables clíniques, bioquímiques, ecogràfiques i paràmetres de coagulació i inflamació) de forma global i exhaustiva permetrà identificar quins son els principals factors de risc associats amb el desenvolupament de TP no tumoral en la cirrosi.

- Les alteracions de la coagulació i d'inflamació que presenten els pacients amb cirrosi hepàtica podrien tenir un paper rellevant augmentant el risc de TP.

- La curació del VHC podria impactar sobre el risc de desenvolupar trombosi portal disminuint-ne el seu risc una vegada eliminat l'agent etiològic mitjançant fàrmacs antivirals d'acció directa.

5. OBJECTIUS

Objectius principals:

1. Estudiar els factors predictius de TP no tumoral en la cirrosi hepàtica.
2. Avaluar l'impacte de la RVS en el desenvolupament de TP no tumoral en els pacients amb cirrosi hepàtica per VHC tractats amb antivirals d'acció directa.

Objectius secundàris:

1. Avaluar la incidència de TP no tumoral en la cirrosi hepàtica.
2. Avaluar el paper de les alteracions de coagulació i inflamació presents en els pacients amb cirrosi sobre el risc de desenvolupar TP.
3. Avaluar l'impacte de la RVS en la supervivència i en el desenvolupament de descompensacions de la cirrosi.

6. RESULTATS

6.1. Estudi 1. Predicting portal thrombosis in cirrhosis: A prospective study of clinical, ultrasonographic and hemostatic factors.

Fanny Turon, Ellen G. Driever, Anna Baiges, Eira Cerda, Ángeles García-Criado, Rosa Gilabert, Concepció Bru, Annalisa Berzigotti, Isabel Nuñez, Lara Orts, Juan Carlos Reverter, Marta Magaz, Genis Camprecios, Pol Olivas, Fabian Betancourt-Sanchez, Valeria Perez-Campuzano, Annabel Blasi, Susana Seijo, Enric Reverter, Jaume Bosch, Roger Borràs, Virginia Hernandez-Gea, Ton Lisman, Juan Carlos Garcia-Pagan

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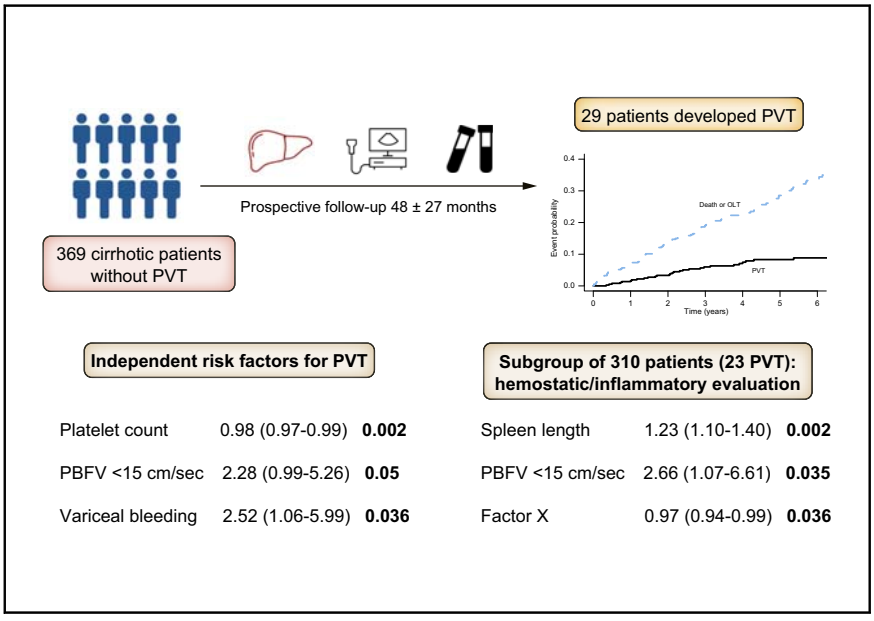
Aquest estudi respon als objectius 1, 2 i 3 d'aquesta tesi.

Research Article
Cirrhosis and Liver Failure

JOURNAL
OF HEPATOLOGY

Predicting portal thrombosis in cirrhosis: A prospective study of clinical, ultrasonographic and hemostatic factors

Graphical abstract



Highlights

- Factors related to more severe portal hypertension are associated with higher risk of PVT in cirrhosis.
- Acquired and inherited alterations of coagulation do not predict PVT development during follow-up.
- Cirrhosis-associated inflammation or generation of NETs are not relevant factors predicting PVT development.

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Lay summary

Patients with cirrhosis and more severe portal hypertension are at higher risk of non-tumoral portal vein thrombosis development. Acquired or inherited hemostatic disorders, as well as inflammatory status, do not seem to predict the development of portal vein thrombosis in patients with cirrhosis.

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Research Article
Cirrhosis and Liver Failure

JOURNAL OF HEPATOLOGY

Predicting portal thrombosis in cirrhosis: A prospective study of clinical, ultrasonographic and hemostatic factors

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Background & Aims: Portal vein thrombosis (PVT) is a relatively frequent event in patients with cirrhosis. While different risk factors for PVT have been reported, such as decreased portal blood flow velocity (PBFV) and parameters related with severity of portal hypertension, these are based on retrospective studies assessing only a discrete number of parameters. The aim of the current study was to evaluate the incidence and risks factors for non-tumoral PVT development in a large prospective cohort of patients with cirrhosis.

Methods: We performed an exhaustive evaluation of clinical, biochemical, inflammatory and acquired/hereditary hemostatic profiles in 369 patients with cirrhosis without PVT who were prospectively followed-up. Doppler ultrasound was performed at baseline and every 6 months or whenever clinically indicated. PVT development was always confirmed by computed tomography.

Results: Twenty-nine patients developed non-tumoral PVT, with an incidence of 1.6%, 6% and 8.4% at 1, 3 and 5 years, respectively. Low platelet count, PBFV <15 cm/sec and history of variceal bleeding were factors independently associated with a high PVT risk. No relationship between PVT development and any other clinical biochemical, inflammatory and acquired or hereditary hemostatic parameter was found.

Conclusions: In patients with cirrhosis, the factors predictive of PVT development were mainly those related to the severity of portal hypertension. Our results do not support the role of hemostatic alterations (inherited or acquired) and inflammatory markers in the prediction of PVT in patients with cirrhosis.

Lay summary: Patients with cirrhosis and more severe portal hypertension are at higher risk of non-tumoral portal vein thrombosis development. Acquired or inherited hemostatic disorders, as well as inflammatory status, do not seem to predict the development of portal vein thrombosis in patients with cirrhosis.

Introduction

Cirrhosis is no longer considered a condition associated with a low risk of developing thrombotic events. Indeed, several studies have shown that patients with cirrhosis are at higher risk of developing splanchnic and extrasplanchnic vein thrombosis.[1,2] Actually, development of non-tumoral portal vein thrombosis (PVT) is a relatively frequent event in patients with cirrhosis; the estimated annual incidence ranges from 4.6 to 26%,[2–6] with the highest incidence in patients with more advanced liver disease.

Most studies evaluating risk factors for PVT are retrospective and transversal, comparing clinical variables between patients with and without PVT. Only a few of these studies are prospective, evaluating the incidence of PVT during follow-up.[3,4,6–8] From these studies, a decreased portal blood flow velocity (PBFV) below 15 cm/sec[4,9] has been described as a major risk factor for PVT development. Additional risk factors for PVT are those related with liver disease severity and the presence of portal hypertension: low platelet count,[4,6,10] low albumin,[11] large esophageal varices[3,7,12] and previous sclerotherapy,[13] previous liver decompensation[6] or presence of large portosystemic collaterals.[5] More recently, it has been suggested that non-selective beta-blockers (NSBBs) may play a role in PVT development.[7,12,14] However, the potential confounding effect of other recognized risk factors for PVT was not adequately evaluated in these studies.

The hemostatic balance in patients with cirrhosis is fragile[15]; it is characterized by a decrease in procoagulant but also anticoagulant factors that are synthesized by hepatocytes, together with an increase in endothelial-derived factors such as Factor VIII (FVIII) and

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von Willebrand factor (VWF). It has been proposed that some hypercoagulable characteristics such as an increased FVIII to protein C ratio (FVIII/PC),[16] increased endogenous thrombin potential (ETP),[17] high FVIII,[18] low ADAMTS13[19] or increased plasma levels of VWF[16] may represent risk factors for developing cirrhosis-associated PVT. Additionally, inherited thrombophilia, notably Factor V Leiden (FVLeiden) and the prothrombin G20210A mutation, has also been suggested to be more frequent in cirrhotic patients with PVT than in those without,[20] but this has not been confirmed in other studies.[3,21] A close relationship between alterations in coagulation and inflammation exists.[22] Neutrophil extracellular traps (NETs) are a web-like structure composed of neutrophil expelled DNA and proteins that have recently been described as a link between inflammation and coagulation and have been implicated in thrombotic diseases.[23] The role of NETs and systemic inflammation in the pathogenesis of PVT in patients with cirrhosis has not been evaluated.

Although clinical, hemostatic and inflammatory parameters may all be involved in development of PVT, all proposed mechanisms continue to be debated and a prospective systematic analysis of all these mechanisms in predicting PVT development has not yet been performed.

The aim of the current study is to evaluate the incidence and risks factors for PVT development in a large cohort of patients with cirrhosis who were prospectively followed-up and in whom an exhaustive clinical, biochemical, inflammatory and acquired/hereditary hemostatic profile was obtained.

Materials and methods

Study population

This is a prospective single center study including consecutive patients with cirrhosis submitted to an abdominal Doppler ultrasound (Doppler-US) for the screening of hepatocellular carcinoma (HCC) between December 2010 and April 2013. Patients between 18 and 80 years with cirrhosis demonstrated by liver biopsy and/or with compatible clinical, laboratory and imaging data were considered eligible for the study. Exclusion criteria were as follows: known HCC, pregnancy, previous orthotopic liver transplantation (OLT), refused to provide informed consent, use of anticoagulation, previous surgical or transjugular intrahepatic portosystemic shunt (TIPS).

Baseline data

Baseline abdominal Doppler-US was performed by 5 ultrasound experts with more than 10 years of experience at our center following a standard protocol[24] and evaluating the patency of the main portal vein trunk and both intrahepatic portal branches, portal blood flow velocity (PBFV – measured as time averaged maximal velocity), portal blood flow direction, portal vein trunk diameter, presence of porto-systemic collaterals, hepatic vein patency, spleen length, splenic artery pulsatility and resistance index and patency of the superior mesenteric and splenic vein if technically possible. For each patient, baseline clinical and laboratory data were collected. Blood samples at baseline were also stored at the Hospital Clinic Biobank facilities for an exhaustive evaluation of hemostatic and inflammatory parameters.

Follow-up

Patients were prospectively followed until February 2019 or until OLT, death, TIPS placement, start of anticoagulation for any reason, evidence of tumoral PVT or hepatic resection surgery. Additionally, because of the potential impact on the natural history of PVT in cirrhosis, patients with HCV who received antiviral treatment during follow-up were censored when sustained virological response (SVR) was achieved. Doppler-US was repeated every 6 months or whenever clinically indicated.

Portal vein thrombosis

PVT was diagnosed by demonstrating the presence of endoluminal material compatible with non-tumoral thrombosis in the portal vein and/or its branches. PVT diagnosis and its extension were always confirmed by computed tomography or magnetic resonance imaging. PVT was defined as occlusive when there was an absence of blood flow in the vein or partial when the lumen was only partially occluded and flow was still present.

Coagulation and inflammation evaluation

Hemostasis and fibrinolysis tests: Coagulation factors II, V, VII; VIII, IX, X, XI, XII, XIII, protein C, S and antithrombin activity, VWF, ADAMTS-13, fragment 1+2 (F1+2), activated factor VII (FVIIa), plasmin-antiplasmin complexes, D-dimer, plasminogen, plasminogen activator inhibitor-1, soluble P-selectin, soluble CD40L were determined as described previously.[25–27] The plasma capacity to generate thrombin was measured using a continuous thrombin-generation assay in an automated system as previously described.[28]

Microparticles were measured by ELISA capture assay based on the ability of annexin V (bonded onto plastic plates) to bind phosphatidylserine on circulating microparticles deposited in pellets (Hyphen BioMed, Neuville-sur-Oise, France). Functional assays were performed to measure the procoagulant activity of microparticles through thrombin generation, as previously described.[29] The measure was based on the activation of prothrombinase activity on the surface of the microparticles following the addition of activated bovine factors X and V and purified human prothrombin. After incubation, the thrombin generated was quantified using a specific chromogenic substrate by measuring absorbance at 405 nm. Microparticles were determined in duplicate and expressed as equivalents of nanomolar of phosphatidylserine (nM PS eq). The laboratory detection limit of the technique was 0.05 nM PS eq. and the intra-assay and inter-assay coefficient of variation were 5% and 8%, respectively.

NETs: complexes of myeloperoxidase and DNA (MPO-DNA) were quantified using a capture ELISA as previously described,[30] using commercially available antibodies (anti-MPO monoclonal antibodies from Sanbio, Uden, Netherlands, and peroxidase-labelled anti-DNA monoclonal antibodies from the cell death detection ELISA kit (Sigma Aldrich, Zwijndrecht, Netherlands). Cell-free DNA (cfDNA) concentration in plasma was measured using the Picogreen Quant-it kit (Fisher Scientific, Landsmeer, Netherlands).

Clot lysis time (CLT): The fibrinolytic capacity of a plasma sample was quantified by CLT assays. CLT was determined by measuring turbidity changes during clot formation and subsequent lysis of the clot, as described before.[31]

Clot retraction: Clot retraction with subsequent red cell extrusion from the clots was performed as described[32] in baseline samples of all PVT cases and 53 randomly selected non-PVT patients. Plasma was mixed with isolated platelets and red blood cells (RBCs) from healthy blood donors with blood group O to

obtain reconstituted blood containing 20,0000 platelets/μl and a hematocrit of 40%.[33] This reconstituted blood was clotted with human alpha-thrombin (Sekisui Diagnostics, Stamford, CT, USA, 0.1U/ml final concentration) and calcium chloride (10 mM final concentration) in siliconized wells for 2 hours at 37 °C. Clots were weighed after removing adherent liquid, and the hemoglobin level in the supernatant (diluted with PBS) was estimated by absorbance measurements at 575 nm. The percentage of extruded RBCs was calculated by comparing the absorbance of reconstituted blood and the supernatant after clot formation and retraction. By mixing patient plasma with healthy blood cells, this assay specifically quantifies the contribution of plasma fibrinogen to clot retraction and thus provides a measure for the functional properties of the fibrinogen molecule that is known to be altered in patients with liver disease.[34]

Permeability: Permeation of fibrin clots from PVT cases and from randomly selected non-PVT patients (n = 53) was measured using a liquid permeation assay as described previously.[35] The permeability coefficient Ks was calculated following Darcy's law. This assay provides a direct measure of fibrin clot quality; the relation between decrease permeability of fibrin clots and thrombotic risk has been well established,[36] as has the increased permeability of plasma clots in patients with cirrhosis.[34]

Fibrinogen concentration was determined on an automated coagulation analyser (ACL TOP 300, Werfen, Barcelona, Spain) with reagents from Werfen (Barcelona, Spain).

Concentrations of C-reactive protein, interleukin 6 (IL-6) and tumor necrosis factor-α were assessed with ELISA kits obtained from R&D Systems (Minneapolis, MN,USA).

Genotyping FVLeiden and Prothrombin 20210A: DNA isolated from whole blood samples was used to genotype using commercially available probes (Thermo Fisher Scientific, Waltham, MA, USA).

An inherited deficiency of protein C, S or antithrombin was excluded by establishing a ratio of protein C, S or antithrombin to (factor II+factor X)/2 of greater than 0.7 and by the study of first degree relatives whenever possible.[37]

Statistical analysis

Data are expressed as frequencies (%) for categorical variables and as mean ± standard deviation for continuous variables. Fisher's exact test was used for categorical variables and the paired Student's *t* test for continuous variables or paired non-parametric test when assumptions of normality could not be verified.

The (event-free) survival of patients was evaluated with Fine-Gray competing risk survival analysis. We estimated the cumulative incidence functions from competing risks data across groups: PVT (event of interest) or competing events (death, OLT, tumoral-PVT or TIPS). The predictors of PVT development were estimated by regression modeling of sub-distribution functions in competing risks analysis. Variables that showed a statistically significant effect on (event-free) survival in univariate analyses (p <0.10) or that were clinically relevant were entered into multivariate models, which were evaluated using clinical criteria and log-likelihood ratio test. For continuous variables, cut-offs were selected either by using the Youden method or based on already validated cut-offs in the literature. The number of variables that could enter the multivariate analysis was limited using the m/10 rule to prevent over-fitting. In addition, in the multivariate analysis, individual parameters were not considered when including scores that contain them (*i.e.* Child-Pugh). Similarly, variables that focus on the same specific hemostatic process were not evaluated together. Hemostatic parameters that were not evaluated in the entire cohort (clot retraction and permeability) were not included in the multivariate analysis.

Additionally, a time-dependent covariate analysis was performed to determine the potential role of NSBB use in PVT development, in a time-varying model adjusted by variceal hemorrhage and presence of large esophageal varices as longitudinal potential confounders. These variables were recorded every 6 months in the scheduled revisions during follow-up.

The level of significance was established at the 2-sided 5% level. Statistical analysis was performed using SPSS 23.0 (SPSS Inc. Chicago,IL) and R software for Windows version 3.6.1 (R project for Statistical Computing; Vienna, Austria).

Ethical aspects

All patients included in the study gave signed written informed consent to participate. The ethical committee of Hospital Clinic Barcelona approved the current study (HCB/2010/6107) in accordance with the International Guideline for Ethical Review of Epidemiological Studies and principles of the Declaration of Helsinki.

Results

Baseline Doppler-US was performed in 437 consecutive patients with cirrhosis initially considered eligible by 1 of the 5 ultrasound experts participating in the study. Thirty-seven patients had ≥1 exclusion criteria. Twenty-three patients (5.8%) had PVT at baseline (21 partial, 2 occlusive). Characteristics of patients with and without baseline PVT are described in Table S1. Eight of the 377 patients without PVT at baseline had no further follow-up at our institution. Thus, finally, 369 patients with cirrhosis without PVT were included in the prospective cohort and were followed-up for a mean of 48 ± 27 months (Fig. 1). 59% were male with a mean age of 59 ± 10 years. The most frequent etiologies of cirrhosis were HCV (56%) and alcohol (27%); 72% of patients had Child-Pugh A and 22% Child-Pugh B cirrhosis. One-hundred forty-five (39%) patients had large varices, 59 (16%) had a history of variceal hemorrhage and 148 (40%) had ascites. Table 1 shows the baseline characteristics of the study population.

During follow-up, 30 (8.1%) patients underwent an OLT, 7 (1.9%) underwent TIPS placement, 4 (1.1%) started anticoagulation, 4 (1.1%) required a large hepatic resection, 60 (16%) developed HCC and 9 (2.4%) tumoral-PVT. Additionally, 100 patients (27.1%) initiated antiviral therapy for HCV and their follow-up was stopped after 45 ± 14months. Twenty patients (5.4%) were lost to follow-up after 28 ± 16 months, mainly due to changing their reference hospital. Seventy-six patients died during follow-up and the overall OLT-free survival was 93.7%, 83% and 73.1% at 1, 3 and 5 years, respectively (Fig. 2).

PVT development

Twenty-nine patients developed non-tumoral PVT during follow-up. The cumulative incidence of PVT was 1.6%, 6% and 8.3% at 1, 3 and 5 years, respectively (Fig. 2). Thrombosis was occlusive in 3 patients and partial in 26. Table S2 provides details on the location and degree of thrombosis. Twenty-one patients (72%) were asymptomatic (2 occlusive; 19 partial) and the PVT was detected on scheduled Doppler-US. The remaining 8 patients (28%) were diagnosed during an extra Doppler-US performed at

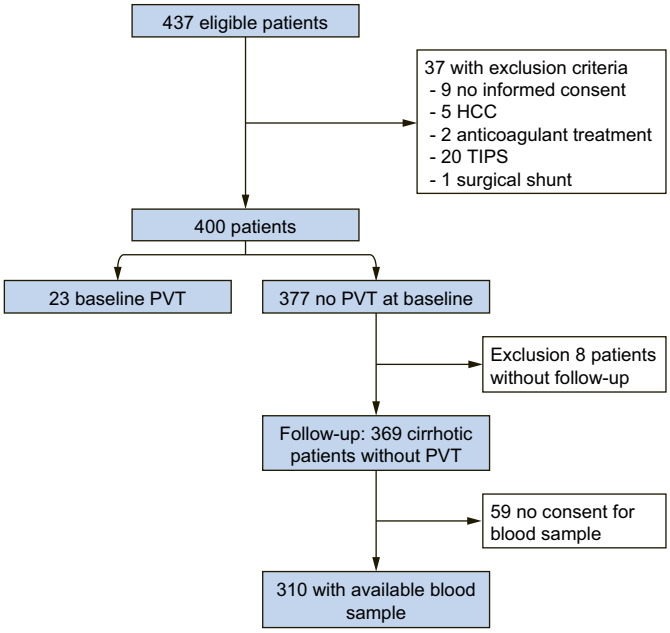

Fig. 1. Inclusion flow chart. PVT, portal vein thrombosis; TIPS, transjugular intrahepatic portosystemic shunt.

hospitalization: Seven with partial PVT presenting with variceal hemorrhage (n = 1), first ascites decompensation (n = 1), jaundice (n = 3), Salmonella gastroenteritis (n = 1), cholecystitis (n = 1) and 1 with occlusive PVT presenting with intestinal ischemia. All patients who developed PVT had a Doppler-US within the 6 previous months with no evidence of PVT. In those patients undergoing OLT without reaching the PVT endpoint, the surgical report was reviewed and no additional PVT was found. None of the 29 patients who developed PVT had HCC at the time of PVT diagnosis. After PVT diagnosis, 10 patients started anticoagulation, 1 received TIPS (as treatment for concomitant variceal bleeding) and 18 were in observation. Among these 18 non-anticoagulated patients, spontaneous recanalization occurred in 2, progression in 5 and PVT maintained stable in the other 11. Regarding their outcome, 12 patients died 22 ± 27 months after PVT and 6 patients underwent OLT after 18 ± 23 months, while the remaining 11 were alive at the end of follow-up.

Table 1. Baseline characteristics of the study population.

Variables	Mean ± SD/n(%)
Age,years	59 ± 10
Sex, male	217 (59%)
Body mass index, Kg/m^2	27.6 ± 4.4
Etiology:	
HCV	209 (56%)
HBV	17 (4.6%)
Alcohol	101 (27%)
MAFLD	13 (3.5%)
Platelets, 10^9/L	116 ± 59
INR	1.23 ± 0.24
Albumin, g/L	37 ± 6
Bilirubin, mg/dl	1.6 ± 1.5
Creatinine, mg/dl	0.88 ± 0.41
MELD	10 ± 4
Child-Pugh score	6 ± 2
Child-Pugh class	
A	264 (72%)
B	82 (22%)
Varices	230 (62%)
Large varices	145 (39%)
Any previous decompensation*:	177 (48%)
Ascites	148 (40%)
Spontaneous bacterial peritonitis	15 (4%)
Variceal bleeding	59 (16%)
Hepatic encephalopathy	51 (14%)
Endoscopic band ligation	46 (12.5%)
NSBBs	149 (40%)
Secondary prophylaxis	51 (13%)
Transient elastography, kPa (n = 253; 22 PVT)	29 ± 17
Spleen length, cm	14.5 ± 2.6
Portal vein diameter, mm (n = 361; 28 PVT)	12.5 ± 2.5
PBFV, cm/sec (n = 357; 28 PVT)	17.7 ± 5.6
PBFV <15 cm/sec	107 (29%)
Splenic artery resistance index	0.71 ± 0.1
Splenic artery pulsatility index (n = 348; 28 PVT)	1.3 ± 0.31
Porto-systemic collaterals (n = 330; 27 PVT)	144 (39%)
HVPG (n = 103;6 PVT)	15.3 ± 5
HVPG ≥10	94 (91%)
HVPG ≥16	45 (12%)
HVPG ≥20	21 (6%)

HVPG, hepatic venous pressure gradient; INR, international normalized ratio; MAFLD, metabolic dysfunction-associated fatty liver disease; MELD, model for end-stage liver disease; NSBBs, non-selective beta-blockers; PBFV, portal blood flow velocity; PVT, portal vein thrombosis.
*Some patients had more than 1 decompensation.

Risk factors for PVT

Factors associated with PVT development at univariate competing risk analysis are shown in Table 2. Multivariate analyses, including those variables significant at univariate analysis with a p value <0.10 identified 2 models: (model 1) platelet count, PBFV <15 cm/sec and variceal bleeding and (model 2) spleen length, PBFV <15 cm/sec and variceal bleeding. MELD and Child-Pugh score were not significant at multivariate analyses (Table 2). Model 1 had the best log-likelihood ratio and a PVT risk score was created by combining the sum of these 3 factors according to their HR to categorize patients based on their risk of developing PVT (Fig. S1).

Additionally, considering that PBFV is a variable not commonly assessed in clinical practice, we explored multivariate models excluding PBVF. The best model excluding PBFV was the combination of history of variceal bleeding (p = 0.0041; HR 3.09; 95% CI 1.43–6.68) and platelet count (p = 0.0026; HR 4.05; 95% CI 1.63–10.05).

Although NSBB use was not identified as a risk factor for PVT in the different multivariate analyses, and because it has been suggested to be involved in PVT development in some studies, an additional time-dependent analysis was performed considering changes in this treatment during follow-up, with evaluations every 6 months according to scheduled medical visits. Indeed, 24 patients started NSBB treatment and 13 patients discontinued treatment during follow-up. The model was adjusted by the confounders variceal bleeding and presence of large esophageal varices. Also, in this time-dependent analysis, NSBB use was not associated with PVT development (p = 0.71; HR 0.745; 95% CI 0.154–3.60]).

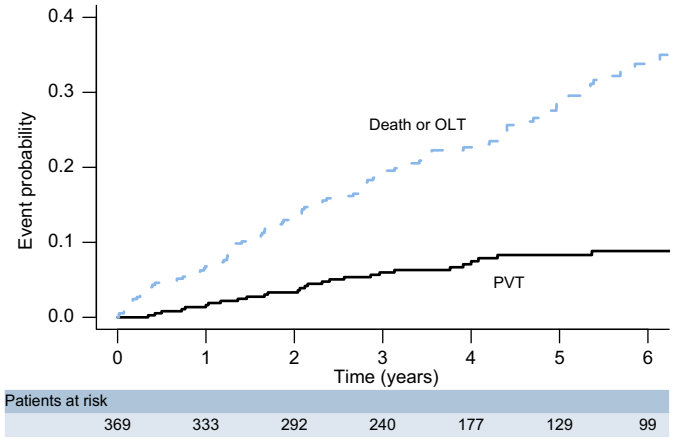

Fig. 2. Cumulative incidence for primary endpoint (PVT) and competitive event. OLT, orthotopic liver transplantation; PVT, portal vein thrombosis.

Hepatic venous pressure gradient evaluation

One-hundred and three patients had a hepatic venous pressure gradient (HVPG) measurement performed within 12 months before inclusion. Six of the 103 patients with an available HVPG developed PVT during follow-up. Median HVPG at baseline was higher in the 6 patients who developed PVT (19.4 ± 4.5 mmHg) compared to the 97 patients who did not (15.4 ± 5 mmHg), although this was not statistically significant ($p = 0.13$). All patients who developed PVT had clinically significant portal hypertension (HVPG above 10 mmHg) at baseline. Interestingly, 4 out of 6 patients (67%) who developed PVT had an HVPG above 20 compared with 17/97 (17%) who did not develop PVT ($p = 0.015$). In other words, 4/21 (19%) patients with HVPG >20 mmHg developed PVT while this only happened in 2/82 (2.4%) of those with an HVPG <20 mmHg. However, HVPG was not included in the multivariate analysis because it was only available in 103 patients.

Evaluation of the hemostatic and inflammatory profile

Three hundred and ten (84% of the included population) patients without PVT at baseline had a blood sample stored at the Biobank in which an exhaustive evaluation of hemostatic and inflammatory factors was performed. Twenty-three of them developed PVT during follow-up. The remaining 16% of patients did not consent to blood sampling.

Hemostatic and inflammatory markers associated with PVT development at univariate competing risk analysis are detailed in Table 3. As shown, patients developing PVT had lower levels of coagulation factors synthesized by the liver (either prothrombotic or antithrombotic factors) and a lower ETP with or without TM. However, these patients had significantly higher levels of soluble P-selectin, F1+2, VIIa, microparticles and a higher FVIII/PC ratio. No differences in NETs or other inflammatory markers were observed. Patients that developed PVT did not have an increased thrombogenicity of fibrin clots generated *in vitro*, except for a slight, but non-significant increase in CLT.

In the multivariate analysis, the variables that independently predicted PVT development were PBFV <15 cm/sec ($p = 0.035$; HR 2.66; 95% 1.07–6.61), spleen length ($p = 0.002$; HR 1.23; 95% CI 1.10–1.40) and levels of the pro-coagulant Factor X ($p = 0.036$; HR 0.97; 95% CI 0.94–0.99).

Table 2. Univariate and multivariate competing risk analysis for PVT development.

Univariate analysis			Multivariate analysis			
Variable	**sHR (95% CI)**	***p* value**	**Variables**	**sHR (95% CI)**	***p* value**	**Log-likelihood ratio test**
Body mass index, Kg/m^2	0.98 (0.89–1.09)	0.79	**Model 1**			
MAFLD	3.09 (0.98– 9.8)	**0.05**	Platelets	0.98 (0.97–0.99)	**0.002**	27
Platelets, 10^9/L	0.98 (0.97–0.99)	**<0.001**	PBFV <15 cm/sec	2.28 (0.99–5.26)	**0.05**	
INR	1.94 (1–3.07)	**0.049**	Variceal bleeding	2.52 (1.06–5.99)	**0.036**	
Albumin, g/L	0.93 (0.89–0.98)	**0.008**	**Model 2**			
Bilirubin, mg/dl	1.10 (0.97–1.24)	0.12	Spleen length	1.26 (1.11–1.42)	**<0.001**	25
Creatinine, mg/dl	0.25 (0.03–1.93)	0.18	PBFV <15 cm/sec	2.31 (1.02–5.26)	**0.046**	
MELD	1.05 (1–1.1)	**0.047**	Variceal bleeding	2.37 (0.99–5.67)	**0.05**	
Child-Pugh score	1.13 (0.99–1.28)	0.062	**Model 3**			
Child-Pugh class B/C	2.36 (1.14–4.88)	**0.021**	Child–Pugh score	1.00 (0.86–1.69)	0.94	22
Large varices	3.61 (1.64–7.94)	**0.001**	PBFV <15 cm/sec	2.92 (1.37–6.19)	**0.005**	
Previous decompensation	4.3 (1.77–10.5)	**0.001**	Platelets	0.98 (0.97–0.99)	**0.002**	
Variceal bleeding	3.37 (1.60–7.13)	**0.001**	**Model 4**			
Ascites	1.89 (0.91–3.96)	0.089	MELD	1.00 (0.93–1.06)	0.86	22
NSBBs	3.44 (1.57–7.53)	**0.002**	Variceal bleeding	2.91 (1.38–6.16)	**0.005**	
Primary prophylaxis	1.47 (0.68–3.16)	0.32	Platelets	0.98 (0.97–0.99)	**0.002**	
Secondary prophylaxis	3.54 (1.65–7.6))	**0.001**				
Spleen length, cm	1.28 (1.15–1.43)	**<0.001**				
Portal vein diameter, mm	1.10 (1.01–1.21)	**0.031**				
PBFV, cm/sec	0.91 (0.81–1.03)	0.15				
PBFV <15 cm/sec	2.70 (1.29–5.68)	**0.008**				
Porto-systemic collaterals	1.05 (0.57–1.91)	0.87				
HVPG, mmHg	1.10 (0.97–1.24)	0.13				
HVPG ≥20	8.08 (1.50–43.6)	**0.015**				

HVPG, hepatic venous pressure gradient; INR, international normalized ratio; MAFLD, metabolic dysfunction-associated fatty liver disease; MELD, model for end-stage liver disease; NSBBs, non-selective beta-blockers; PBFV, portal blood flow velocity; PVT, portal vein thrombosis; sHR, subdistribution hazard ratio.
Values in bold denote statistical significance.
Predictors were estimated by regression modeling of subdistribution functions in competing risks scenarios. The statistical test used to define these variable associations is the Wald test, which under the null hypothesis follows an asymptotic $\chi 2$-distribution with 1 degree of freedom.

Inherited thrombophilic disorders

The prothrombin G20210A mutation and FVLeiden were tested in 266 patients, 23 of them developing PVT during follow-up. None of these 23 patients had the prothrombin G20210A variant or were carriers of FVLeiden. Of the 243 patients that did not develop PVT, 5 patients were heterozygous and 1 was homozygous for the prothrombin 20210A variant, whereas 9 patients were heterozygous FVLeiden carriers.

Additionally, in the 21 patients who were excluded because of PVT at baseline, only 1 was heterozygous for the prothrombin 20210A variant and none were FVLeiden carriers. None of the 310 patients had a confirmed hereditary deficiency of protein C, S or antithrombin.

Discussion

This is the first study to prospectively address, in a large cohort of patients with cirrhosis, the risk factors for non-tumoral PVT development including a comprehensive number of clinical and ultrasonographic parameters together with an exhaustive evaluation of coagulation and inflammatory markers.

The observed cumulative incidence of PVT is in the lower range of that previously published[2–6] and our 5 year incidence of

Table 3. Competing risk univariate analysis of hemostatic and inflammatory evaluation.

Variables	PVT (n = 23)	No PVT (n = 287)	sHR (95% CI)	*p* value
Hemostatic proteins				
Primary hemostasis				
VWF (Ag,%)	120.9 ± 32	125.6 ± 27.3	0.99 (0.97–1.01)	0.54
VW ristocetin co-factor (functional,%)	108.8 ± 22.4	119.1 ± 26.3	0.98 (0.97–1)	0.051
ADAMTS13,%	98.6 ± 18.5	96.9 ± 17.3	1 (0.98–1.03)	0.69
Secondary hemostasis				
sTF,ng/ml	135.9 ± 25.5	143.5 ± 27.9	0.99 (0.98–1)	0.19
Factor II, %	52.8 ± 15	62.9 ± 16.6	0.96 (0.94–0.99)	**0.001**
Factor V, %	52 ± 12.9	62.2 ± 15.8	0.95 (0.93–0.98)	**0.001**
Factor VII, %	43.9 ± 12.6	52.6 ± 15.6	0.96 (0.95–0.99)	**0.002**
Factor VIII, %	125.8 ± 19.1	135.2 ± 23.3	0.981 (0.96–0.99)	**0.036**
Factor IX, %	57.5 ± 18.8	59.8 ± 19.1	0.99 (0.97–1.01)	0.49
Factor X, %	50.4 ± 12.1	61.2 ± 15.3	0.95 (0.92–0.97)	**<0.001**
Factor XI, %	62.5 ± 17	76.9 ± 19.6	0.96 (0.95–0.98)	**<0.001**
Factor XII, %	58.4 ± 14.4	65 ± 15.1	0.97 (0.95–0.99)	**0.024**
Factor XIIIa, %	61.2 ± 28.5	75.0 ± 36.1	0.99 (0.97–1.01)	0.18
Fibrinogen, mg/ml	2.24 ± 0.71	2.47 ± 0.84	0.68 (0.39–1.17)	0.16
Protein C, %	60.3 ± 20.7	79.0 ± 23.9	0.97 (0.95–0.98)	**<0.001**
Protein S, %	66.7 ± 17.9	81.5 ± 20.4	0.97 (0.95–0.98)	**<0.001**
Antithrombin, %	75.5 ± 17.9	84.1 ± 18.8	0.98 (0.96–0.99)	**0.02**
Fibrinolysis				
Plasminogen, %	53.7 ± 11.5	60.8 ± 13.1	0.96 (0.94–0.99)	**0.002**
PAI-1, ng/ml	26.7 ± 7.6	23.3 ± 7.1	1.05 (1.01–1.09)	**0.017**
Markers of activation of hemostasis				
Soluble P-Selectin, ng/ml	73.7 ± 18	63.3 ± 24.2	1.02 (1–1.03)	**0.012**
Soluble CD40L, ng/ml	98.3 ± 19.7	104.5 ± 21.9	0.98 (0.97–1)	0.14
Fragment 1+2, nmol/ml	1.8 ± 0.8	1.4 ± 0.5	2.51 (1.47–4.27)	**<0.001**
Factor VIIa, ng/ml	3.4 ± 1.8	2.4 ± 1.5	1.29 (1.11–1.51)	**0.001**
Factor XIIa, ng/ml	3.8 ± 1.7	3.3 ± 1.6	1.17 (0.96–1.43)	0.11
D-dimer, ng/ml	466.8 ± 225.8	460.9 ± 221.2	1 (0.99–1)	0.95
PAP, μg/ml	1024.1 ± 268.4	949.6 ± 338.46	1(1–1)	0.21
Microparticles	24.3 ± 10.4	18.0 ± 9.1	1.05 (1.02–1.08)	**<0.001**
Global functional tests				
ETP (without TM), nM IIa*min	322.9 ± 27.7	349.8 ± 60.6	0.98 (0.97–0.99)	**<0.001**
ETP (with TM), nM IIa*min	268.6 ± 24.2	282.9 ± 44.3	0.98 (0.97–0.99)	**0.009**
Clot lysis time, min	82 ± 40	71 ± 25	1.01 (0.99–1.02)	0.072
Permeability, Ks	$4.2 \times 10^{-9} \pm 1.9 \times 10^{-9}$	$4.7 \times 10^{9} \pm 8 \times 10^{-9}$	0.99 (0.95–1.03)	0.61
Clot weight, mg	55 ± 10	60 ± 9	0.95 (0.90–0.99)	**0.042**
Ratios				
Von Willebrand ratio (VWF co-factor/Ag)	0.96 ± 0.33	0.98 ± 0.27	0.76 (0.11–5.01)	0.78
Ratio FVIII/Protein C	2.34 ± 0.98	1.89 ± 0.75	1.58 (1.17–2.14)	**0.0028**
ETP ratio (with/without TM)	0.83 ± 0.08	0.81 ± 0.06	1.70 (0.77–3.72)	0.20
Inflammatory markers				
Cell-free DNA, ug/ml	0.89 ± 0.16	0.89 ± 0.22	0.97 (0.22–4.27)	0.97
MPO-DNA (AU)	0.21 ± 0.29	0.29 ± 0.46	0.68 (0.28–1.67)	0.40
IL-6, pg/ml	7.7 ± 7.9	8.4 ± 12.5	0.99 (0.97–1.02)	0.70
TNF-α,pg/ml	12.4 ± 5.1	11.6 ± 10.5	0.01 (0.99–1.03)	0.32
CRP, ng/ml	5315 ± 8044	3584 ± 6631	1 (1–1)	0.24

CRP, C-reactive protein; ETP, endogenous thrombin potential; IL-6, interleukin-6; MPO-DNA, complexes of myeloperoxidase and DNA; PAI-1, plasminogen activator inhibitor-1; PAP, plasmin/antiplasmin complex; sTF, soluble tissue factor; TM, thrombomodulin; TNF, tumor necrosis factor; VWF, von Willebrand factor.
Values in bold denote statistical significance.
Predictors were estimated by regression modeling of subdistribution functions in competing risks scenarios. The statistical test used to define these variable associations is the Wald test, which under the null hypothesis follows an asymptotic χ2-distribution with 1 degree of freedom.

8.3% compares with the previously study from Nery *et al.*, especially when considering the 6-month ultrasound screening strategy (8%).[3] We are confident that this low incidence is real and not due to false negative ultrasound studies. Indeed, this is a prospective study specifically aimed at ruling out PVT and US-Doppler was always performed by the same 5 US experts.

Our study shows that the severity of portal hypertension, estimated by history of variceal bleeding and low platelet count, together with low PBFV are the main factors associated with PVT development. Different studies have already described previous decompensation and low platelet count as potential risk factors for PVT.[4,6,10] Controversies were raised about the reproducibility of PBFV measurements and therefore their potential role as a determinant factor for PVT development.[7] Our study clearly shows that accurate measurement of PBFV, by an experienced professional, is a useful predictive tool for PVT development. Indeed, our study confirms the previously described threshold of 15 cm/sec[4,9] as an independent risk factor for PVT.

Patients with cirrhosis have been shown to have a fragile rebalanced hemostatic system with a decrease in both pro and anticoagulant factors.[15] It has been suggested that, in different situations, this balance can be lost and a hypo or hypercoagulable state develops. Some studies have evaluated the potential role of the hemostatic state of patients with cirrhosis in promoting PVT development. Indeed, FVIII,[18] low ADAMTS13,[19] thrombomodulin resistance in a thrombin generation test[17] and an increased FVIII/protein C ratio[16] have been proposed as risk factors for PVT. However, previous studies have not comprehensively analyzed a full hemostatic profile or considered its potential independent role of other relevant variables such as severity of liver disease or PBFV in relation to PVT development. Our study confirms that patients with cirrhosis developing PVT had significantly reduced levels of several anticoagulant factors as well as a significant increase in the FVIII/protein C ratio, suggesting that a hypercoagulable state may also play a role in PVT development. When these results were adjusted, in different statistical models, by clinical and ultrasonographic variables, low levels of the liver synthesized procoagulant factor X were the only coagulation parameter that independently predicted PVT. Therefore, these data suggest that alterations of most of these acquired defects are a consequence of the more advanced liver disease state.

A well-known relationship exists between inflammation and coagulation.[38] Indeed, increased IL-6 levels have been reported to be associated with the presence of PVT in cirrhosis[39] and NETs have previously been implicated in thrombotic disease, such as deep vein thrombosis or myocardial infarction,[23] but their specific role in PVT development in the setting of cirrhosis has never been evaluated. In addition, NETs have been implicated in progression of various liver diseases[40] and have been shown to be associated with activation of coagulation during liver transplant surgery.[30] With this background, we decided to evaluate the potential role of several inflammatory markers in predicting PVT development. Our study did not find any association between the inflammatory markers evaluated, including IL-6 or NETs markers (MPO-DNA or cfDNA), and the risk of PVT development. Thus, our results do not support that cirrhosis-associated inflammation or NETs predict PVT development in cirrhosis. However, it cannot be excluded that an acute inflammatory response or an acute increase in the generation of NETs rather than baseline inflammatory status, or even a local increase of circulating inflammatory markers in the portal vein,[41] may drive PVT development.

In our broad evaluation of factors promoting blood hypercoagulability, we also evaluated the potential role of inherited coagulation disorders. Previous studies, including a recent meta-analysis, suggested that inherited thrombophilic factors such as FVLeiden and prothrombin G20210A mutation might play a role in PVT development in patients with cirrhosis.[20] Nevertheless, this has not been confirmed in other studies.[3,21] Most of these studies were retrospective and with a high probability of selection bias, therefore the potential role of the inherited prothrombotic factors was uncertain. In the current study, none of the 23 patients developing PVT of the 266 patients in whom inherited disorders were tested were carriers of FVLeiden or the prothrombin G20210A variant. The results of our study therefore argue against a role for these inherited disorders in PVT development in patients with cirrhosis.

Endothelial dysfunction/injury, reduction in blood flow and hypercoagulability are the 3 main pathophysiological mechanisms leading to venous thrombosis. Although we did not specifically evaluate the severity of endothelial dysfunction/injury, several clinical and experimental studies have shown a clear relationship between endothelial dysfunction and the severity of portal hypertension in cirrhosis.[42,43] History of variceal bleeding and low platelets are highly likely a reflection of a more severe degree of portal hypertension and of endothelial dysfunction. It is important to remark that in the subgroup of patients in whom HVPG was available, it was significantly higher in those patients who developed PVT. All patients developing PVT had an HVPG ≥10 mmHg and the risk seems to be much increased when HVPG is >20 mmHg. Additionally, it is clear that in the portal venous system there is a close relationship between endothelial dysfunction and portal hypertension, since endothelial dysfunction is one of the main mechanisms leading to an increased hepatic resistance to portal blood flow.[44] Increased portal pressure promotes the development of porto-collateral circulation (resulting in clinically relevant esophageal varices) diverting part of the portal blood flow to the systemic circulation, bypassing the liver and therefore reducing PBFV in the portal vein. Therefore, these 2 components, although independent, synergize with each other. Therefore, the results of our study suggest that PVT in patients with cirrhosis is mainly related to changes in portal blood flow and to endothelial dysfunction/injury while the role of hypercoagulability, either acquired or inherited, seems to be minor.

To shed more light on the controversy surrounding the use of NSBBs, we evaluated the role of NSBBs on PVT risk in a time-dependent analysis. Taking into consideration NSBB use, presence or development of esophageal varices and variceal bleeding, no association was found between NSBBs and PVT development. This observation goes against that from a recently published meta-analysis that reported a 4.6-fold increase in the risk of PVT with NSBB use.[14] However, it included 9 heterogeneous studies, mainly retrospective, with limited follow-up and without considering dynamic changes in NSBB use, variceal bleeding and variceal size. Thus, according to our results, the presence of risk factors for PVT development should not preclude the use of NSBBs if indicated.

Other factors previously suggested as potential risk factors for PVT such as obesity,[45] large esophageal varices, previous variceal endoscopic treatment[8,13,14] or etiology of liver disease, especially

metabolic dysfunction-associated fatty liver disease (MAFLD)[8,13,46,47] were not confirmed in our prospective study. However, only 13 patients in our cohort had MAFLD, so no firm conclusions can be drawn.

Combining the 3 variables with independent predictive value we were able to build a PVT risk score that identified 2 different populations (Fig. S1). Using our score, for every 4 high-risk patients that develop PVT after 4 years of follow-up, 1 low-risk patient will develop PVT. However, this model should be validated in future prospective cohorts.

We have to acknowledge some limitations of the study. First, the low number of *de novo* cases of PVT during follow-up reduces the number of parameters that can be included in the multivariate analysis and therefore some additional parameters related to PVT development may be missed. Although our study shows that the severity of portal hypertension is one of the strongest parameters related to PVT development, most patients included in the study were Child-Pugh A. Whether the risk factors for PVT are the same in Child-Pugh B and C patients is not known. Additionally, this is a single-center study and, although a multicenter study would have allowed for a larger sample, the design of our study had the advantage of maximizing the homogeneity of the data. Secondly, blood samples (to determine hemostatic and inflammatory status) were taken at inclusion rather than immediately before PVT development. Thus, while these factors are not predictive of PVT development according to our results, it cannot be excluded that they may play a pathophysiological role in a given situation (*i.e.* acute increase of inflammatory markers). Third, patients who achieve SVR after HCV antiviral treatment were censored due to its potential impact on the natural history of cirrhosis and PVT. This was inevitable since it would have been unethical to delay or withhold effective treatment for HCV patients after it became broadly available. However, this only occurred in 100 patients and their follow-up (45 ± 14 months) did not differ significantly from the follow-up of the whole cohort; thus, we think this is likely to have had minimal impact on our results.

In conclusion, factors related to more severe portal hypertension, including PBFV <15 cm/sec, low platelet count and history of variceal bleeding are independently associated with a higher risk of developing PVT in the setting of cirrhosis. Our results do not support that acquired hypercoagulability alterations observed in patients with cirrhosis, inherited disorders of coagulation or inflammatory status were predictive of PVT development. In addition, NSBB use was not independently associated with PVT risk.

Abbreviations

cfDNA, cell-free DNA; CLT, clot lysis time; Doppler-US, Doppler ultrasound; EBL, endoscopic band ligation; ETP, endogenous thrombin potential; FVIII, Factor VIII; FVLeiden, Factor V Leiden; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; IL-6, interleukin 6; MAFLD, metabolic dysfunction-associated fatty liver disease; MELD, model for end-stage iver disease; MPO-DNA,complexes of myeloperoxidase and DNA; NETs, neutrophil extracellular traps; NSBBs, non-selective beta-blockers; OLT, orthotopic liver transplantation; PBFV, portal blood flow velocity; PI, pulsatility index; PVT, portal vein thrombosis; RI, resistance index; sHR, subdistribution hazard ratio; sTF, soluble tissue factor; SVR, sustained virological response; TIPS, transjugular intrahepatic portosystemic shunt; TM, thrombomodulin; VWF, von Willebrand factor.

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Conflict of interest

Prof JB is a consultant for Gilead Science, Surrozen, Actelion, BMS, Biovie and BLB. Prof JCGP is a consultant for GORE and research grants from NOVARTIS. AGC receives speaker fees from BTG and Terumo.

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

Authors' contributions

Fanny Turon: study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, drafting of the manuscript. Ellen G. Driever: Performed laboratory tests, analysis and interpretation of data, critical revision of the manuscript for important intellectual content. Anna Baiges: acquisition of data, drafting of the manuscript. Eira Cerda: acquisition of data, drafting of the manuscript. Ángeles Garcia-Criado: ultrasound acquisition, drafting of the manuscript. Rosa Gilabert: ultrasound acquisition, drafting of the manuscript. Concepció Bru: ultrasound acquisition, drafting of the manuscript. Annalisa Berzigotti: ultrasound acquisition, drafting of the manuscript. Isabel Nuñez: ultrasound acquisition, drafting of the manuscript. Lara Orts: acquisition of data, obtaining blood samples for biobank, monitoring patients. Juan Carlos Reverter: Performed and supervised laboratory tests, analysis and interpretation of data, critical revision of the manuscript for important intellectual content. Marta Magaz: acquisition of data, drafting of the manuscript. Genis Camprecios: acquisition of data, drafting of the manuscript. Pol Olivas: acquisition of data, drafting of the manuscript. Fanian Betancourt-Sanchez: acquisition of data, drafting of the manuscript. Valeria Perez-Campuzano: acquisition of data, drafting of the manuscript. Annabel Blasi: analysis and interpretation of data, drafting of the manuscript. Susana Seijo: acquisition of data, drafting of the manuscript. Enric Reverter: acquisition of data, drafting of the manuscript. Jaume Bosch: obtained funding, critical revision of the manuscript for important intellectual content. Roger Borràs: Analysis and interpretation of data, drafting of the manuscript. Virginia Hernandez-Gea: critical revision of the manuscript for important intellectual content. Ton Lisman: Supervised laboratory tests, analysis and interpretation of data, obtained funding, critical revision of the manuscript for important intellectual content. Juan Carlos Garcia-Pagan: study concept and design, analysis and interpretation of data, statistical analysis, drafting of the manuscript, critical revision of the manuscript for important intellectual content, obtained funding and study supervision.

All the authors read and approved the final version of the paper.

Data availability statement

The data used in the manuscript are available in case of need.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.07.020.

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Author names in bold designate shared co-first authorship

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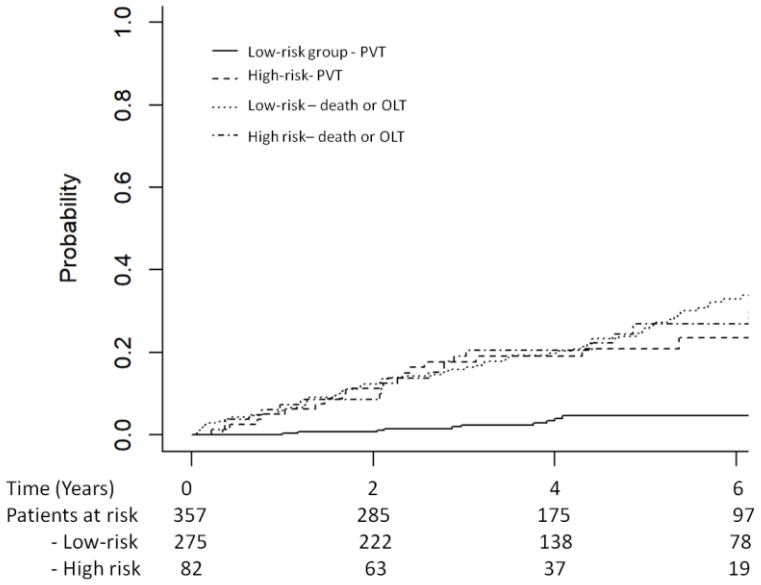
Supplementary Data

Supplementary figure 1. Cumulative incidence functions for primary endpoint (PVT) and competitive event (death/OLT/TIPS) stratified by optimal cutoff points.



Legend supplementary figure 1:

A PVT risk score was created by combining the sum of the three identified factors in the multivariate converted into categorical variables: Platelet count<100.000 (p=0.004; HR 3.90 [1.56-9.77]), PBFV<15 cm/sec (p=0.06; HR 2.11 [0.94-4.83]) and history of variceal bleeding (p=0.029; HR 2.61 [1.10-6.16]). Points were assigned for every variable in the model according to the HR: Platelet count<100.000 (4 points), PBFV<15 cm/sec (2 points) and variceal bleeding (3 points). A cutoff point of 5 was determined by the Youden index as the best cutoff to identify high-risk (5 or more) and low-risk (up to 4) patients for developing PVT. Accordingly, this score could be simplified by classifying patients with none or one risk factor into low-risk group and patients with two or three into high risk group because patients with more than one risk factor would always have at least 5 points. The model had an AUROC curve of 0.778(0.692-0.863) for predicting PVT. The incidence of PVT in patients with low-risk was 0.7, 4 and 4.6% at 2, 4 and 6 years while it was 11.2, 19.1 and 23.5% respectively in patients with high-risk (p<0.001; HR 6.46 [2.98-14]).

Supplementary table 1. Comparison between patients with and without PVT at baseline.

Variables (Mean ± SD ; n (%)	**Baseline PVT (n= 23)**	**No baseline PVT (n= 377)**	**P Value**
Age, years	58 ± 12	59 ± 10	0.497
Sex, male	16 (70%)	220 (58%)	0.383
BMI, Kg/m^2	26.8 ± 3.9	27.6 ± 4.4	0.447
Etiology - VHC - VHB - Alcohol - MAFLD - Autoimmune - Others	 9 (39.1%) 3 (13%) 9 (39.1%) 0 1 (4.3%) 1 (4.3%)	 214 (57%) 17 (4.5%) 103 (27%) 13 (3.4%) 12 (3.2%) 18 (4.8%)	 0.129 0.100 0.235 0.457 0.543 0.701
Hemoglobin, g/L	117 ± 18	130 ± 22	**0.005**
Leucocytes, $10^9/L$	4.22 ± 2.5	5.23 ± 2.3	**0.040**
Platelets, $10^9/L$	74 ± 39	115 ± 58	**0.000**
INR	1.5 ± 0.3	1.2 ± 0.2	**0.000**
Albumin, g/L	33 ± 5	38 ± 6	**0.000**
Bilirubin, mg/dL	2.6 ± 1.9	1.6 ± 1.5	**0.003**
ALT, U/L	35 ± 18	64 ± 58	**0.000**
GGT, U/L	88 ± 99	114 ± 146	0.390
Creatinine, mg/dL	0.84 ± 0.18	0.88 ± 0.40	0.598
Sodium, mEq/L	140 ± 3	140 ± 3	0.682
Colesterol, mg/dL	131 ± 38	156 ± 43	**0.006**
MELD	14± 3	10 ± 4	**0.000**
Child-Pugh score	8 ± 2	6 ± 2	**0.000**
Transient Elastography, Kpa (n=270)	36 ± 20	29 ± 17	0.115
Spleen length, cm	17.4 ± 3.5	14.5 ± 2.6	**0.000**
Portal vein diameter, mm	12.2 ± 3.8	12.5 ± 2.5	0.699
Varices Large varices	21 (91%) 19 (82%)	231 (62%) 145 (39%)	**0.003** **0.000**
Ascites Variceal bleeding	19 (83%) 10 (44%)	150 (40%) 59 (16%)	**0.000** **0.002**

EBL	12 (52%)	46 (12%)	**0.000**
NSBB use	17 (74%)	149 (40%)	**0.001**

Abbreviations: BMI, body mass index; MAFLD, metabolic-associated fatty liver disease; INR, international normalized ratio; MELD, Model for End-stage Liver Disease; EBL, endoscopic band ligation; NSBB, Non selective beta-blockers

Supplementary table 2. Location and degree of 29 incident PVT as shown by a confirmatory CT or MRI.

	Occlusive PVT (n=3)	**Partial PVT (n=26)**
Only Intrahepatic Portal Vein Branches		5
+ Mesenteric Vein		1
Main portal Vein		
Only		8
+ Intrahepatic portal branches	1	5
+ Superior Mesenteric vein		4
+ Intrahepatic portal branches + mesenteric + Splenic Veins	1	
+ Intrahepatic portal branches + mesenteric vein	1	3

6.2. Estudi 2. Risk of non-tumoural portal vein thrombosis in patients with HCV-induced cirrhosis after sustained virological response.

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ORIGINAL ARTICLE

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Risk of non-tumoural portal vein thrombosis in patients with HCV-induced cirrhosis after sustained virological response

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Abstract

Background & Aims: Sustained virological response (SVR) to direct-acting antivirals ameliorates portal hypertension, improves hepatic function and may reverse the procoagulant state observed in patients with cirrhosis. However, an unexpected incidence of portal vein thrombosis (PVT) immediately after antiviral therapy has recently been reported. Therefore, we analysed the long-term impact of SVR on the development of non-tumoural PVT.

Methods: Our study comprised of two well-characterized prospective cohorts (hepatitis C virus '(HCV)-Cured': n = 354/'HCV-Active': n = 179) of patients with HCV cirrhosis who underwent standardized ultrasound surveillance. In the main analysis, the event of interest was de novo non-tumoural PVT and events known to modify its natural history (orthotopic liver transplantation, transjugular intrahepatic portosystemic shunt, death, tumoural PVT and anticoagulation) were considered as competing risk. Adjusted models were built using propensity scores for baseline covariates. Moreover, predictive factors were investigated by conventional multivariate analysis.

Results: Ten (2.8%) patients in the 'HCV-Cured' cohort developed a non-tumoural PVT during a median follow-up of 37.1 months, while 8 (4.5%) patients in the 'HCV-Active' cohort were diagnosed with non-tumoural PVT during a median follow-up

M. M. and F. T. contributed equally to the work.

of 42.2 months. High Child-Pugh score was the only independent risk factor for non-tumoural PVT development and stage A patients were at low risk. Importantly, HCV cure did not decrease the risk of non-tumoural PVT in inverse probability of treatment-weighted (IPTW) analysis (subdistribution hazard ratio: 1.31 (95% confidence interval [95% CI]: 0.43-3.97); $P = .635$). In contrast, SVR was associated with a substantial reduction in mortality (IPTW-adjusted sHR: 0.453 [95% CI: 0.287-0.715]; $P < .001$).

Conclusions: The risk of non-tumoural PVT persists after HCV cure in patients with cirrhosis, despite improving survival. Even after aetiological cure, severity of liver disease remains the main determinant of non-tumoural PVT development.

KEYWORDS

direct-acting antivirals, hepatitis C, portal vein thrombosis

1 | INTRODUCTION

Even in previous difficult-to-cure patient populations such as patients with cirrhosis and portal hypertension,[1,2] direct-acting antiviral agent (DAA)-based regimens for chronic hepatitis C are highly effective. Accordingly, the focus of attention has shifted to liver disease regression and its implications on clinical outcomes as well as the long-term management of patients with cirrhosis.[3,4] Because of its beneficial effects on portal hypertension,[3,5-10] the cure of hepatitis C virus (HCV) infection decreases the risk of hepatic decompensation[9] and there is increasing evidence that the observed improvements in liver function[11-13] translate into substantially decreased mortality.[14]

A common complication of patients with cirrhosis is development of portal vein thrombosis (PVT), especially when liver function deteriorates and portal hypertension arises, and it may have a detrimental impact in prognosis.[15,16] Whether this risk persists after aetiological treatment remains a matter of debate. Indeed, despite reducing the hypercoagulable state, improving liver function and reducing decompensation, the risk of developing de novo PVT after achieving sustained virological response (SVR) as a result of DAA treatment has been suggested to be increased.[17]

Therefore, we aimed to analyse the impact of SVR on the development of non-tumoural PVT and mortality on two large, well-characterized cohorts of patients with HCV cirrhosis undergoing standardized imaging surveillance.

Key points

- In patients with cirrhosis, curing hepatitis C improves survival.
- However, the risk of developing blood clots in the veins between the gut and the liver persists.
- Impaired liver function remains the main determinant of this complication.

2 | PATIENTS AND METHODS

2.1 | Study setting and population

Our study comprised of two patient cohorts who were followed up prospectively at the Liver Unit of the Hospital Clínic in Barcelona, Spain:

1. Patients with HCV-induced cirrhosis achieving SVR (ie HCV treatment failures were not considered) as a result of DAA-based regimens (excluding boceprevir and telaprevir) between 2011 and 2016 ('HCV-Cured') were included and followed until the occurrence of competing events, an event of interest (ie depending on the analysis, non-tumoural PVT or death) or June 2019.
2. Viraemic patients with HCV-induced cirrhosis who were enrolled in a prospective study on PVT incidence between 2010 and 2013 ('HCV-Active'). Of note, patients with a history of hepatocellular carcinoma (HCC) were excluded from this study. These patients were followed until HCV treatment initiation, the occurrence of other competing events, an event of interest or June 2019. Of note, patients from this group achieving SVR were not re-included in the 'HCV-Cured' cohort.

Patients with a previous history of transjugular intrahepatic portosystemic shunt (TIPS), orthotopic liver transplantation (OLT) or PVT, as well as patients in whom anticoagulation have been initiated before the baseline of the study were excluded.

2.2 | Diagnosis of cirrhosis

Pretreatment cirrhosis was diagnosed based on liver histology or conclusive clinical findings: Evidence of clinically significant portal

hypertension (ascites, gastro-oesophageal varices or portosystemic collaterals), hepatic venous pressure gradient (HVPG) ≥6 mm Hg, liver stiffness ≥12.5 kPa as assessed by vibration-controlled transient elastography (Fibroscan; Echosens, Paris, France) or the presence of more than one ultrasound (US) finding indicative of cirrhosis.

2.3 | US surveillance protocol

All patients underwent abdominal US at the same institution every 6 months for both HCC and PVT screening. Diagnosis and extension of PVT was confirmed/assessed by computed tomography or magnetic resonance imaging, as recommended by current clinical practice guidelines.[18]

2.4 | HCV treatments

See the supplementary information.

2.5 | Statistical analyses

Continuous variables were reported as mean ± standard deviation or median (25th to 75th percentile). Categorical variables were presented as absolute frequencies (n) and proportions (%) of patients. Comparisons of categorical and continuous characteristics at treatment initiation/end of treatment were performed using chi-squared test and Mann-Whitney *U* test respectively.

Patients in the 'HCV-Cured' cohort entered the time-to-event analyses (time zero) at the end of antiviral therapy, while 'HCV-Active' patients entered the time-to-event analysis when they were included in the prospective study.

We conducted competing risk analyses with three different events of interest:

1. Firstly, for the analysis on non-tumoural PVT (event of interest), events known to modify its natural history (OLT, TIPS, death, tumoural PVT and anticoagulation) were considered as competing risks. Patients were censored at the time when the last imaging was done. Patients with no imaging available in a time frame of 6 (+3) months were considered as 'lost to follow-up'.
2. Secondly, in the analyses of death (event of interest), OLT was considered as the only competing risk.
3. Thirdly, for the analyses of HCC development (event of interest), OLT and death were considered as competing risks.

Standardized differences (STD), defined as differences between groups divided by pooled standard deviation, were calculated to assess the heterogeneity between groups for covariables. For the 'HCV-Cured' cohort, information obtained at treatment initiation was used. In order to investigate the impact of HCV treatment on clinical outcomes, the inverse probability of the treatment weighting (IPTW) approach[19] was applied to create a pseudo-population in which the two groups ('HCV-Cured' and 'HCV-Active') were balanced across relevant covariates. The stabilized weights were calculated using propensity score[20] obtained from a logistic regression model aimed to minimize the STD between groups.[21] Covariate balance was assessed by the STD with the goal to achieve *P* values >.1 that defined insignificant differences in potential confounders. Categorical and continuous data were compared using the chi-squared test and analysis of variance with rank-transformed data, for both raw and IPTW-adjusted analyses. Raw and IPTW-adjusted competing risk models were used to estimate subdistribution hazard ratios (sHR) with 95% confidence intervals (95% CI).

To investigate predictive factors for non-tumoural PVT development, additional Fine and Gray competing risk regression models (cmprsk: subdistribution analysis of competing risks, https://CRAN.R-project.org/package=cmprsk)[22] were calculated. Besides HCV treatment group, variables which we considered as relevant for the development of non-tumoural PVT based on the previous literature (history of bleeding, non-selective beta-blocker [NSBB] therapy and Child-Turcotte-Pugh [CTP] score) were included as covariates. These analyses were performed accounting for the characteristics of the 'HCV-Cured' cohort at treatment initiation when the HCV infection was still active and at the end of treatment, as hepatic function may improve after successful antiviral therapy.[11-13]

Statistical analyses were performed using IBM SPSS Statistics 26 version (IBM, Armonk, NY, USA), SAS version 9.4 or higher (SAS Institute Inc, Cary, NC, USA) and R version 3.4.1 (R Core Team, R foundation for Statistical Computing, Vienna Austria). The level of significance was set at two sided 5% (ie 0.05).

2.6 | Ethics

The data analysis was approved by the institutional review committee, which waived the requirement of a written informed consent.

3 | RESULTS

3.1 | Patient selection

1. 'HCV-Cured' cohort: A total of 365 consecutive patients with HCV-induced cirrhosis achieving SVR (ie excluding HCV treatment failures) to DAA-based regimens (excluding boceprevir and telaprevir) were considered (Figure 1). The characteristics of the antiviral regimens are denoted in Table S1. Two patients were excluded because of previous OLT, 6 as a result of anticoagulation for extrahepatic comorbidities before/at end of antiviral treatment and 3 because of the presence of PVT at the end of antiviral treatment. Finally, 354 patients were included.

2. 'HCV-Active' cohort: Of 210 consecutive patients with HCV-induced cirrhosis, 30 have to be excluded because of HCV-RNA negativity at baseline, and 1 patient was excluded as a result of missing clinical information. After applying these exclusion criteria, 179 patients were finally included.

3.2 | Comparison of baseline characteristics

Patients with large varices or a history of variceal bleeding were more common in the 'HCV-Active' cohort, which resulted in a higher proportion of patients being treated with NSBB or endoscopic variceal ligation (EVL) (Table 1). Moreover, patients in the 'HCV-Active' cohort were slightly younger. Importantly, hepatic dysfunction (as assessed by CTP and model for end-stage liver disease [MELD] scores and their components) was more pronounced in the 'HCV-Active' cohort, as compared to the 'HCV-Cured' cohort, regardless of whether data obtained at the time of treatment initiation or at the end of treatment were used. Accordingly, analyses of clinical outcomes were adjusted for these differences in patient characteristics.

3.3 | Follow-up events

Median follow up was 37.1 months in the 'HCV-Cured' cohort. Ten (2.8%) patients developed non-tumoural PVT, while OLT or death each occurred in five (1.4%) patients. Nine (2.5%) patients had tumoural PVT, while anticoagulation for another reason than PVT was initiated in 5 (1.4%) patients. In patients not developing any of these outcomes, 287 (80.8%) were followed until their last US required for HCC surveillance. Thirty-three (9.3%) patients were lost to follow up after a mean of 21.2 ± 14.5 months.

In the 'HCV-Active' cohort, median follow up was 42.2 months. Eight (4.5%) patients were diagnosed with non-tumoural PVT, while TIPS placement, OLT and death occurred in 3 (1.7%), 12 (6.7%) and 27 (15.1%) patients respectively. Six (3.4%) patients developed tumoural PVT and anticoagulation for another reason than PVT was started in 3 (1.7%) patients. HCV treatment was initiated in 103 (57.5%) patients. Eleven (6.1%) patients were lost to follow up after a mean of 42.5 ± 22.7 months.

The cumulative incidences of non-tumoural PVT were 1.4%, 1.7%, 2.7%, 3.9% and 3.9% at 1, 2, 3, 4 and 5 years after the end of antiviral therapy in the 'HCV-Cured' cohort, respectively, while in the 'HCV-Active' group, the cumulative incidences were 1.7%, 2.3%, 3.5%, 5.4% and 5.4%. In patients who developed non-tumoural PVT, the mean time periods between the end of HCV treatment/study inclusion and the diagnosis of non-tumoural PVT were 17.6 ± 14.7 and 22.8 ± 15.9 months in the 'HCV-Cured' and 'HCV-Active' cohorts respectively.

3.4 | Non-tumoural PVT-related characteristics

Detailed information on non-tumoural PVT-related characteristics is provided in Table S2. Of note, no complete occlusion of the main portal vein (PV) at the time of non-tumoural PVT diagnosis was observed in this study.

3.5 | Balance of covariates after IPTW adjusting

As shown in Table S3, the treatment groups of the resulting pseudo-population were very well matched for all characteristics with STD <|10%| in all cases. Even though the differences in the individual variables serum creatinine and international normalized ratio attained statistical significance, the STD were very small in both cases (|1.5%|). In addition, MELD (which comprises the latter two variables) did not differ (P =.556) and the STD was also very small (−4.9%), indicating that our IPTW-adjusted analyses were not confounded by the severity of underlying liver disease.

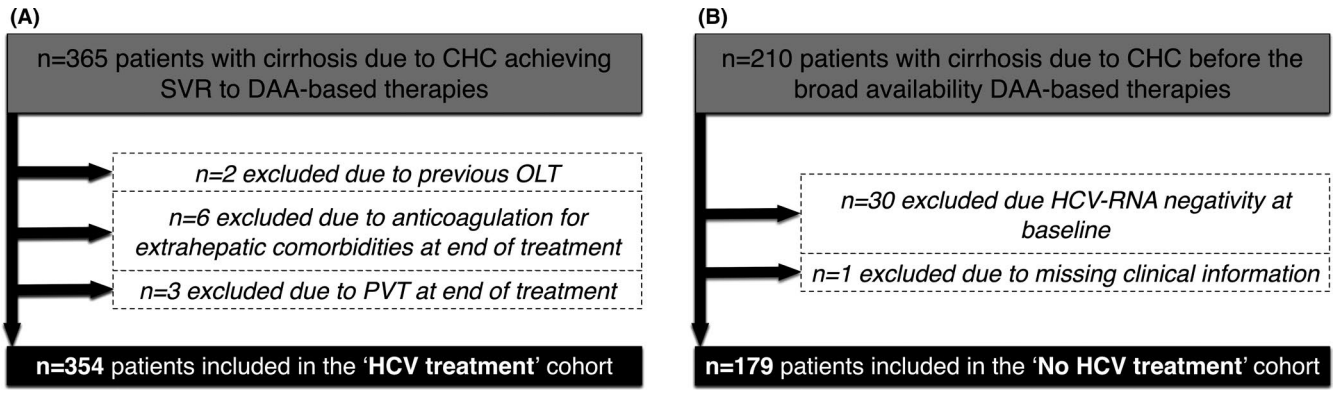

FIGURE 1 Study flow chart for the (A) 'HCV-treatment' and (B) 'No-HCV-treatment' cohorts. CHC, chronic hepatitis C; DAA, direct-acting antiviral; HCV, hepatitis C virus; OLT, orthotopic liver transplantation; PVT, portal vein thrombosis; SVR, sustained virological response

TABLE 1 Patient characteristics at study inclusion at HCV treatment initiation and end of treatment. Continuous variables were reported as mean ± standard deviation or median (25th to 75th percentile)

Patient characteristics	'HCV-Cured', n = 354	'HCV-Active', n = 179	*P* value
Sex, male	194 (54.8%)	84 (46.9%)	.086
Varices[a]			
No	104 (39%)	66 (36.9%)	.038
Small	90 (33.7%)	45 (25.1%)	
Large	73 (27.3%)	68 (38%)	
History of bleeding	15 (4.2%)	20 (11.2%)	.002
NSBB	66 (18.6%)	69 (38.5%)	<.001
EVL	20 (5.6%)	19 (10.6%)	.038
Treatment initiation			
Age, years	64.3 (55-70.5)	61.5 (52-70.2)	.017
Ascites	51 (14.4%)	50 (27.9%)	<.001
HE	16 (4.5%)	18 (10.1%)	.014
CTP score, points	5 (5-6)	5 (5-7)	<.001
A	313 (88.4%)	128 (71.5%)	<.001
B	35 (9.9%)	43 (24%)	
C	6 (1.7%)	8 (4.5%)	
MELD score, points	8 (7.84-10)	9 (8-11)	.007
Albumin, $g \times L^{-1}$	41 (38-44)	37 (33-40)	<.001
Bilirubin, $mg \times dL^{-1}$	1 (0.7-1.4)	1.1 (0.8-1.7)	.005
Creatinine, $mg \times dL^{-1}$	0.72 (0.63-0.86)	0.79 (0.7-0.91)	<.001
INR	1.15 (1.08-1.23)	1.17 (1.09-1.28)	.082
Platelet count, $G \times L^{-1}$	112 (80-144)	90 (67-126)	<.001
End of treatment			
Age, years	64.6 (55.3-70.7)	61.5 (52-70.2)	.008
Ascites	39 (11%)	50 (27.9%)	<.001
HE	15 (4.2%)	18 (10.1%)	.008
CTP score, points	5 (5-6)	5 (5-7)	<.001
A	306 (86.4%)	128 (71.5%)	<.001
B	46 (13%)	43 (24%)	
C	2 (0.6%)	8 (4.5%)	
MELD score, points	8 (8-11)	9 (8-11)	.143
Albumin, $g \times L^{-1}$	42 (39-45)	37 (33-40)	<.001
Bilirubin, $mg \times dL^{-1}$	1 (0.7-1.6)	1.1 (0.8-1.7)	.02
Creatinine, $mg \times dL^{-1}$	0.76 (0.65-0.88)	0.79 (0.7-0.91)	.009
INR	1.14 (1.08-1.22)	1.17 (1.09-1.28)	.037
Platelet count, $G \times L^{-1}$	124 (93-173)	90 (67-126)	<.001

Note: Categorical variables were presented as absolute frequencies (n) and proportions (%) of patients. Comparisons of categorical and continuous characteristics were performed using chi-squared test and Mann-Whitney *U* test respectively

Abbreviations: CTP, Child-Turcotte-Pugh; EVL, endoscopic variceal ligation; HCV, hepatitis C virus; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, model for end-stage liver disease; NSBB, non-selective beta-blocker.

[a]Information available for 447 patients.

3.6 | Determinants of non-tumoural PVT development

Hepatitis C virus cure did not modify the risk of non-tumoural PVT, neither in the raw ('HCV-Cured' vs 'HCV-Active': sHR: 0.744; 95% CI: 0.292-1.898; *P* =.536) nor in the IPTW-adjusted analysis (sHR: 1.31 [95% CI: 0.43-3.97]; *P* =.635; Figure 2, Tables 2 and 3, Figures S1-S3).

In univariate analyses, development of non-tumoural PVT was associated with portal hypertension severity, as reflected by history of bleeding as well as NSBB and EVL treatment (Table 3). Moreover,

indicators of hepatic dysfunction (ie CTP and MELD scores as well as most of their components) were associated with non-tumoural PVT development. Of note, the association between hepatic dysfunction and non-tumoural PVT development was confirmed using both baseline and end-of-treatment information of patients who underwent antiviral therapy (ie 'HCV-Cured' cohort). We also performed a multivariate analysis including factors that were potentially relevant for non-tumoural PVT development, as well as HCV-treatment status (Table 3). First, we calculated a model comprising history of bleeding, NSBB use (which, on the one hand, is an indicator of portal hypertension severity and, on the other hand, may directly impact non-tumoural PVT development[23]) and CTP score at treatment initiation. Next, we calculated a similar model including CTP score at the end of treatment. With adjusted sHR ('HCV-Cured' vs 'HCV-Active') of 1.38 (95% CI: 0.52-3.63); $P = .51$ and 1.38 (95% CI: 0.51-3.71); $P = .52$, HCV cure did not modify the risk of non-tumoural PVT in these two models (Table 3). High pre-treatment CTP score (per point; adjusted sHR: 1.32 [95% CI: 1.04-1.66]; $P = .021$) was the only independent risk factors for non-tumoural PVT development. Of note, HCV cure did not seem to modulate the impact of CTP score on non-tumoural PVT risk as the addition of the interaction term did not yield a statistically significant result ($P = .24$). Next, we analysed the incidence of non-tumoural PVT according to CTP stage in the overall study population (using end-of-treatment CTP score in the 'HCV-Cured' cohort). Since only 10 CTP C patients were included in our study, we decided to merge CTP B and C in order to provide meaningful results. The cumulative incidences of non-tumoural PVT in CTP A patients were 0%, 0.2%, 1.6% and 3.5% at 1, 2, 3 and 4 years, respectively, while the rates in those with CTP B/C were 7.2%, 8.3%, 9.9% and 9.9% (sHR: 0.213 [95% CI: 0.085-0.535]; $P < .001$; Figure S1). Moreover, we also analysed the 'HCV-Cured' cohort separately, in which we observed a similar picture (sHR: 0.218 [95% CI: 0.061-0.773]; $P = .011$; Figure S2).

Finally, we evaluated whether a CTP score decrease ≥1 point at end of treatment translated into a decreased risk of non-tumoural PVT in the 'HCV-Cured' cohort (Figure S3). Detailed information is provided in the supplementary material.

3.7 | Impact of antiviral therapy on mortality

Sustained virological response to HCV treatment was linked to a decreased risk of mortality in raw ('HCV-Cured' vs 'HCV-Active'; sHR: 0.323 [95% CI: 0.174-0.598]; $P = .003$) analysis and the treatment effect was confirmed in IPTW-adjusted analysis, which indicated a sHR of 0.453 (95% CI: 0.287-0.715); $P < .001$ (Table 2 and Figure 3) (Table S4).

While 'HCV-Cured' was associated with decreased mortality in multivariate analyses, high pre- or post-treatment CTP scores were linked to an increased risk of death (Table S4).

3.8 | HCC development according to HCV treatment group

Detailed information is provided in the supplementary material (Figure S4).

4 | DISCUSSION

The present study evaluates for the first time the incidence of non-tumoural PVT in a large prospective cohort of patients with HCV-induced cirrhosis after achieving SVR as a result of DAA. The results indicate that the risk PVT does not decrease after DAA-induced SVR as would be expected, and this happens despite improvements in

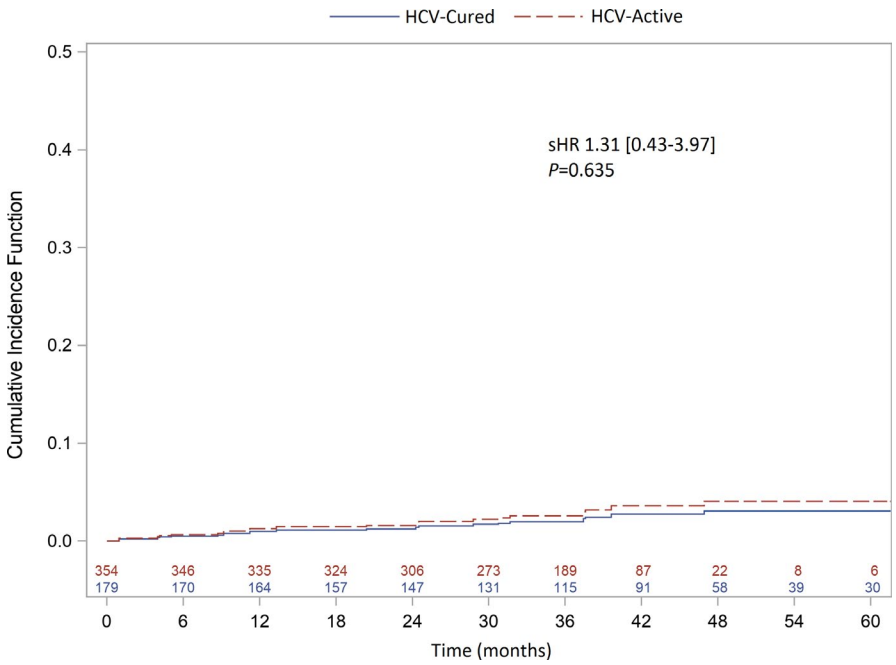

FIGURE 2 IPTW-adjusted impact of HCV cure on the risk of non-tumoural PVT. Events known to modify the natural history (OLT, TIPS, death, tumoural PVT and anticoagulation) of the event of interest were considered as competing risks. HCV, hepatitis C virus; IPTW, inversed probability of treatment weighting; PVT, portal vein thrombosis; OLT, orthotopic liver transplantation; TIPS, transjugular intrahepatic portosystemic shunt; sHR, subdistribution hazard ratio

TABLE 2 (A) Raw and (B) IPTW-adjusted competing risk analyses on the impact of HCV cure (ie 'HCV-Active' [reference category] vs 'HCV-Cured') on clinical outcomes

	A		B	
Patient characteristics	**sHR (95% CI)**	***P* value**	**Adjusted sHR (95% CI)**	***P* value**
Non-tumoural PVT	0.744 (0.292-1.898)	.536	1.31 (0.43-3.97)	.635
Death	0.323 (0.174-0.598)	.003	0.453 (0.287-0.715)	<.001

Abbreviations: 95 CI, 95% confidence interval; HCV, hepatitis C virus; IPTW, inversed probability of treatment weighting; PVT, portal vein thrombosis; sHR, subdistribution hazard ratio.

other liver-related complications and survival. We evaluated the impact of antiviral therapy on the risk of non-tumoural PVT development adopting the concept of competing risks[24] using three different approaches. First, to evaluate the impact of HCV clearance on non-tumoural PVT development estimating causal treatment effects, we performed IPTW-adjusted analyses using a propensity score that considered pre-treatment characteristics. The two groups of the resulting pseudo population were well matched for relevant covariates. Second, we performed a conventional multivariate analysis to investigate other potential risk factors for non-tumoural PVT. Third, as HCV cure improves hepatic function and the patient characteristics/risk factors, we performed another conventional multivariate analysis considering characteristics at the end of treatment. This analysis was adjusted by the degree of portal hypertension (as estimated by history of variceal bleeding and NSBB treatment as an indicator of high-risk varices) and hepatic dysfunction (as assessed by CTP score).

All three statistical approaches yielded nearly identical results, indicating the robustness of our findings. After HCV cure, the risk for developing non-tumoural PVT persists and severity of hepatic dysfunction is a crucial risk factor for non-tumoural PVT.[16] Indeed, each 1-point increase in CTP score translated into a 32% increased risk of PVT. Accordingly, the risk of non-tumoural PVT was very low in CTP A patients. However, since HCC surveillance by US is recommended for all patients with pretreatment advanced liver fibrosis (ie ACLD), who achieved SVR according to European guidelines,[25] we still think that a concomitant assessment of the splenoportal axis should be performed, as it does not add additional cost/burden to the patient. However, if future studies identify subpopulations of patients in whom HCC surveillance can be safely stopped, no specific surveillance for non-tumoural PVT is warranted in CTP A patients.

When mortality was evaluated through the same 3 analytical strategies, the beneficial effect of HCV cure on survival was confirmed.[4]

Intriguingly, despite improving liver function, survival and also being linked with a decreased risk of de novo HCC, SVR did not impact on the risk of de novo PVT. Venous thrombosis is promoted by a triad of pathophysiological factors: hypercoagulability, changes in the blood flow and endothelial dysfunction/injury (Virchow's triad). The impact of HCV cure on the components of the Virchow's triad is only partially understood.

HCV clearance has been shown to improve routine coagulation parameters and reduce the procoagulant imbalance, possibly leading to a more stable haemostatic equilibrium.[26,27] However, according to preliminary data from our group,[28] alterations of the haemostatic profile that are related to hypercoagulability are not risk factors for de novo non-tumoural PVT. Therefore, the individual contribution of 'cirrhotic coagulopathy' to PV clotting remains an open question.

Several studies have identified portal blood flow velocity as the main risk factor for PVT development. The majority of patients achieving SVR show a decrease in HVPG,[3,5-10] which may be explained by a decline in intrahepatic hepatic vascular resistance and an amelioration of hyperdynamic circulation/splanchnic vasodilatation and both mechanisms would result in opposing changes in portal venous blood flow. The only data on the impact of SVR on PV blood flow velocity available so far is a recent study based on paired phase-contrast magnetic resonance imaging assessments, which reported that it is unaffected by SVR.[29] Of note, our study cannot provide robust information on the impact of (changes in) portal blood flow velocity on non-tumoural PVT risk, as it has not been systematically assessed in the 'HCV-Cured' cohort. Studies investigating the dynamics of PV blood flow velocity after aetiological therapies in relation to non-tumoural PVT risk are warranted.

Finally, although HCV eradication has been shown to improve systemic endothelial dysfunction, this effect seemed to be less marked or even absent in subjects with cirrhosis.[30-32] Importantly, it is still unknown whether splanchnic endothelial dysfunction may be modified after SVR.

Taken together, current evidence suggests that in patients with HCV cirrhosis, SVR may not have a clear beneficial effect on the different components of the Virchow's triad involved in thrombotic risk.

As previously discussed, SVR improves liver function[4] and liver function is the main predictive factor for PVT,[15,16] as confirmed by our data. Therefore, contrary to what was observed, a reduction in PVT incidence after HCV cure would be expected. We can only speculate whether a direct effect caused by HCV eradication or by DAA-based regimens may counterbalance the positive effect of SVR-induced improvement of liver function on PVT risk. In this regard, development or worsening of pulmonary arterial hypertension (PAH) has been observed in patients treated for HCV infection either with interferon-[33] or DAA-based[34] regimens. Although the real

TABLE 3 sHR for non-tumoural portal PVT, considering tumoural PVT, anticoagulation, transjugular intrahepatic portosystemic shunt, OLT and death as competing risks. (A) Univariate analysis. Besides HCV treatment group, variables that we considered as relevant for the development of non-tumoural PVT based on the previous literature (history of bleeding, NSBB therapy and CTP score) were included as covariates in the multivariate models. These analyses were performed accounting for the characteristics of the 'HCV-Cured' cohort (B) at treatment initiation when the HCV infection was still active and (C) at the end of treatment. Fine and Gray competing risk regression models (cmprsk: subdistribution analysis of competing risks, https://CRAN.R-project.org/package=cmprsk)[22] were calculated

	A (univariate)		B (multivariate; treatment initiation)		C (multivariate; end of treatment)	
Patient characteristics	sHR (95% CI)	*P* value	Adjusted sHR (95% CI)	*P* value	Adjusted sHR (95% CI)	*P* value
Sex, male vs female	1.35 (0.596-3.08)	.2	–	–	–	–
History of bleeding, yes vs no	5.18 (1.88-14.3)	.005	2.58 (0.89-7.48)	.081	2.71 (0.918-7.99)	.071
NSBB, yes vs no	4.06 (1.64-10)	.002	2.32 (0.79-6.84)	.13	2.39 (0.77-7.42)	.13
EVL, yes vs no	8.05 (3.19-20.3)	.002	–	–	–	–
Treatment initiation						
Age, per 10 years	1.46 (0.93-2.29)	.1	–	–	–	–
Ascites, yes vs no	10.2 (3.9-26.6)	<.001	–	–	–	–
HE, yes vs no	1.55 (0.35-7.6.92)	.56	–	–	–	–
CTP score, per point	1.45 (1.25-1.7)	<.001	1.32 (1.04-1.66)	.021	–	–
MELD score, per point	1.09 (1.03-1.15)	.004	–	–	–	–
Albumin, per g × L^{-1}	0.885 (0.843-0.94)	<.001	–	–	–	–
Bilirubin, per mg × dL^{-1}	1.33 (1.12-1.59)	.001	–	–	–	–
Creatinine, per mg × dL^{-1}	0.284 (0.011-7.09)	.44	–	–	–	–
INR, per 0.1	1.21 (1.09-1.34)	<.001	–	–	–	–
Platelet count, per 10 G × L^{-1}	0.904 (0.777-1.05)	.19	–	–	–	–
End of treatment						
Age, per 10 years	1.46 (0.92-2.3)	.11	–	–	–	–
Ascites, yes	11.9 (4.6-31)	<.001	–	–	–	–
HE, yes	0.795 (0.106-5.99)	.82	–	–	–	–
CTP score, per point	2.81 (1.66-4.76)	<.001	–	–	1.91 (0.93-3.94)	.079
MELD score, per point	1.12 (1.06-1.18)	<.001	–	–	–	–
Albumin, per g × L^{-1}	0.872 (0.826-0.921)	<.001	–	–	–	–
Bilirubin, per mg × dL^{-1}	1.03 (1-1.06)	.086	–	–	–	–
Creatinine, per mg × dL^{-1}	0.289 (0.009-8.86)	.48	–	–	–	–
INR, per 0.1	7.33 (2.81-19.1)	<.001	–	–	–	–
Platelet count, per 10 G × L^{-1}	0.99 (0.979-1)	.08	–	–	–	–
'HCV-Cured' vs 'HCV-Active'	0.744 (0.292-1.898)	.536	1.38 (0.52-3.63)	.51	1.38 (0.51-3.71)	.52

Abbreviations: 95 CI, 95% confidence interval; CTP, Child-Turcotte-Pugh; EVL, endoscopic variceal ligation; HCV, hepatitis C virus; INR, international normalized ratio; MELD, model for end-stage liver disease; NSBB, non-selective beta-blocker; OLT, orthotopic liver transplantation; PV, portal vein; PVT, portal vein thrombosis; sHR, subdistribution hazard ratio.

incidence of this complication and the potential mechanism involved are not clear, it has been suggested that suppression of HCV-RNA could lead to a rapid decrease in levels of vasodilatory mediators, thereby triggering PAH.[33,34] Whether this phenomenon also effects the portal venous circulation and may promote non-tumoural PVT development requires further study.

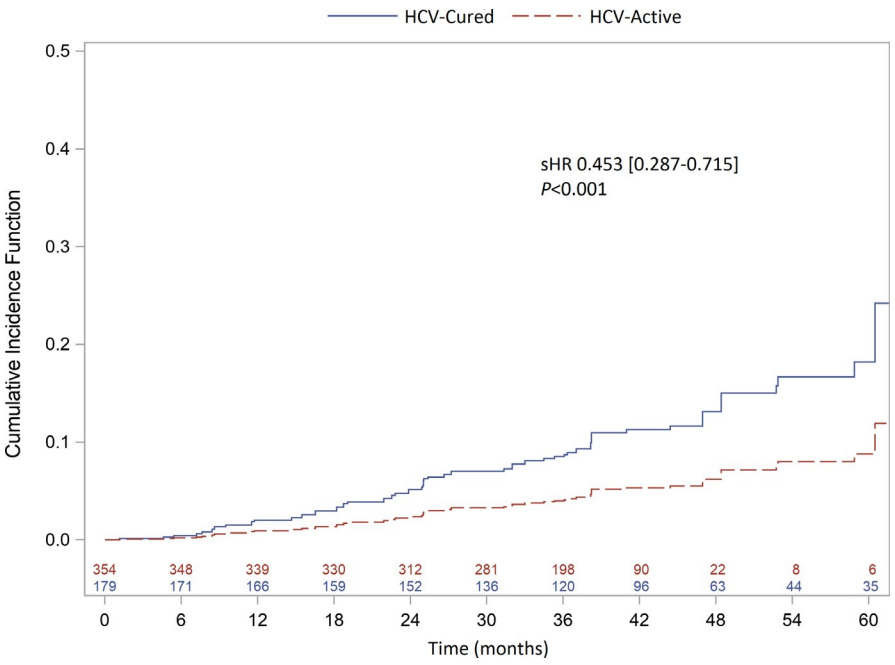

FIGURE 3 IPTW-adjusted impact of HCV cure on the risk of death considering OLT as competing risks. HCV, hepatitis C virus; IPTW, inversed probability of treatment weighting; OLT, orthotopic liver transplantation; PVT, portal vein thrombosis; sHR, subdistribution hazard ratio

We have to acknowledge several limitations. Firstly, our study combines two cohorts of patients who were recruited in different, but partially overlapping time periods. This cannot be avoided as it would have been unethical to delay or withhold effective treatment in the 'HCV-Active' cohort after it became broadly available. Moreover, there were also slight differences in the duration of follow up, which is explained by the more recent inclusion, and thus, the shorter potential follow up of 'HCV-Cured' patients. Secondly, the low number of de novo PVT occurring during follow up may limit the strength of our analysis. However, a modelling study indicated that approximately 5 events per predictor variable seem to be sufficient for Cox regression (ie a form of analysis that is close to the statistical approach applied for the analysis of risk factors non-tumoural PVT development in our study),[35] thereby relaxing the still commonly applied rule of thumb of 10 events per predictor variable. We would like to emphasize that for testing the main objective of our study (ie impact of HCV cure on non-tumoural PVT), we performed IPTW that creates a pseudo-population in which the two groups ('HCV-Cured' and 'HCV-Active') were balanced across relevant covariates, thereby avoiding the need for multivariate adjustment and eliminating the above-mentioned concern. The cumulative incidence of PVT in our cohort is slightly lower, but still quite close to a previous report from Nery et al (approximately 6% at 3 years with the 6-monthly US screening strategy).[36] This is not surprising, as our study comprised 83% CTP A patients who are at comparatively low risk of PVT, while the proportion in the study by Nery and colleagues was only 69%. Moreover, we would like to point out that both cohorts included in the present study were followed up prospectively. While 'HCV-Active' patients were assessed within a study that specifically aimed to diagnose PVT, 'HCV-Cured' patients were investigated within clinical routine. However, all investigations in the latter group were performed by abdominal US experts from the same department, who were usually the same as those involved in 'HCV-active' cohort, with a long-lasting expertise in vascular liver diseases including PVT.[37-41] Therefore, we are confident that PVT was accurately detected and not underdiagnosed. Of note, this is the only large longitudinal study evaluating the impact of SVR to DAA-based regimens on the incidence of PVT to date. Since the incidence of non-tumoural PVT is low regardless of treatment, a considerably higher sample size would have been required to provide profound increases in statistical power; however, it seems unlikely that a cohort of such size with a similarly thorough imaging follow up will become available in the near future. Finally, the two cohorts differed in patient characteristics, implicating that raw/unadjusted estimates have to be interpreted with caution. However, we performed conventional multivariate and also IPTW-adjusted analyses using a propensity score to obtain unbiased estimates of the impact of HCV cure. After these adjustments, the risk of non-tumoural PVT was even numerically increased by 31% in the 'HCV-Cured' cohort, providing convincing evidence for the absence of a risk reduction, as it has been observed for mortality.

In conclusion, we reported for the first time that, although – particularly in CTP A patients – the incidence is low, the risk of non-tumoural PVT persists at least 3 years after achieving SVR in HCV cirrhosis. Importantly, this occurs despite an improvement in survival and liver function, suggesting that the latter benefit may be counteracted by a direct effect of HCV cure or the drugs used to achieve it on the portal venous/hepatic vasculature.

CONFLICTS OF INTEREST

M. Man. served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Gilead, Collective Acumen and W. L. Gore & Associates, and received travel support from AbbVie, Bristol-Myers Squibb and Gilead. F. Tu. served as speaker and/or consultant and/or advisory board member for W. L. Gore & Associates. S. L. served as speaker and/or consultant and/

or advisory board member for AbbVie, Gilead and MSD. A. B. has nothing to disclose. A. G.-C. served as a speaker and/or consultant and/or advisory board member for BTG and Terumo. A. D. served as a speaker and/or consultant and/or advisory board member for and received travel support from Bayer. E. B. received travel support from BTG. J. F-A. has nothing to disclose. M. Mag. has nothing to disclose. V. P.-C. has nothing to disclose. P.O. has nothing to disclose. D. B. received travel support from Gilead. G.C. has nothing to disclose. F. To. has nothing to disclose. Z. M. served as a speaker and/or consultant and/or advisory board member for AbbVie, Gilead and MSD. X. F. served as a speaker and/or consultant for AbbVie and Gilead. V. H-G. served as a speaker and/or consultant and/or advisory board member for W. L. Gore & Associates. J.C. G.-P. served as a speaker and/or consultant and/or advisory board member for Cook and W. L. Gore & Associates, and received grants/research support from Conatus, Exalenz, Novartis and Theravance.

AUTHORS' CONTRIBUTIONS

All authors contributed to conceptualization (M. Man., F. Tu. and J.C. G-P.), data curation (all authors except for D. B., G. C. and F. To.), formal analysis and visualization (M. Man., D. B., G. C. and F. To.), writing of the original draft (M. Man.), reviewing and editing (all authors) or supervision (J.C. G-P.). All authors approved the final version of the article.

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SUPPORTING INFORMATION

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

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Supplementary information

HCV-treatment

In the '**HCV-Cured**' cohort, all patients were treated with DAA-based therapies. The choice of the regimen was at the physicians' discretion and depended on the availability of early access programs, reimbursement policies, as well as international (1-3) clinical practice guidelines at the time of treatment initiation. Treatment durations ranged from 12-48 weeks. Detailed information regarding the treatment regimens is provided in **Supplementary table 1**.

*Impact of HCV-treatment-induced changes in CTP score on non-tumoral-PVT risk in the '**HCV-Cured**' cohort **(Supplementary figure 3)***

Among 90 '**HCV-Cured**' patients with a pre-treatment CTP score ≥6 points, 39 (43.3%) showed an improvement of CTP score at end of treatment. Among those with a CTP score decrease ≥1 point, the cumulative incidences of non-tumoral-PVT were 0%, 0%, 2.6%, and 6.5% at 1, 2, 3, and 4 years after the end of antiviral therapy, respectively, while the rates in those without a CTP decrease were 8%, 8%, 10.8%, and 10.8% (**Supplementary figure 3**). Although the difference did not attain statistical significance (sHR: 0.454 (95%CI: 0.01-2.18); *P*=0.331), the incidence of non-tumoral-PVT was numerically lower in patients who had an improvement in hepatic function during treatment, warranting the consideration of pre-/post-treatment hepatic function in our analyses on the risk factors for non-tumoral-PVT development.

*HCC development according to HCV-treatment group **(Supplementary figure 4)***

In the 'HCV-Cured' cohort, 31 patients had a history of HCC before HCV-treatment initiation and 2 patients developed HCC before the end of treatment. In contrast, no patients with a history of HCC were included in the '**HCV-Active**' cohort, since patients with a history of HCC have been excluded from the prospective study. Importantly, only one of these patients (i.e., 3%) developed non-tumoral PVT, which is in line with the rate of non-tumoral-PVT-development in the '**HCV-Cured**' cohort (6.2%). However, although history of HCC did not impact the incidence of our main event of interest (non-tumoral-PVT development), as expected, it was associated with a high rate of HCC development during follow-up (34.4%

vs. 10.6% in those without a history of HCC). Accordingly, we have excluded patients with a history of HCC (and thus, at risk of HCC recurrence) from our analysis and focused on *de novo* HCC development. In the remaining 500 patients, the cumulative incidences of *de novo HCC* in '**HCV-Cured**' were 2.8%, 5.1%, 6.9%, and 6.9% at 1, 2, 3, and 4 years, respectively, while the rates in '**HCV-Active**' were 1.7%, 5.2%, 13.4%, and 15.8% (sHR: 0.442 (95%CI: 0.253-0.769); *P*=0.005; **Supplementary figure 4**).

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Supplementary tables

Supplementary table 1

DAA	No. of patients	PEGIFN	RBV	Treatment duration (weeks)
SOF/LDV	125 (35.5%)	0 (0%)	101 (80.8%)	12: 88 (70.4%)
				24: 37 (29.6%)
2D or 3D	112 (31.5%)	0 (0%)	68 (60.7%)	12: 103 (92%)
				24: 9 (8%)
SOF/SMV	49 (13.8%)	0 (0%)	43 (87.5%)	12: 48 (98%)
				16: 1 (2%)
SOF/DCV	28 (7.9%)	0 (0%)	23 (82.1%)	24: 28 (100%)
SOF	13 (3.7%)	1 (7.7%)	13 (100%)	12: 2 (15.4%)
				16: 1 (7.7%)
				24: 9 (69.2%)
				48: 1 (7.7%)
EBV/GZV	9 (2.5%)	0 (0%)	8 (88.9%)	12: 5 (55.6%)
				16: 1 (11.1%)
				24: 3 (33.3%)
SMV/DCV	7 (2%)	0 (0%)	3 (42.9%)	24: 7 (100%)
ASV/DCV	3 (0.8%)	0 (0%)	0 (0%)	24: 3 (100%)
SOF/VEL	3 (0.8%)	0 (0%)	1 (33.3%)	12: 2 (66.7%)
				16: 1 (33.3%)
SMV	3 (0.8%)	3 (100%)	3 (100%)	24: 3 (100%)
G/P	1 (0.3%)	0 (0%)	0 (0%)	12: 1 (100%)
FDV	1 (0.3%)	0 (0%)	1 (100%)	24: 1 (100%)

Supplementary table 1. Summary of HCV-treatment regimens.

Abbreviations: 2D ombitasvir/paritaprevir/ritonavir; 3D ombitasvir/paritaprevir/ritonavir plus dasabuvir; ASV asunaprevir; DAA direct-acting antiviral*;* DCV daclatasvir*;* EBV elbasvir*;* FDV faldaprevir*;* G/P glecaprevir/pibrentasvir*;* GZV grazoprevir; *HCV hepatitis C virus;* LDV ledipasvir*;* PEGIFN pegylated interferon; *PVT portal vein thrombosis;* RBV ribavirin*;* SMV simeprevir*;* SOF sofosburvir.

Supplementary table 2

Non-tumoral-PVT-related characteristics	'HCV-Cured', n=10*/354	'HCV-Active', n=8*/179
Symptomatic	3 (30%)	3 (37.5%)
Completely occlusive	1 (10%)	0 (0%)
Right intrahepatic PV	1 (10%)	0 (0%)
Partially occlusive	9 (90%)	8 (100%)
Right or left intrahepatic PV	2 (20%)	1 (12.5%)
Main PV		
Only vessel affected	3 (30%)	3 (37.5%)
+ right or left intrahepatic PV	0 (0%)	3 (37.5%)
+ both intrahepatic PV	2 (20%)	0 (0%)
+ both intrahepatic PV + superior mesenteric vein	0 (0%)	1 (12.5%)
Superior mesenteric vein	1 (10%)	0 (0%)
Splenic vein	1 (10%)	0 (0%)

*If not stated otherwise, proportions are reported as percentage of patients with PVT in the respective group.

Supplementary table 2. Information on portal blood flow velocity (derived from the last ultrasound prior to non-tumoral-PVT diagnosis), symptoms, as well as the location and extent of PVT in patients developing non-tumoral-PVT.

Abbreviations: PV portal vein; *PVT portal vein thrombosis.*

Supplementary table 3

Patient characteristics	A Raw 'HCV-Cured'	A Raw 'HCV-Active'	A Raw STD	A Raw *P* value	B IPTW-adjusted 'HCV-Cured'	B IPTW-adjusted 'HCV-Active'	B IPTW-adjusted STD	B IPTW-adjusted *P* value
Age	64.3 [55-70.5]	61.5 [52-70.2]	24.8%	0.008	62.9 [54.1-70]	62.6 [53.3-72.3]	-5.7%	0.795
Sex, female	160 (45.2%)	95 (53.1%)	-16.4%	0.080	168 (47.3%)	84 (49.6%)	-2.8%	0.623
History of bleeding	15 (4.2%)	20 (11.2%)	-26.3%	0.002	22 (6.2%)	13 (7.6%)	-1.4%	0.54
NSBB	66 (18.6%)	69 (38.5%)	-44.6%	<0.001	91 (25.5%)	47 (27.7%)	-1.1%	0.594
EVL	20 (5.6%)	19 (10.6%)	-19.4%	0.038	28 (7.9%)	13 (7.5%)	5.4%	0.859
Ascites	51 (14.4%)	50 (27.9%)	-32.9%	<0.001	70 (19.5%)	36 (21.3%)	-2.2%	0.637
HE	16 (4.5%)	18 (10.1%)	-20.7%	0.013	26 (7.4%)	14 (8.1%)	0.7%	0.794
CTP score, points	5 [5-6]	5 [5-7]	-42.5%	<0.001	5 [5-6]	5 [5-6]	-0.5%	0.983
MELD score, points	8 [7.84-10]	9 [8-11]	-24.3%	0.009	9 [8-10]	8.00 [7-11]	-4.9%	0.556
Albumin, g x L^{-1}	41 [38-44]	37 [33-40]	76.7%	<0.001	40 [36-43]	39 [36-42]	0.5%	0.112
Bilirubin, mg x dL^{-1}	1 [0.7-1.4]	1.1 [0.8-1.7]	-28.9%	0.004	1 [0.7-1.5]	1.1 [0.7-1.50]	-1.3%	0.957
Creatinine, mg x dL^{-1}	0.72 [0.63-0.86]	0.79 [0.7-0.91]	5.8%	<0.001	0.72 [0.62-0.85]	0.81 [0.7-0.92]	-1.5%	<0.001
INR	1.15 [1.08-1.23]	1.17 [1.09-1.28]	-25.8%	0.084	1.16 [1.09-1.26]	1.13 [1.07-1.24]	1.5%	0.043
Platelet count, G x L^{-1}	112 [80-144]	90 [67-126]	29.7%	0.001	101 [68-138]	95 [68-131]	7.3%	0.285

Supplementary table 3. Patient characteristics (in the '**HCV-Cured**' group, pre-treatment characteristics were used) of the study population of the raw and IPTW-adjusted analysis. Sample sizes for the study cohorts '**HCV-Cured**' and '**HCV-Active**' are 354 and 179, respectively. Continuous variables were reported as mean±standard deviation or median [25th to 75th percentile]. Categorical variables were presented as absolute frequencies (n) and proportions (%) of patients. STD, defined as differences between groups divided by pooled standard deviation were calculated to assess the heterogeneity between groups for covariables. Categorical and continuous data were compared using the Chi-squared test and analysis of variance (ANOVA) with rank-transformed data, for both raw and IPTW-adjusted analyses.

Abbreviations: CTP Child-Turcotte-Pugh; EVL endoscopic variceal ligation; HCV hepatitis C virus; HE hepatic encephalopathy; INR international normalized ratio; IPTW inverse probability of treatment weighting; MELD model for end-stage liver disease; NSBB non-selective beta-blocker; STD standardized differences

Supplementary table 4

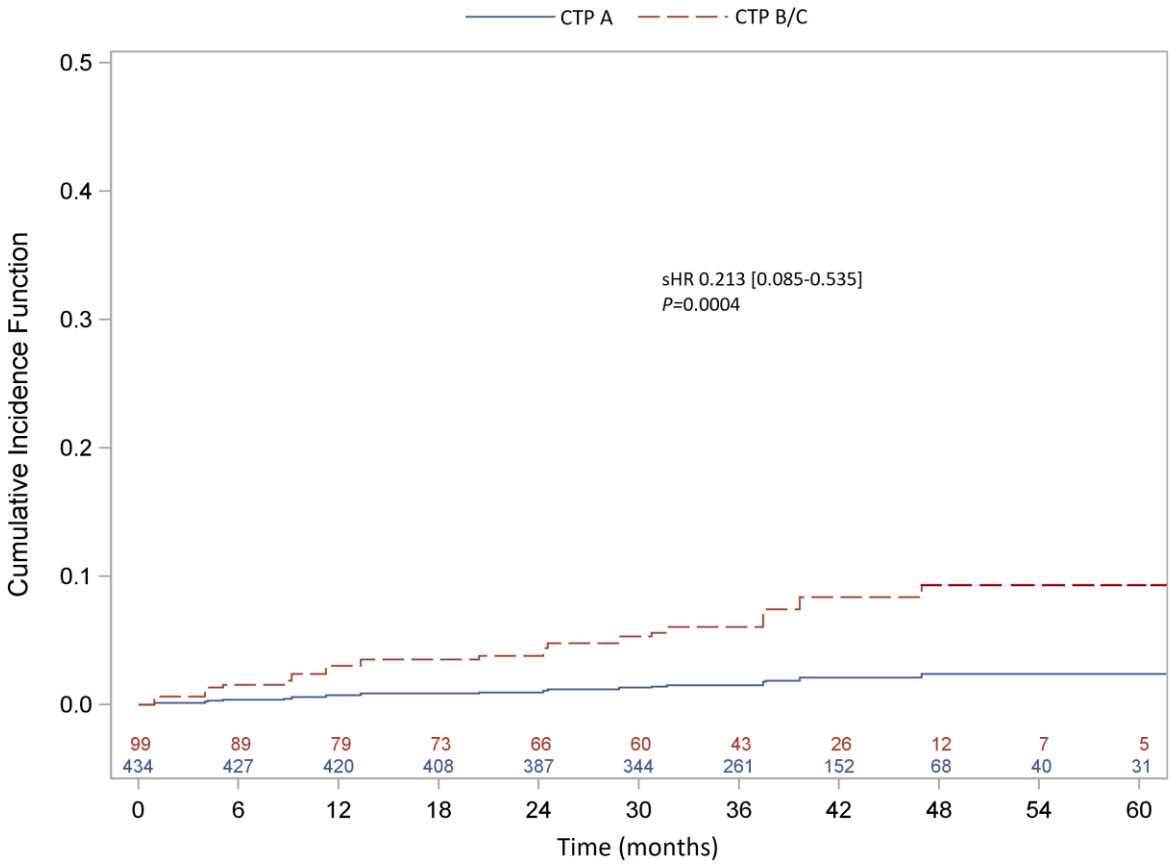
Patient characteristics	A		B	
	Adjusted sHR (95%CI)	***P* value**	**Adjusted sHR (95%CI)**	***P* value**
History of bleeding, yes vs. no	1.58 (0.7-3.55)	0.27	1.62 (0.73-3.63)	0.237
NSBB, yes vs. no	1.22 (0.65-2.28)	0.544	1.17 (0.62-2.2)	0.634
Treatment initiation				
CTP score, per point	1.27 (1.08-1.49)	0.004	-	-
End of treatment				
CTP score, per point	-	-	1.29 (1.1-1.52)	0.002
'**HCV-Cured**' vs. '**HCV-Active**'	0.394 (0.204-0.758)	0.005	0.4 (0.207-0.774)	0.007

Supplementary table 4. Adjusted sHR for death considering OLT as competing risk. Multivariate analyses considering values at **A** treatment initiation and **B** end of treatment in patients undergoing antiviral therapy. Fine and Gray competing risk regression models (cmprsk: subdistribution analysis of competing risks, https://CRAN.R-project.org/package=cmprsk) (4) were calculated. Besides HCV-treatment group, variables which we considered as relevant for the mortality based on the previous literature (history of bleeding and NSBB therapy as surrogates of portal hypertension and CTP score) were included as covariates in the multivariate models. These analyses were performed accounting for the characteristics of the '**HCV-Cured**' cohort **A** at treatment initiation when the HCV-infection was still active and **B** at the end of treatment.

Abbreviations: 95CI 95% confidence interval; CTP Child-Turcotte-Pugh; HCV hepatitis C virus; NSBB non-selective beta-blocker; OLT orthotopic liver transplantation; sHR subdistribution hazard ratio.

Supplementary figures and figure legends

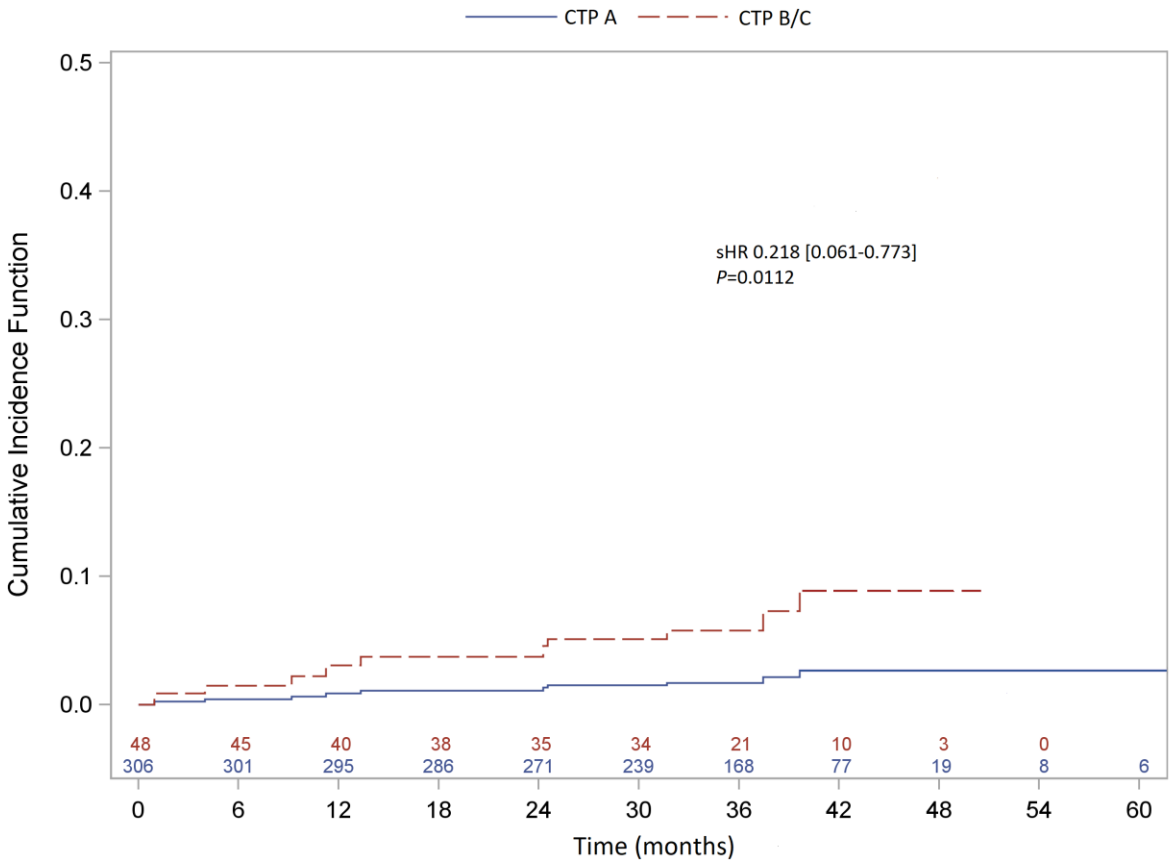
Supplementary figure 1



Supplementary figure 1. Cumulative incidence of non-tumoral-PVT according to the CTP stage (A vs. B/C). For the '**HCV-Cured**' cohort, CTP score at end of treatment was used. Events known to modify the natural history (OLT, TIPS, death, tumoral-PVT and anticoagulation) of the event of interest were considered as competing risks.

Abbreviations: PVT portal vein thrombosis; CTP Child-Turcotte-Pugh; OLT orthotopic liver transplantation; TIPS transjugular intrahepatic portosystemic shunt.

Supplementary figure 2



Supplementary figure 2. Cumulative incidence of non-tumoral-PVT according to the CTP stage (A vs. B/C) in the '**HCV-Cured**' cohort. CTP score at end of treatment was used. Events known to modify the natural history (OLT, TIPS, death, tumoral-PVT and anticoagulation) of the event of interest were considered as competing risks.

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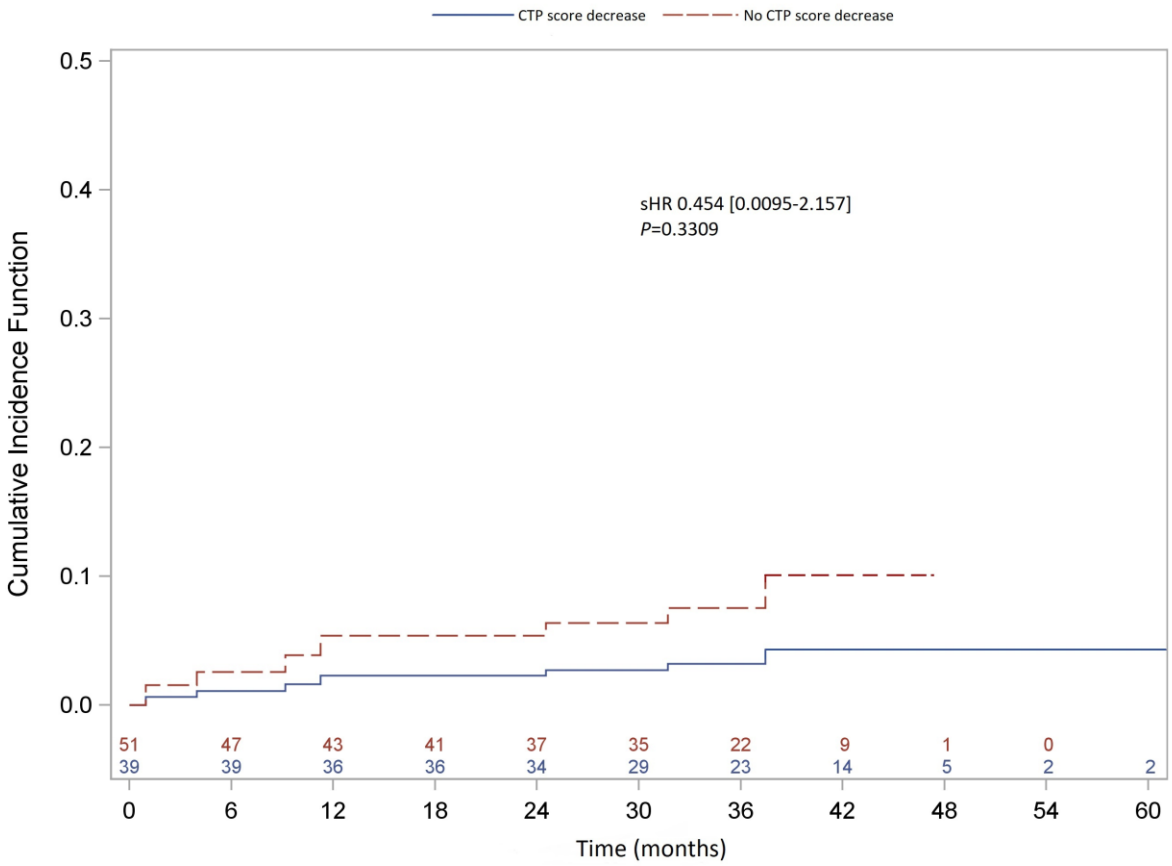
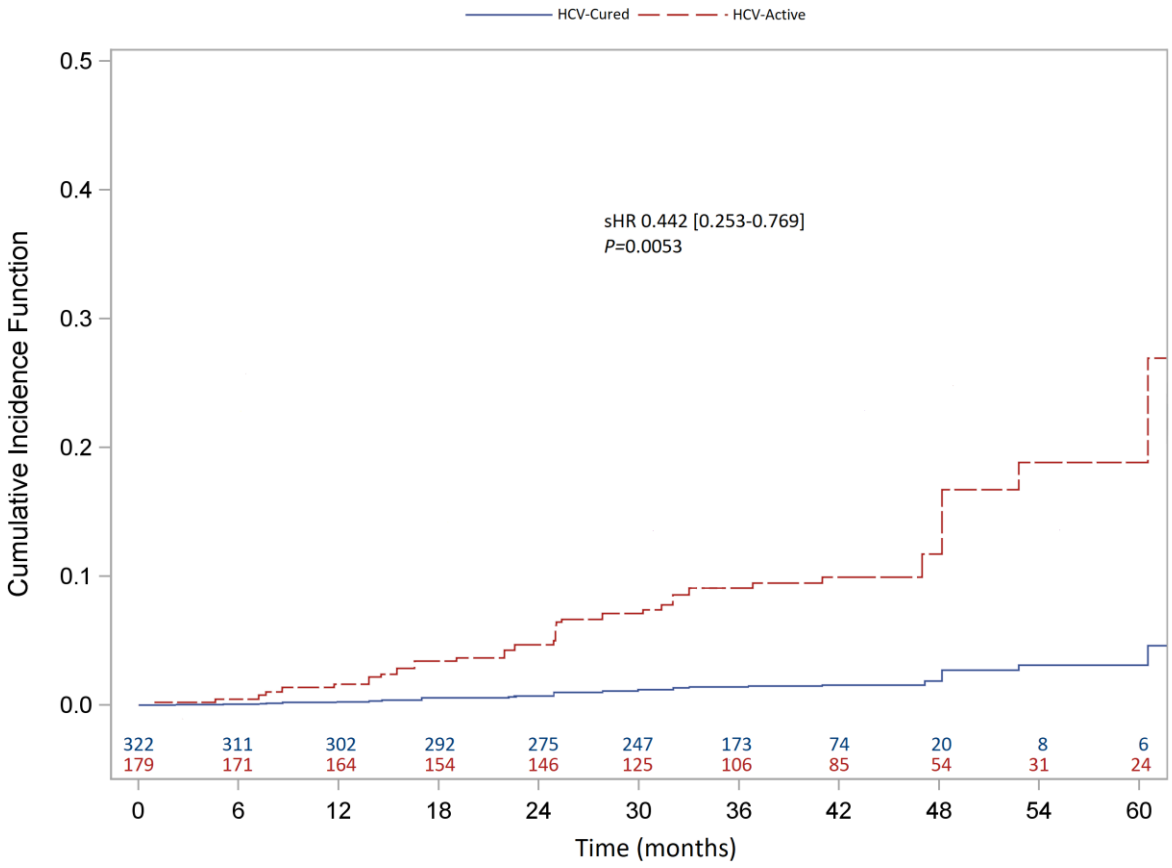
Supplementary figure 3



Supplementary figure 3. Cumulative incidence of non-tumoral-PVT in the '**HCV-Active**' cohort according to the presence or absence of a CTP score decrease ≥1 point at end of treatment. Patients with a pre-treatment CTP score of 5 points were excluded from this analysis. Events known to modify the natural history (OLT, TIPS, death, tumoral-PVT and anticoagulation) of the event of interest were considered as competing risks.

Abbreviations: HCV hepatitis C virus; PVT portal vein thrombosis; OLT orthotopic liver transplantation; TIPS transjugular intrahepatic portosystemic shunt.

Supplementary figure 4



Supplementary figure 4. Cumulative incidence of *de novo* HCC according to the HCV-treatment status in the subgroup of n=500 patients without a history of HCC. OLT and death were considered as competing risks.

Abbreviations: HCC hepatocellular carcinoma; HCV hepatitis C virus; PVT portal vein thrombosis; OLT orthotopic liver transplantation.

7. DISCUSSIÓ

La cirrosi hepàtica s'associa a una alta morbi-mortalitat i suposa un greu problema de salut a nivell mundial (1,2). Una de les complicacions que pot succeir durant el curs de la malaltia és el desenvolupament d'una trombosi portal no tumoral, que és una complicació relativament freqüent sobretot en pacients que presenten una malaltia hepàtica més avançada i en pacients en llista d'espera per a transplantament hepàtic (6–10). No està ben establert l'impacte de la TP en la història natural de la cirrosi hepàtica però s'ha demostrat en diferents estudis que la TP en context del trasplantament hepàtic s'associa a una major morbi-mortalitat en el post trasplantament, sobretot quan la trombosi és extensa i impedeix realitzar una anastomosi portal anatòmica (13–17). En algunes ocasions la TP pot contraindicar el trasplantament hepàtic limitant així les opcions terapèutiques d'aquests pacients.

Com ja s'ha mencionat anteriorment, la fisiopatologia de la TP no tumoral en la cirrosi hepàtica no es coneguda i els factors de risc que n'afavoreixen el seu desenvolupament no estan establerts. Els estudis previs que ho han avaluat només han abordat el problema de forma parcial sense tenir en compte tots els principals factors implicats.

El primer estudi que conforma aquesta tesi, és el primer estudi prospectiu que valora d'una forma global, en una cohort gran de pacients amb cirrosi, els diferents factors potencialment implicats en el desenvolupament de la TP: característiques clíniques, bioquímiques i ecogràfiques conjuntament amb un estudi exhaustiu que avalua l'estat de la coagulació i l'estat inflamatori dels pacients.

La incidència de TP observada al nostre estudi és de 1.6, 6 i 8.4% a 1, 3 i 5 anys, respectivament. Aquesta incidència està en el rang inferior respecte els estudis publicats prèviament (6–10), però, la incidència als 5 anys, és comparable a la incidència de 8% obtinguda a l'estudi de Nery et al. (quan es compara amb l'estratègia de seguiment semestral), un dels estudis longitudinals que ha inclòs més pacients (7). Estem segurs que la baixa incidència de TP no és deguda a falsos negatius, ja que el nostre estudi ha estat específicament dissenyat per identificar TP

mitjançant seguiment ecogràfic realitzat sempre pels 5 mateixos exploradors amb gran experiència.

L'estudi mostra que la història prèvia d'hemorràgia digestiva per varius, un recompte plaquetari baix i la velocitat portal < 15 cm/segons son factors de risc independents pel desenvolupament de TP. Aquests factors estan relacionats amb un major grau d'hipertensió portal i per tant, la severitat de la hipertensió portal sembla el factor més important a l'hora de determinar un major risc de TP. Les descompensacions prèvies i el baix recompte plaquetari també s'havien descrit en estudis previs com a factors de risc de TP (8,10,52). La velocitat portal disminuïda per sota de 15 cm/segon també ha estat descrita prèviament com un factor determinant en el risc de TP (8,20). No obstant, ha estat qüestionada la reproductibilitat de la seva mesura per ecografia-Doppler posant en dubte el seu valor per predir el desenvolupament de TP. En aquest aspecte, el nostre estudi mostra clarament que la mesura precisa de la velocitat portal és possible i fiable en mans experimentades i confirma el paper de la velocitat portal < 15 cm/segon com a factor de risc independent per al desenvolupament de TP.

Hem avaluat de forma molt exhaustiva el possible paper de les alteracions hemostàtiques presents en la cirrosi. Estudis previs han suggerit el paper del factor VIII (34), nivells baixos d'ADAMTS-13 (35), la resistència a la trombomodulina en el test de generació de trombina (33) i una ratio factor VIII/proteïna C elevada (32) com a factors predictius en el desenvolupament de TP. No obstant això, els estudis disponibles han analitzat els factors de risc d'una forma aïllada, avaluant els factors de coagulació per separat i sense tenir en compte altres potencials factors de risc com la velocitat portal o factors relacionats amb la severitat de la malaltia hepàtica. El nostre estudi mostra que els pacients que desenvolupen una TP al llarg del seguiment, tenen significativament nivells més baixos de factors anticoagulants i la ratio factor VIII/proteïna C disminuïda, suggerint així el possible paper de la hipercoagulabilitat. No obstant, quan aquests resultats s'ajusten en diferents models multivariats incloent variables clíniques i ecogràfiques, el Factor X disminuït, factor de coagulació de síntesi hepàtica, és l'únic factor de coagulació que s'associa de forma independent a un major risc de desenvolupar TP. Aquest resultat suggereix

que la TP es un producte més de la malaltia hepàtica més avançada on els nivells dels factors de coagulació sintetitzats pel propi fetge es troben disminuïts, i no pas que els factors de coagulació tinguin un paper pel seu potencial efecte hipercoagulant.

En quant a la inflamació, no hem trobat associació ni dels NETs (MPO-DNA or cfDNA) ni de la resta de marcadors inflamatoris avaluats (IL-6, TNF-α, PCR) amb el risc de desenvolupar trombosis portal. Per tant, aquests resultats suggereixen que ni l'estat inflamatori associat a la cirrosi ni la generació de NETs tenen un paper rellevant en el risc de TP. No obstant, amb les nostres dades, no es pot excloure que un estat proinflamatori agut o un augment agut en la generació de NETs, o inclús un increment local de marcadors inflamatoris a la vena porta (72), puguin influir en el desenvolupament de TP.

Dins del ampli estudi de paràmetres d'hipercoagulabilitat que hem avaluat, també hem avaluat el potencial rol de les alteracions de trombofília hereditàries (Factor V Leiden i la mutació del gen de Protrombina G20210A), que han estat suggerits com a factors de risc de TP en alguns estudis previs (36) però no ha sigut confirmat en d'altres (7,37). La majoria d'aquests estudis però, son retrospectius amb una alta probabilitat de un biaix de selecció i per tant, no està clar el paper d'aquestes alteracions. En el nostre estudi, cap dels 23 pacients que desenvolupen TP dels 266 pacients que han estat testats per aquestes mutacions, son portadors del FV Leiden ni de la mutació de protrombina, suggerint així que aquests trastorns hereditaris de la coagulació no juguen un paper primordial en el risc de TP en context de la cirrosi.

Apart de la reducció de la velocitat del flux portal i dels trastorns de coagulació, un altre dels pilars de la tríada de Virchow és el dany o disfunció endotelial. Encara que no hem avaluat específicament la severitat d'aquesta disfunció, diferents estudis clínics i experimentals han demostrat relació entre la disfunció endotelial i la severitat de la hipertensió portal en els pacients amb cirrosi (73,74). L'existència prèvia d'hemorràgia per varius i la plaquetopènia són dos factors que clarament són el reflex d'una major severitat del grau d'hipertensió portal i de disfunció endotelial. Es important remarcar, que en el subgrup de 103 pacients dels que disposàvem de la

mesura del gradient de pressió venós hepàtic (GPVH), aquest era significativament més elevat en els pacients que desenvolupaven TP. Tots els pacients que desenvolupaven TP tenien un GPVH ≥10mmHg i el risc sembla que era molt més alt amb un GPVH ≥20 mmHg. Addicionalment, la disfunció endotelial té un rol molt important en el sistema venós portal ja que és un dels principals mecanismes que porten a un increment de la resistència intrahepàtica al flux portal i per tant, a la hipertensió portal (4). A més, l'increment de la pressió portal promou el desenvolupament de circulació col·lateral portosistèmica (resultant en les varius esofàgiques) amb el conseqüent pas de part del flux de sang portal a la circulació sistèmica sense passar pel fetge i per tant, reduint encara més la velocitat portal. Per tant, aquests dos components, encara que són independents, es potencien l'un a l'altre. Tot això suggereix que la TP en la cirrosi es relaciona majoritàriament amb canvis en el flux portal i la disfunció endotelial mentre que el paper de la hipercoagulabilitat, tant adquirida com hereditària, sembla minoritari.

Addicionalment, ja que els últims anys ha sorgit controvèrsia sobre el possible increment del risc de TP relacionat amb l'ús de beta-bloquejants no cardioselectius (BBNS), ho hem analitzat en la nostra cohort. Hem fet un anàlisi addicional temps-depenent tenint en compte els canvis en el tractament (inici o discontinuació) durant el seguiment), presència o aparició de varius de risc i hemorràgia per varius durant el seguiment sense observar cap associació entre el tractament amb BBNC i el desenvolupament de TP. Aquests resultats són oposats a un metanàlisi publicat recentment en el que es conclou que aquest tractament incrementa 4.6 vegades el risc de desenvolupar TP. No obstant, cal destacar que en aquest metanàlisi es van incloure 9 estudis molt heterogenis, majoritàriament retrospectius, amb un seguiment limitat i sense tenir en compte els canvis dinàmics en el tractament, tamany de les varius i hemorràgia variceal com en el nostre estudi. Per tant, segons els nostres resultats, si el tractament amb BBNS es considera indicat, no s'hauria de modificar malgrat la presència de factors de risc per desenvolupar TP.

Respecte d'altres factors que prèviament s'han suggerit com a potencials factors de risc de TP en la cirrosi com la obesitat (75), presència de varius esofàgiques o tractament endoscòpic previ de les varius (8,13,14) o l'etiologia de la cirrosi,

especialment el fetge gras (MAFLD o metabolic associated fatty liver disease) (19,55,71,76), no han estat confirmats en el nostre estudi. Cal mencionar però, que només 13 pacients de la nostra cohort tenien MAFLD com a etiologia i per tant, no podem extreure conclusions fermes en aquest sentit.

Addicionalment, hem pogut crear un score de risc de TP combinant les tres variables que tenen un valor predictiu independent amb un AUROC of 0.778 per la predicció de TP. Aquest score discrimina dues poblacions diferents on els pacients d'alt risc tenen 4 vegades més risc de desenvolupar TP que la població de baix risc. Aquest score però, hauria de ser validat en futures cohorts prospectives.

No obstant, el nostre estudi té algunes limitacions que cal mencionar. Primerament, el baix número de TP que s'han produït durant el seguiment i que limiten l'anàlisi estadístic. La majoria de pacients inclosos al nostre estudi son Child-Pugh A i, tenint en compte que la severitat de la hipertensió portal és un dels factors principals implicats en el desenvolupament de TP, això podria explicar la incidència de TP de la nostra cohort. No obstant, no podem determinar si els factors de risc de TP que hem identificat es mantindrien en el cas de avaluar una cohort que inclogués més pacients Child-Pugh B i C. En segon lloc, destacar que aquest es un estudi unicèntric i, encara que un estudi multicèntric permetria ampliar el tamany de la mostra, el disseny del nostre estudi té l'avantatge de maximitzar l'homogeneïtat de les dades. Addicionalment, és important remarcar també que les mostres de sang s'han obtingut al moment d'inclusió a l'estudi representant així un estat hemostàtic i inflamatori diferent que aquell que podríem trobar en el moment de produir-se la TP. Per tant, d'acord amb els nostres resultats, els paràmetres hemostàtics i inflamatoris avaluats no són predictius de TP però no podem excloure completament que puguin jugar un paper fisiopatològic en alguna situació determinada (per exemple, en el cas d'un increment agut dels marcadors inflamatoris). Mencionar també que els pacients amb cirrosi per VHC que van realitzar tractament antiviral durant el seguiment van ser censorats de l'estudi pel potencial impacte que la curació del VHC podia tenir en la història natural de la cirrosi i el desenvolupament de la TP. Aquest fet ha sigut inevitable ja que no hauria estat ètic no oferir o posposar aquest tractament després de que fos accessible pels pacients amb cirrosi.

Malgrat tot, només han sigut 100 pacients, el seguiment dels quals (45 ± 14 mesos) no ha sigut significativament diferent de la resta de cohort i per tant, pensem que aquest fet ha tingut un mínim impacte en els resultats del estudi.

En resum, els resultats del primer estudi d'aquesta tesi indiquen que els factors relacionats amb una major severitat de la hipertensió portal, incloent la velocitat portal < 15 cm/segon, el baix recompte de plaquetes i l'hemorràgia prèvia per varius són factors que s'associen de forma independent a un major risc de TP en la cirrosi. Els nostres resultats no recolzen que les alteracions de la coagulació adquirides que s'observen en els pacients amb cirrosi, les alteracions de coagulació hereditàries (FV Leiden i mutació Protrombina G20210A) i l'estat inflamatori siguin factors predictors de TP en context de la cirrosi. Addicionalment, l'ús de BBNS no s'associa de forma independent a un risc incrementat de TP.

D'altra banda, hem avaluat per primera vegada la incidència de TP no tumoral en una gran cohort prospectiva de pacients amb cirrosi pel VHC després de realitzar tractament amb AAD i assolir RVS. Els resultats indiquen que el risc de TP no disminueix després de la RVS i això passa malgrat si que s'observa una disminució en el risc d'altres complicacions i una millora en la supervivència.

Hem avaluat l'impacte de la teràpia antiviral sobre el risc de TP mitjançant un anàlisi de riscos competitius amb tres enfocaments diferents: 1) En primer lloc, per avaluar l'impacte de la curació del VHC en el desenvolupament de TP estimant els efectes causals del tractament, hem realitzat un anàlisi ajustat per IPTW (Inverse probability of treatment weighting) atorgant una puntuació de propensió i considerant les característiques dels pacients prèvies al tractament. Aquest mètode estadístic crea dos grups ajustats per les covariables més rellevants per l'anàlisi. 2) En segon lloc, hem realitzat un anàlisi multivariat convencional (també tenint en compte les característiques dels pacients prèvies al tractament) per avaluar altres potencials factors de risc per a desenvolupar TP. 3) Per últim, tenint en compte que la funció hepàtica sol millorar després del tractament antiviral i RVS, hem realitzat un tercer anàlisi multivariat convencional però en aquest cas tenint en compte les característiques al final del tractament, i ajustat pel grau d'hipertensió portal (segons

història prèvia d'hemorràgia variceal i tractament amb BBNC com a indicador de varius d'alt risc) i disfunció hepàtica (segons la puntuació Child-Pugh).

Amb aquests tres anàlisis hem obtingut resultats gairebé idèntics, reforçant així la solidesa dels nostres resultats. Després de la curació del VHC, el risc de desenvolupar una TP no tumoral persisteix i la severitat de la disfunció hepàtica és un factor de risc crucial per al seu desenvolupament. De fet, l'augment d'un punt en la puntuació Child-Pugh es tradueix en un augment del 32% del risc de TP. En conseqüència, la incidència de TP no tumoral en els pacients Child-Pugh A ha estat molt baixa. No obstant, les guies clíniques actuals recomanen el cribratge d'hepatocarcinoma a tots els pacients amb cirrosi o fibrosi hepàtica avançada previs al tractament independentment de l'estadi Child-Pugh (77) i donat que no afegeix cap cost o càrrega addicional pel pacient o sistema sanitari, pensem que s'hauria de realitzar una avaluació concomitant de l'eix esplenoportal. Probablement, si estudis futurs identifiquen subpoblacions de pacients en les quals no calgui realitzar vigilància de l'hepatocarcinoma, l'avaluació sistemàtica i específica per a la TP podria deixar de realitzar-se de forma segura en pacients compensats en estadi Child-Pugh A.

Al avaluar l'impacte de la cura del VHC sobre els components de la tríada de Virchow, només s'entén parcialment el possible mecanisme fisiopatogènic involucrat en el desenvolupament de la TP. En quant a la coagulació, estudis previs han demostrat que l'eliminació del VHC millora els paràmetres rutinaris de coagulació en la cirrosi disminuint la hipercoagulabilitat cosa que pot conduir a un equilibri hemostàtic més estable (69,70). Tanmateix, l'estudi 1 d'aquesta tesi suggereix que les alteracions del perfil hemostàtic relacionades amb la hipercoagulabilitat no son factors de risc per desenvolupar TP en la cirrosi. Per tant, la potencial contribució de la "coagulopatia cirròtica" a la formació de la TP en aquest context continua sent una qüestió no resolta.

D'altra banda, la velocitat del flux sanguini portal disminuïda ha estat identificada en prèviament com un dels principals factors de risc per al desenvolupament de TP (8). S'ha demostrat que la majoria dels pacients que aconsegueixen la curació del VHC

després del tractament mostren una disminució del GPVH (61,62,64,67). Aquesta disminució en la severitat de l'hipertensió portal s'explica per una disminució en la resistència vascular intrahepàtica i una millora de la circulació hiperdinàmica i de la vasodilatació esplàncnica, mecanismes que donarien lloc a canvis oposats al flux venós portal augmentant-ne la velocitat. Les úniques dades disponibles sobre l'impacte de la RVS sobre la velocitat del flux sanguini portal provenen d'un estudi recent basat en avaluacions d'imatges de ressonància magnètica, que descriu que la velocitat portal no s'afectava per la RVS (78). Cal destacar però, que el nostre estudi no pot proporcionar informació sòlida sobre l'impacte de la velocitat portal sobre el risc de TP ja que no s'ha avaluat sistemàticament en tots els pacients.

Finalment, s'ha demostrat que l'eliminació del VHC millora la disfunció endotelial sistèmica, encara que aquest efecte sembla menys marcat o absent en pacients amb cirrosi (79–81) però es desconeix l'efecte que pugui tenir la RVS sobre la disfunció endotelial a nivell esplàncnic. En conjunt, l'evidència actual suggereix que l'assoliment de la RVS en pacients amb cirrosi per VHC, podria no tenir un clar efecte beneficiós sobre els diferents components de la tríada de Virchow implicats en el risc trombòtic.

Com hem comentat anteriorment, la RVS millora la funció hepàtica i la hipertensió portal i aquests son els principals factors predictius de la TP d'acord amb els nostres resultats. Per tant, esperaríem una reducció en la incidència de TP després de la curació del VHC però no s'observa en el present estudi. Es pot especular sobre si podria ser un efecte directe causat per l'eliminació del VHC o pels tractaments basats en AAD que puguin contrarestar l'efecte positiu en la millora de la funció hepàtica i hipertensió portal després de la RVS. En aquest sentit, també s'ha descrit prèviament l'empitjorament o aparició de hipertensió arterial pulmonar en pacients que han rebut tractament antiviral, ja sigui amb règims d'interferó (82) o basats en AAD (83). Tot i que la incidència real d'aquesta complicació i mecanisme implicat no estan clars, s'ha suggerit que la supressió de l'RNA-VHC podria provocar una ràpida disminució dels nivells de mediadors vasodilatadors, provocant així la hipertensió pulmonar (82,83). Una hipòtesi seria que aquest fenomen també pugui afectar la circulació venosa portal afavorint així l'aparició de TP.

L'estudi té algunes limitacions que cal mencionar. En primer lloc, aquest estudi combina dues cohorts de pacients reclutats en períodes de temps diferents, però parcialment superposats. Això no es va poder evitar ja que no hauria estat ètic retardar el tractament pel VHC després de que estigués disponible. A més, també hi ha lleugeres diferències en la durada del seguiment, cosa que es pot explicar per la inclusió més recent dels pacients "VHC-curats". En segon lloc, cal mencionar la baixa incidència de TP durant el seguiment que limita la força del nostre anàlisi. No obstant, hem pogut realitzar l'anàlisi incloent 5 esdeveniments per variable predictora, criteri que sembla ser suficients per a l'anàlisi multivariat amb regressió de Cox (84). Cal destacar que per avaluar l'objectiu principal del nostre estudi (l'impacte de la curació del VHC post tractament amb AAD en la TP no tumoral) hem realitzat l'anàlisi IPTW que crea una pseudo-població en la què els dos grups d'estudi ("HC-curats" i "VHC-actius") queden equilibrats per les covariables més rellevants, evitant així la necessitat d'un ajust multivariant i eliminant per tant, el problema esmentat degut al baix número d'events durant el seguiment. En quant a la baixa incidència de TP de la nostra cohort es pot explicar perquè un 83% de pacients inclosos tenen un estadi Child-Pugh A i aquests pacients presenten un risc més baix de TP. La nostra incidència es lleugerament inferior, però propera, a la incidència reportada anteriorment per Nery et al. que inclou un 69% de pacients Child-Pugh A (7). Addicionalment, tot i que els pacients de la cohort "VHC-actius" provenien d'un estudi dissenyat amb l'objectiu específic d'avaluar la incidència de TP i que els pacients de la cohort "VHC-curats" van ser avaluats dins la pràctica clínica habitual, ambdues cohorts es van seguir prospectivament i totes les exploracions ecogràfiques van ser realitzades per els mateixos ecografistes experts en l'avaluació de malalties vasculars hepàtiques. Per tant, estem segurs que la TP no ha estat infradiagnosticada en cap dels dos grups. Finalment, les dues cohorts difereixen en les característiques dels pacients, la qual cosa implica que les estimacions no ajustades s'han d'interpretar amb precaució. No obstant això, hem realitzat tan anàlisis convencionals multivariants com l'anàlisi ajustat per IPTW per obtenir estimacions més precises de l'impacte de la curació del VHC. Després d'aquests ajustos, el risc de TP es veu incrementat un 31% a la cohort "VHC-curats", proporcionant així proves

convincents de l'absència d'una reducció del risc, tal com si que s'observa per a la mortalitat.

En resum, en aquest estudi es demostra per primera vegada que, tot i que amb una incidència baixa (sobretot en pacients Child-Pugh A), el risc de TP no tumoral persisteix almenys 3 anys després d'assolir RVS per AAD en pacients amb cirrosi per VHC. És important destacar que això es produeix malgrat una millora de la supervivència i la funció hepàtica, cosa que suggereix que aquest darrer benefici podria estar contrarestat per un efecte directe de la curació del VHC o del tractament que s'utilitza per aconseguir-ho sobre la vasculatura venosa portal.

8. CONCLUSIONS

1. La incidència de TP observada és de 1.6, 6 i 8.4% a 1, 3 i 5 anys, respectivament.
2. Els factors relacionats amb una hipertensió portal més severa, incloent la velocitat portal < 15 cm/seg, recompte de plaquetes baix i història d'hemorràgia variceal, són els factors de risc independents associats a un major risc de desenvolupar TP en la cirrosi.
3. Ni les alteracions de la coagulació tant adquirides com hereditàries, ni els marcadors inflamatoris i NETS no són factors de risc rellevants per predir el desenvolupament de TP en la cirrosi.
4. L'ús de BBNC no s'associa de forma independent a un risc incrementat de TP.
5. La RVS després de tractament antiviral d'acció directa no modifica la incidència de TP no tumoral en els pacients amb cirrosi per VHC.
6. El risc de TP persisteix a pesar de que hi ha una reducció en el risc de descompensacions hepàtiques i una reducció en la mortalitat.

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10. ALTRES PUBLICACIONS

Altres publicacions realitzades durant el període de la tesi:

Articles originals

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Revisions

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