Avances en el diagnóstico, etiopatogenia y pronóstico de la hipertensión portal no cirrótica

Susana Seijo Ríos

Tesis doctoral
Universidad de Barcelona
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SUSANA SEIJO RÍOS

Para optar al grado de Doctor
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UNIVERSITAT DE BARCELONA

Informe de los directores de tesis

Barcelona, 9 de Abril del 2013

Juan Carlos García-Pagán, consultor Sénior del Servicio de Hepatología del Hospital Clínic de Barcelona y Jaume Bosch Genover, catedrático de la Facultad de Medicina de la Universidad de Barcelona y Consultor Sénior del Servicio de Hepatología del Hospital Clínic de Barcelona,

Certifican:

Que la tesis doctoral Avances en el diagnóstico, etiopatogenia y pronóstico de la Hipertensión Portal No Cirrótica, presentada por Susana Seijo Ríos para optar al título de Doctor de la Universidad de Barcelona se ha realizado bajo nuestra dirección y cumple todos los requisitos necesarios para ser defendida delante del Tribunal de evaluación correspondiente.

Juan Carlos García-Pagán

Jaime Bosch Genover

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Abreviaturas

AUC: área bajo la curva
BCIS score: *BCS intervention-free survival prognostic score*
BCS-TIPS PI score: *BCS-TIPS prognostic index score*
BMPR2: receptor de la proteína morfogenética ósea 2
CSPH: hipertensión portal sinusoidal clínicamente significativa
EN-Vie: *European Network for Vascular Disorders of the Liver*
ET: elastografía de transición
GPVH: gradiente de presión venosa hepática
CVVH: comunicantes vena-vena hepáticas
HAP: hipertensión arterial pulmonar
HAPI: hipertensión arterial pulmonar idiopática
HTPI: hipertensión portal idiopática
HTPNC: hipertensión portal no cirrótica
kPa: kilo Pascales
MLPA: *Multiple Ligation Probe Amplification*
PLS-DA: *Partial Least Squares Projection to Latent Structures regression with Discriminant Analysis*
PP: presión portal
PSHE: presión suprahepática enclavada
PSHL: presión suprahepática libre
SBC: Síndrome de Budd-Chiari
SNPs: polimorfismos de un solo nucleótido
TARGA: terapia antiretroviral de gran actividad
TGF-β: factor de crecimiento transformante beta
TH: trasplante hepático
TIPS: *transjugular intrahepatic portosystemic shunting*
TVPNC: trombosis venosa portal no cirrótica
VCAM-1: *vascular cell adhesion molecule-1*
VCI: vena cava inferior
VIH: virus de la inmunodeficiencia humana
VIP: *variable importance in the projection*

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Introducción

INTRODUCCIÓN

La hipertensión portal es un síndrome clínico que se caracteriza por un incremento patológico del gradiente de presión venosa hepática (GPVH) por encima del su valor normal de 1-5 mmHg. La cirrosis hepática es la principal causa de hipertensión portal en el mundo occidental y se define como una alteración difusa de la arquitectura del hígado con fibrosis y nódulos de regeneración[1]. El gran número de pacientes con cirrosis ha permitido el avance en el conocimiento de la fisiopatología, historia natural y tratamiento de la hipertensión portal asociada a la cirrosis hepática[2, 3]. Sin embargo, existen otras enfermedades hepáticas diferentes de la cirrosis que también pueden ocasionar hipertensión portal.

Etiología y clasificación de la hipertensión portal

Cualquier enfermedad que interfiera con el flujo sanguíneo hepático (a cualquier nivel entre el bazo y la aurícula derecha) puede causar hipertensión portal. Por ello las enfermedades que ocasionan hipertensión portal se clasifican en base a su localización anatómica en: *prehepáticas* (afectan al eje venoso esplenoportomesentérico), *intrahepáticas* y *posthepáticas*[3-5] (tabla 1). La cirrosis es la causa más frecuente de hipertensión portal en el mundo occidental. Las otras causas de hipertensión portal se engloban bajo el término de hipertensión portal no cirrótica (HTPNC)[2, 3]. Dentro de la HTPNC se encuentran los trastornos vasculares hepáticos[6, 7]. Los trastornos vasculares hepáticos engloban una serie de condiciones raras (con una prevalencia inferior a 5/10.000 habitantes) que en conjunto representan una importante causa de HTPNC que conlleva una alta morbilidad y mortalidad en todo el mundo. Las tres principales son el Síndrome de Budd-Chiari (SBC), la trombosis portal no cirrótica (TVPNC) y la hipertensión portal idiopática (HTPI). Estos trastornos comparten varias características: la hipertensión portal como la manifestación clínica principal; los trastornos protrombóticos subyacentes frecuentes; los cambios arquitecturales en el lecho vascular, y las trombosis venosas[8-10].

Tabla 1. Clasificación de las enfermedades que ocasionan hipertensión portal

Lugar de la obstrucción al flujo hepático	Causas principales	GPVH
Prehepática	Trombosis del eje venoso esplenoportomesentérico Compresión extrínseca del eje venoso esplenoporto-mesentérico Estenosis congénita de la vena porta Fístula arteriovenosa	GPVH normal (PSHE y PSHL normales)
Intrahepática *Presinusoidal*	Esquistosomiasis Hiperplasia nodular regenerativa Fibrosis hepática congénita Hipertensión portal idiopática Peliosis hepática Enfermedad hepática poliquística Sarcoidosis Tuberculosis Amiloidosis Cirrosis biliar primaria Intoxicación por arsénico, sulfato de cobre o vinilo	GPVH Normal (PSHE y PSHL normales)
Intrahepática *Sinusoidal*	Cirrosis Hepatitis aguda grave (virus y alcohol) Hepatitis crónica activa Fibrosis/hepatotoxicidad por metotrexato, azatio-prina o amiodarona Enfermedad aguda grasa del embarazo Mastocitosis Enfermedad de Gaucher Neoplasias vasculares primarias Amiloidosis Hipervitaminosis A Síndrome de obstrucción sinusoidal (SOS)	GPVH aumentado (PSHE aumentado)
Intrahepática *Postsinusoidal*	Síndrome de Budd-Chiari	GPVH normal o discretamente aumentado (aumento de la PSHE, PSHL y de la presión de la VCI)
Posthepática	Síndrome de Budd-Chiari Malformaciones congénitas y trombosis de la VCI Pericarditis constrictiva Valvulopatía tricuspídea	GPVH normal o discretamente aumentado (aumento de la PSHE, PSHL y de la presión de la VCI)

Abreviaturas: GPVH: gradiente de presión venosa hepática; PSHE: presión suprahepática enclavada; PSHL: presión suprahepática libre; VCI: vena cava inferior.
Modificado de *Berzigotti et al.* Expert Rev Gastroenterol Hepatol 2013; *Bosch et al.* Nat Rev Gastroenterol Hepatol 2009; *Bosch et al.* J Hepatol 2008; *Roskams et al.* Histopatology 2003. 2-5.

La TVPNC es la principal causa de *hipertensión portal prehepática*. En los adultos, las enfermedades protrombóticas, tanto congénitas (tales como el déficit de antitrombina, proteína C o proteína S) como adquiridas (como las neoplasias mieloproliferativas), y/o los factores locales como la cirugía abdominal, procesos inflamatorios/infecciosos abdominales (pancreatitis, colecistitis, diverticulitis, etc) o sepsis son responsables del 70% de los casos. En el 30% restante, tras un estudio exhaustivo no se encuentra ninguna causa protrombótica o local subyacente, y la TVPNC es considerada idiopática. En los niños, la trombosis portal suele estar relacionada con onfalitis o cateterización de la vena umbilical. La TVPNC puede presentarse como dos escenarios clínicos distintos, la TVPNC aguda o la TVPNC crónica (cavernomatosis portal), y su diagnóstico se basa en técnicas de imagen [6].

La *hipertensión portal intrahepática* puede clasificarse en presinusoidal, sinusoidal y postsinusoidal en base al sitio de mayor resistencia y los resultados de la cateterización de las venas suprahepáticas (tabla 1). La hipertensión portal presinusoidal se caracteriza por valores GPVH normales o levemente aumentados. La hipertensión portal idiopática, la esquistosomiasis, la sarcoidosis, la tuberculosis, y las primeras etapas de la cirrosis biliar primaria son las principales causas de hipertensión portal intrahepática presinusoidal. La hipertensión portal sinusoidal es característica de la mayoría de las enfermedades hepáticas crónicas y se caracteriza por un aumento del GPVH. La cirrosis es la causa más común de hipertensión portal sinusoidal [3, 5].

El síndrome de Budd-Chiari (SBC) es la causa más frecuente de *hipertensión portal postsinusoidal*. La obstrucción del flujo venoso hepático puede estar presente desde las vénulas hepáticas de pequeño calibre (y ser verdaderamente intrahepática) hasta la entrada de la vena cava inferior (VCI) en la aurícula derecha (posthepática). El SBC suele estar causado por trastornos protrombóticos subyacentes (fundamentalmente neoplasias mieloproliferativas) y su diagnóstico se hace generalmente mediante técnicas de imagen [6, 11]. En la hipertensión portal postsinusoidal el GPVH suele ser normal.

En algunas enfermedades el cateterismo venoso hepático puede mostrar la evolución de la patología. Por ejemplo, la esquistosomiasis en la fase inicial induce la formación de granulomas y fibrosis portal causando una hipertensión portal presinusoidal (GPVH es normal en esta fase). Más tarde, la fibrosis puede extenderse fuera del tracto portal y causar hipertensión portal sinusoidal, con GPVH alto, con un patrón hemodinámico y clínico muy similar a la cirrosis [5].

Medición del gradiente de presión venosa hepática (GPVH): utilidad en el diagnóstico de enfermedades que causan hipertensión portal

La medición del GPVH es la mejor forma de la evaluar la presión portal y ayuda clasificar las diferentes causas de hipertensión portal (tabla 1). Es una técnica segura y reproducible. La medición del GPVH se realiza bajo una ligera sedación consciente (midazolam 0.02 mg/kg iv)[12] y con monitorización no invasiva de constantes vitales (electrocardiograma, presión arterial y pulsioximetría). Se cateteriza la vena yugular derecha (o la vena femoral o antecubital) con anestesia local y bajo control ecográfico[13]. A continuación se coloca un introductor, y bajo control fluoroscópico, se introduce un catéter a través de la aurícula derecha y la vena cava inferior, en la vena suprahepática derecha.

El GPVH se define como la diferencia entre la presión suprahepática enclavada (PSHE) y la presión suprahepática libre (PSHL). Se basa en el concepto de que cuando se bloquea el flujo de sangre de una vena suprahepática mediante la oclusión con un catéter enclavado, la columna estática de la sangre transmite la presión de los sinusoides hepáticos. Así, la PSHE es una medición de la presión sinusoidal hepática y no de la presión portal en sí. Como en la cirrosis las comunicaciones entre los sinusoides se pierden debido a la formación de fibrosis, septos y nódulos de regeneración, la presión sinusoidal se equilibra con la presión portal. Está demostrado que la PSHE refleja adecuadamente la presión portal en la hepatopatía de origen alcohólico, hepatitis C y/o hepatitis B[14], que son las causas más frecuentes de enfermedad hepática crónica.

La presión suprahepática libre (PSHL) se mide mediante el mantenimiento de la punta del catéter «libre» en la vena suprahepática, a 2-4 cm de su abertura en la VCI (Figura 1). Si la diferencia entre la PSHL y la presión de la VCI es mayor de 2 mmHg, es probable que el catéter esté colocado de forma inadecuada. Cabe señalar que la presión de la aurícula derecha no puede sustituir la PSHL en la medición de GPVH[15]. La PSHE se mide mediante la oclusión de la vena suprahepática mediante el inflado del globo de la punta del catéter o de forma manual avanzando el catéter hasta la zona más distal de la vena suprahepática (Figura 1). La correcta oclusión de la vena suprahepática se confirma inyectando lentamente 5 ml de medio de contraste en la vena con el balón inflado. Este procedimiento debe mostrar un típico patrón en ‹cuña›, sin reflujo del medio de contraste o de lavado a través de comunicaciones con otras venas hepáticas (comunicantes veno-venosas, CVVH) lo que podría infravalorar la verdadera PSHE.

La PSHE debe medirse hasta que el valor se mantiene estable (normalmente más de 60 segundos). Todas las medidas deben ser tomadas por triplicado y los trazados deben obtenerse utilizando una grabadora multicanal y transductores adecuadamente calibrados.

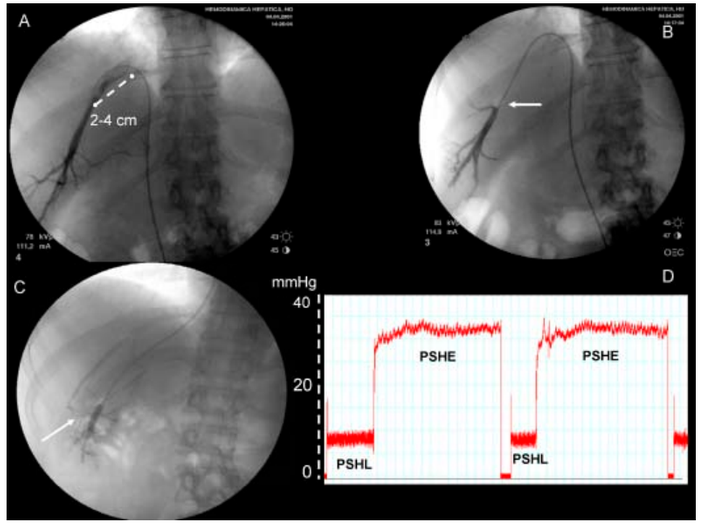

FIGURA 1. Medición del GPVH. (A) La PSHL se mide manteniendo la punta del catéter «libre» en la vena suprahepática, a 2-4 cm de su abertura en la vena cava inferior. (B) La presión PSHE se mide mediante la oclusión de la vena suprahepática con el inflado del globo de la punta del catéter. La oclusión adecuada de la vena suprahepática se confirma inyectando lentamente 5 ml de contraste en la vena con el balón inflado. (C) Se observa lavado de material de contraste a través de una comunicante con otras venas suprahepáticas (flecha) lo que impide una correcta medición de la PSHE. (D) Imagen típica de registro de las presiones suprahepáticas usando una grabadora multicanal y transductores adecuadamente calibrados.
De *Berzigotti et al.* Expert Rev Gastroenterol Hepatol 2013

La hipertensión portal sinusoidal suele presentar un aumento en la PSHE con PSHL normal, resultando en un GPVH alto. La hipertensión portal presinusoidal se caracteriza por valores GPVH normales o levemente aumentados, con PSHE normal o ligeramente aumentada y una PSHL normal. En la hipertensión portal postsinusoidal, GPVH es también normal, pero sin embargo, a diferencia con la hipertensión portal presinusoidal, tanto PSHE y PSHL están incrementados (tabla 1).

En la cirrosis, cuando el GPVH se incrementa por encima de ≥10 mmHg se conoce como hipertensión portal sinusoidal clínicamente significativa (CSPH) y es el dintel para la aparición de las complicaciones de la hipertensión portal como la formación de colaterales portosistémicas y varices, hemorragia digestiva alta por rotura de varices y gastropatía de la hipertensión portal, ascitis, disfunción renal, encefalopatía hepática, entre otros[3]. Los valores de GPVH entre 5-9 mmHg se conoce como hipertensión portal clínicamente no significativa.

Las complicaciones de esta técnica son poco frecuentes (<1% de los casos), la mayoría de ellas están relacionados con una lesión local en la zona del acceso venoso (dolor, hematoma, punción accidental de la arteria carótida). Este riesgo se reduce notablemente con el uso de ultrasonido para guiar la punción venosa. El paso del catéter a través de la aurícula derecha raramente causa arritmias, que son generalmente transitorias.

Medición de la rigidez hepática en las enfermedades que causan hipertensión portal

La medición de la rigidez hepática por elastografía de transición (ET) (Fibroscan®, Echosens, París, Francia) es una técnica validada para la evaluación no invasiva de la fibrosis hepática[16]. Las mediciones se realizan con un transductor de ultrasonidos construido en el eje de un vibrador que transmite una vibración de amplitud suave y de baja frecuencia que causa una onda que se propaga a través del tejido hepático. El Fibroscan® mide la velocidad de propagación de dicha onda a través del parénquima hepático, que está directamente relacionada con la rigidez del tejido. Dado que la fibrosis es el principal determinante de la rigidez hepática y de la resistencia al flujo sanguíneo portal (el principal determinante de la presión portal en las primeras etapas de la hipertensión portal), en los últimos años se ha evaluado el potencial de la ET para estimar de forma no invasiva el GPVH[16].

En la mayoría de los estudios publicados los valores de rigidez por encima de 12.5-17.6 kPa se asociaban a cirrosis hepática con elevada sensibilidad (77-92%) y especificidad (91-97%)[17-20]. Se han descrito también varios dinteles para el diagnóstico de CSPH: una ET <13.6 kPa descarta y una ET ≥21.1 kPa confirma de manera fiable la ausencia y presencia de CSPH, respectivamente. Un estudio reciente mostró que tanto el GPVH ≥10 mmHg como la ET ≥21.1 kPa fueron buenos predictores de descompensación clínica en la cirrosis[21]. Por otra parte, los pacientes con valores intermedios (ET entre 13.6 kPa y 21.1 kPa) no pueden ser clasificados respecto a tener o no CSPH[22].

Las principales limitaciones técnicas del Fibroscan® incluyen la falta de visualización del parénquima hepático, y la imposibilidad de obtener mediciones o resultados poco fiables en 3-16% de los casos, sobre todo debido a la obesidad o a la presencia de ascitis [23].

Existen múltiples estudios que han evaluado el papel del Fibroscan® en el diagnóstico de la cirrosis y de la CSPH; así como la evaluación de la fibrosis en hepatitis virales crónicas. Sin embargo apenas hay datos sobre el papel que puede jugar esta técnica en la evaluación de pacientes con HTPNC.

Hipertensión portal idiopática

La hipertensión portal idiopática representa una de las causas intrahepáticas de hipertensión portal no cirrótica (HTPNC) causando una alta morbi-mortalidad[24]. Los avances en el conocimiento de esta enfermedad se ven obstaculizados por el hecho de ser una enfermedad rara y por la dificultad en su diagnóstico ya que no existe ninguna prueba o test que permitan establecer el diagnóstico de forma positiva, por lo que es un diagnóstico de exclusión[25]. Así, actualmente el diagnóstico requiere los siguientes criterios[25]: (a) la existencia inequívoca de signos de hipertensión portal (varices gastroesofágicas, ascitis, esplenomegalia), (b) una biopsia hepática que descarte otras enfermedades hepáticas que causan hipertensión portal, y (c) ausencia de trombosis del eje esplenoportal y de las venas suprahepáticas.

La biopsia hepática es imprescindible para el diagnóstico de la HTPI. Sin embargo no existe una alteración histológica patognomónica en la HTPI por lo que el objetivo de la biopsia es descartar la presencia de cirrosis o de otras enfermedades hepáticas que causen hipertensión portal. Los hallazgos histopatológicos comunes de la HTPI incluyen la fibrosis de los espacios porta, con engrosamiento de la capa íntima e hipertrofia de la

capa muscular que ocasiona un estrechamiento y la obliteración del lumen de las vénulas portales, la dilatación y fibrosis sinusoidal, la presencia de vasos portales aberrantes, microtrombosis o la presencia de hiperplasia nodular regenerativa[9, 26-28] (figura 2).

La HTPI es conocida también como esclerosis hepatoportal[29], fibrosis portal no cirrótica[30], cirrosis septal incompleta[31], hiperplasia nodular regenerativa[32, 33] o trasformación nodular parcial[34, 35]. Esta variabilidad en la nomenclatura y los hallazgos histopatológicos sugieren que bajo la definición de HTPI podrían englobarse varias entidades con origen fisiopatológico diferente. Todo esto hace que el proceso diagnóstico de estos pacientes sea largo, costoso, y que además incluya la realización de exploraciones invasivas.

La dificultad en el diagnóstico de la HTPI ocasiona además una serie de problemas: (a) el hecho de que sea requisito indispensable para el diagnóstico la presencia de signos inequívocos de hipertensión portal, ocasiona que estos pacientes sean comúnmente diagnosticados en fases muy avanzadas de su enfermedad, frecuentemente coincidiendo con un episodio de hemorragia por rotura de varices. Ello restringe el tratamiento actual al de las complicaciones de la enfermedad y limita la posibilidad de implementar estrategias de prevención de desarrollo de las mismas. (b) En un paciente con

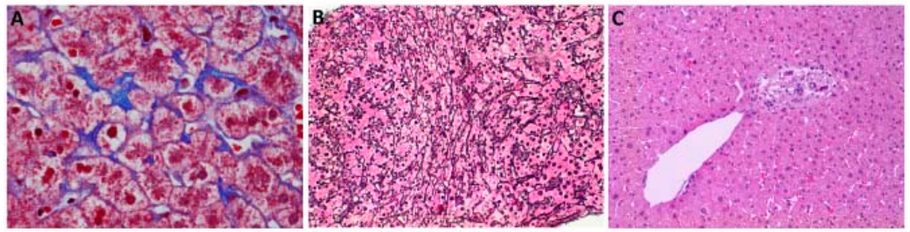

FIGURA 2. Hallazgos histopatológicos comunes de la HTPI. A) Tricrómico de Masson donde se pone de manifiesto la presencia de fibrosis perisinusoidal. B) Tinción de reticulina, mostrando la imagen típica de la hiperplasia nodular regenerativa. C) Tinción de hematoxilina-eosina, presencia de un espacio porta con vascularización anómala en el que existe una gran estructura vascular de pared fina, anormalmente dilatada, excéntrica. *Cortesía de la Dra. Miquel. Servicio de Anatomía Patológica. Hospital Clínic de Barcelona.*

hipertensión portal grave, el hallazgo en una biopsia hepática de mínimas alteraciones histológicas es interpretada en muchas ocasiones como un posible error de muestreo y el paciente es diagnosticado erróneamente de cirrosis criptogénica y no de HTPI [36]. Es de vital importancia realizar un diagnóstico correcto para poder establecer el pronóstico del paciente ya que la prevalencia de hipertensión portopulmonar, síndrome hepatopulmonar[37, 38] y hepatocarcinoma[39, 40] es más baja en pacientes con HTPI que en pacientes con cirrosis y la supervivencia es mejor[24, 41, 42] (c). Dado que la trombosis del eje esplenoportal es una complicación relativamente frecuente en la historia natural de la HTPI[43, 44], si estos pacientes son identificados cuando la trombosis ya está presente dificulta, y en ocasiones imposibilita, el diagnóstico de HTPI.

Por lo tanto, es de vital importancia encontrar características específicas que permitan diferenciar la HTPI de otras entidades que cursan con hipertensión portal (p.ej. la cirrosis o la TVPNC), o mejor aún la identificación de un marcador diagnóstico positivo de HTPI, permitiría un diagnóstico precoz de la enfermedad. Las mediciones de GPVH mediante cateterismo hepático y las mediciones de elastografía de transición mediante Fibroscan® son técnicas frecuentemente empleadas en la evaluación de pacientes con enfermedades hepáticas e hipertensión portal[3, 45]. Sin embargo, no existen apenas datos de estas técnicas en la HTPI y podrían tener un papel relevante en el diagnóstico de esta entidad.

En los últimos años ha habido un incremento en el uso de las tecnologías de última generación; como la metabolómica, transcriptómica o la proteómica; en el estudio de la fisiopatología, clasificación y pronóstico de diferentes enfermedades. La metabólomica es una tecnología precisa y rápida que proporciona una información valiosa sobre el fenotipo químico de un individuo mediante el análisis de un fluido o tejido biológico. Se han publicado múltiples aplicaciones clínicas de la metabolómica en el estudio de enfermedades hepáticas [46, 47 48 49 50, 51]. Por similitud, el análisis metabolómico de muestras de plasma y/o suero podrían ser herramientas útiles para el diagnóstico y la clasificación no invasiva de los pacientes con HTPI.

Los datos existentes sugieren que la HTPI puede ser la fase final común de entidades nosológicas diferentes, con origen fisiopatológico distinto y que por tanto podrían tener un pronóstico diferente y beneficiarse de pautas terapéuticas distintas.

Se han formulado varias hipótesis en relación con la etiología de la HTPI[25, 38, 52, 53]. Sin embargo dada la baja prevalencia de esta enfermedad y la dificultad en su

diagnóstico, los estudios son escasos e incluyen un número pequeño de pacientes. La mayoría de teorías patogénicas de la HTPI sugieren un daño inicial a nivel endotelial que acabaría desencadenando una serie de alteraciones estructurales y de la perfusión hepática como la venopatía portal obliterativa o el desarrollo de nódulos de hiperplasia nodular regenerativa que darían lugar al desarrollo de hipertensión portal. Las diferentes hipótesis descritas en la etiología de la HTPI se describen a continuación.

Trastornos protrombóticos. Esta hipótesis señala la existencia de una mayor frecuencia de trastornos protrombóticos adquiridos o hereditarios en la HTPI, que conllevaría a la formación repetida de microtrombos en las vénulas portales de pequeño calibre[28]. Esta venopatía portal obliterativa resultante podría ocasionar alteraciones de la arquitectura hepática con el subsecuente depósito de fibrosis y formación de vasos aberrantes. Asimismo, el aumento de la activación de las células estrelladas hepáticas por la trombina puede resultar en el desarrollo de la fibrosis perisinusoidal[54]. A favor de esta hipótesis, se ha demostrado que los trastornos protrombóticos pueden identificarse hasta en el 50% de los casos de una pequeña cohorte de pacientes europeos con HTPI[43]. Por otra parte, también se sabe que durante el curso de la enfermedad un número significativo de pacientes con HTPI desarrollan trombosis de la vena porta[24], [43, 44]. Sin embargo, la evidencia es escasa y el mecanismo patogénico exacto por el cual un estado de trombofilia podría inducir HTPI no se conoce.

Alteraciones inmunológicas. Se ha descrito también una asociación de la HTPI con enfermedades que tienen un claro origen autoinmune como el lupus eritematoso sistémico, la esclerosis sistémica, tiroiditis autoinmune, artritis reumatoide, escleroderma y enfermedad celíaca, entre otras[25, 55-57]. Asimismo diversos estudios han hallado una mayor prevalencia de ciertos autoanticuerpos en comparación con la población control (p. ej. anticuerpos anti-ADN, anticuerpos antinucleares, anticuerpos microsomales, anticuerpos antitiroglobulina)[26]. En la misma línea, otros estudios han mostrado otras alteraciones inmunológicas como una sobreexpresión de VCAM-1 (vascular cell adhesion molecule 1)[58], un aumento de los niveles séricos de IL-6 e IFN-γ[59] o un aumento significativo en el ratio Th1 y Th2 en linfocitos aislados de sangre periférica o de bazo de pacientes con HTPI en comparación con sujetos controles[60].

Infecciones. Las infecciones repetidas del tracto digestivo y la sepsis umbilical también han sido propuestas como posibles causas de HTPI, al ocasionar trombosis, esclerosis y obstrucción de las ramas portales de pequeño y mediano tamaño. Estudios recientes han relacionado la infección por el virus de la inmunodeficiencia humana (VIH) en la patogenia de esta enfermedad, fundamentalmente aquellos pacientes que habían recibido tratamiento con terapia antiviral, en especial con didanosina y estavudina[61, 62].

Alteraciones genéticas. Se ha sugerido que la genética podría tener un papel en la etiopatogenia de la HTPI. Se han observado lesiones similares a la HTPI en pacientes son Síndrome de Turner[63, 64] o Síndrome de Adams Oliver[65]. También, estudios familiares han sugerido una agregación familiar de la HTPI[66-68]. Sin embargo, estudios exhaustivos que incluyan un número amplio de familias con varios miembros afectos son escasos. Así mismo las lesiones vasculares observadas en el hígado de pacientes con HTPI también comparten varias similitudes morfológicas con las observadas en los pulmones de pacientes con hipertensión arterial pulmonar (HAP)[69-71]. Además, en ambas enfermedades se ha observado agregación familiar. Las mutaciones en el gen del receptor de la proteína morfogenética ósea 2 (BMPR2) constituyen un factor de riesgo conocido para la hipertensión arterial pulmonar idiopática (HAPI) y hipertensión arterial pulmonar familiar [72-75]. El gen BMPR2 codifica un receptor de membrana del factor de crecimiento transformante beta (TGF-β), que ocasiona un aumento en la actividad TGF-β que promueve la fibrogénesis[76, 77]. Hasta la fecha no se ha evaluado si las mutaciones del gen BMPR2 también pueden contribuir a la patogénesis de la HTPI.

Tóxicos y drogas. La exposición a arsénico ha sido considerada una causa de HTPI en India[78, 79]. Se han publicado casos de exposición a vinilio[80], sulfato de cobre, tratamiento con metrotrexato, 6-mercaptopurina, azatioprina, pednisolona en pacientes trasplantados renales[81], irradiación y quimioterapia en pacientes con tumor de Wilms [82].

La multiplicidad de teorías patogénicas en la HTPI es una prueba clara del desconocimiento actual de los mecanismos que llevan a su desarrollo. Por ello son

precisos más estudios que permitan mejorar el conocimiento de la etiopatogenia de la enfermedad. Esto permitiría desarrollar terapias dirigidas a interferir en los mecanismos fisiopatológicos de la misma y no sólo realizar tratamiento de las complicaciones, como se hace en la actualidad.

Síndrome de Budd-Chiari

El Síndrome de Budd-Chiari (SBC) es una causa rara de HTPNC que afecta fundamentalmente a adultos jóvenes y causa una alta morbilidad[11]. El SBC se define como el conjunto de manifestaciones derivadas de la obstrucción al flujo venoso hepático, independientemente del nivel de la obstrucción, que puede localizarse desde las vénulas hepáticas de pequeño tamaño hasta la entrada de la VCI en la aurícula derecha[8]. La forma de presentación es muy heterogénea y puede variar desde la ausencia de signos y síntomas, hasta un cuadro de fallo hepático fulminante con encefalopatía [8, 11]. No obstante la forma más frecuente de presentación es la descompensación en forma de ascitis [11]. El diagnóstico de SBC se realiza únicamente tras evidenciar, de forma inequívoca, la existencia de una obstrucción al flujo venoso hepático.

El SBC se puede clasificar en primario (causado por trombosis o membrana) o secundario (causado por invasión tumoral o por compresión por una lesión ocupante de espacio). En Occidente la causa más habitual de éste es la trombosis de las venas suprahepáticas. En Oriente y en el sur de África se debe más frecuentemente a la obstrucción de la VCI a nivel suprahepático, bien por trombosis de la misma o por presencia de membranas en la luz probablemente secuela de una trombosis previa. En más del 90% de los pacientes con SBC primario existe un factor protrombótico subyacente (fundamentalmente neoplasias mieloproliferativas) que, en alrededor de un 25% de casos pueden coexistir varios de ellos[6, 83, 84] (tabla 2). Por ello, siempre debe realizarse un estudio etiológico exhaustivo a pesar de que se ya haya detectado un posible factor.

Tabla 2. Factores etiológicos asociados a Síndrome de Budd-Chiari primario.

Enfermedades hereditarias	Prev	Enfermedades adquiridas	Prev
Mutación del factor V Leiden	6-32%	Neoplasias mieloproliferativas: Policitemia vera Trombocitemia esencial Mielofibrosis idiopática	28-49%
Mutación G20210A del gen de la protrombina	3-7%	Síndrome antifosfolípido	4-25%
Déficit de proteína C	0-30%	Hemoglobinuria paroxística nocturna	0-19%
Déficit de proteína S	0-20%	Enfermedad de Behçet	0-33%
Déficit de antitrombina	0-23%	Hiperhomocisteinemia	0-37%
Mutación gen C677T MTHFR	13-52%	Otros factores* - Embarazo - Anticonceptivos orales	0-15% 6-60%

Adaptada de Seijo et al. Capítulo Budd-Chiari Syndrome del libro Vascular liver deseases. Editores Deleve, Garcia-Tsao. Editorial Springer 201185. Referencias: 6, 11, 83, 84, 86.
(*) Es frecuente hallar otro factor protrombótico.
Abreviaturas: Prev: prevalencia; MTHFR: Metilenetetrahidrofolato reductasa.

La gravedad del SBC viene determinada por el número de venas afectadas, así como por la velocidad de instauración de la obstrucción. La tendencia natural de la enfermedad es presentar varios episodios de trombosis separados en el tiempo, cuyo daño sobre el parénquima hepático se va sumando. Entre los distintos episodios las áreas de parénquima con obstrucción del flujo venoso pueden desarrollar colaterales veno-venosas que descomprimen las zonas afectadas, de tal modo que dichos episodios pueden

pasar desapercibidos desde el punto de vista clínico hasta que el daño hepático es ya importante. En otros casos la enfermedad evoluciona de un modo brusco desde una forma leve a una grave debido a la retrombosis de lesiones antiguas o a la trombosis de la vena porta.

Los objetivos del tratamiento del SBC son: (1) reconocer y tratar la enfermedad o condición protrombótica subyacente, (2) mantener las venas suprahepáticas permeables mediante un adecuado tratamiento anticoagulante, (3) aliviar la congestión hepática para minimizar el impacto sobre la función hepática y los síntomas de la hipertensión portal y (4) tratar las complicaciones derivadas del desarrollo de hipertensión portal, fundamentalmente la ascitis y la prevención primaria o secundaria de la hemorragia por varices esofágicas.

El tratamiento de cada paciente debe individualizarse en función de: (1) la extensión de la trombosis, (2) la severidad de la disfunción hepática, (3) la existencia de complicaciones derivadas de la hipertensión portal y (4) la enfermedad protrombótica subyacente. Con esta finalidad hoy en día se recomienda un tratamiento progresivamente invasivo en el SBC (figura 3)[6, 8].

Todos los pacientes deben recibir tratamiento anticoagulante lo antes posible para prevenir la aparición de nuevos eventos trombóticos. Los pacientes en los que se identifique una estenosis corta, la angioplastia con o sin colocación de stents constituyen el mejor tratamiento. Aquellos pacientes que presentan un progresivo deterioro clínico (ascitis refractaria, signos de insuficiencia hepática u otras complicaciones derivadas de

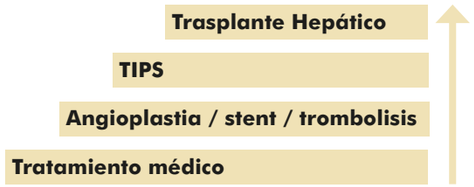

FIGURA 3. Tratamiento escalonado en el manejo del Síndrome de Budd-Chiari.
Adaptado de *Plessier et al.* Sem Liv Dis 2008. *Valla* Gut 2008 8, 87.

la hipertensión portal) necesitarán el restablecimiento del flujo venoso hepático. Aunque no existe ningún estudio aleatorizado y controlado que compare el TIPS frente a los shunts portosistémicos, el TIPS con prótesis recubiertas es una técnica segura y eficaz: evita la elevada morbi-mortalidad de la cirugía en estos pacientes y descomprime de un modo eficaz y duradero el territorio portal. En los casos en los que la descompresión no sea efectiva y la enfermedad progrese hacia la cirrosis hepática deben ser evaluados para trasplante hepático (TH). Así mismo el TH debe considerarse en todos aquellos pacientes con formas fulminantes.

Se han realizado diversos intentos de obtener parámetros o combinaciones de los mismos que puedan predecir el pronóstico de estos pacientes (tabla 3). El primer índice pronóstico en el SBC fue descrito en 1999 y mostró que la edad, la creatinina sérica, la ascitis refractaria y la puntuación de Child-Pugh fueron factores independientes que relacionados con el pronóstico[88]. Un índice revisado por el mismo grupo incorporó a las variables previas la presencia de características que indican una lesión aguda superpuesta a lesiones crónicas como indicadores pronósticos independientes[89]. Sin embargo, esta nueva variable añadía una mayor complejidad y subjetividad al índice pronóstico. Posteriormente, *Murad et al.*, propuso el índice de Rotterdam, que permitía la estratificación de los pacientes en tres grupos de riesgo, con una supervivencia estimada a los 5 años de 89%, 74% y 42% respectivamente[90]. Sin embargo estos índices pronósticos tienen limitaciones que condicionan su uso en la práctica clínica habitual actual: los estudios son retrospectivos, todos estos índices pronósticos se desarrollaron en la época previa a la implantación del TIPS dentro del tratamiento del SBC y dado que es una enfermedad rara los pacientes han sido reclutados durante largo de periodo de tiempo en el que la estrategia terapéutica ha sido diferente. Recientemente se ha desarrollado un nuevo índice pronóstico en pacientes con SBC tratados con TIPS llamado el BCS-TIPS PI score[91]. Una puntuación superior a 7 puntos de este score predice la mortalidad libre de trasplante tras 1 año del TIPS con una sensibilidad del 58% y una especificidad del 99%[91]. La limitación de este índice pronóstico es que sólo es aplicable en pacientes con SBC que hayan sido sometidos a TIPS. Son necesarios por tanto nuevos índices pronósticos desarrollados en cohortes contemporáneas de SBC para identificar pacientes con mal pronóstico que se beneficiarían de un tratamiento más invasivo.

Tabla 3. Índices pronósticos publicados en el Síndrome de Budd-Chiari

Autor	Variables	Outcome
Zeitoun et al. Hepatol 1999 [88]	Ascitis, score de Child-Pugh, edad, creatinina sérica	Supervivencia
Langlet et al. J Hepatol 2003 [89]	Ascitis, score de Child-Pugh, edad, creatinina sérica, tipo de BCS*	Supervivencia
Murad et al. Hepatol 2004 [90]	Encefalopatía, ascitis, tiempo de protrombina, bilirrubina	Supervivencia
Garcia-Pagan et al. Gastroenterol 2008 [91]	Bilirrubina, edad, INR	Supervivencia libre de trasplante tras TIPS

* BCS tipo I se caracteriza por la ausencia de características crónicas; el BCS tipo II por la presencia de al menos una de las características crónicas en ausencia de rasgos agudos, y el tipo III cuando al menos hay una características aguda y una crónica. Se definen como características agudas: dolor abdominal agudo en el cuadrante superior derecho; ALT igual o superior a cinco veces el límite superior de la normalidad, la pérdida de células del hígado en la biopsia hepática cuando esté disponible; y como características crónicas: hospitalización previa por síntomas inexplicables que revierten espontáneamente y que posteriormente se relacionaban con el SBC (por ejemplo, dolor agudo en el cuadrante superior derecho, ascitis, ictericia, función hepática anormal); esplenomegalia; complejo atrofia / hipertrofia; fibrosis centrolobulillar o cirrosis en la biopsia hepática cuando esté disponible.

Justificación, hipótesis y objetivos

JUSTIFICACIÓN, HIPÓTESIS Y OBJETIVOS

Justificación y objetivos generales:

El Síndrome de Budd-Chiari (SBC) y la hipertensión portal idiopática (HTPI) son enfermedades hepáticas que causan hipertensión portal[2, 3]. Los avances en el conocimiento de estas enfermedades se ven obstaculizados por el hecho de ser enfermedades raras.

El conocimiento de la HTPI se ve dificultado también por el hecho de que no existe ninguna prueba o test que permitan establecer el diagnóstico de forma definitiva[25]. Todo esto hace que el proceso diagnóstico de estos pacientes sea largo, costoso, y que además incluya la realización de exploraciones invasivas, como la biopsia hepática. La HTPI puede causar complicaciones relacionadas con la hipertensión portal, como la hemorragia por varices o ascitis en ausencia de cirrosis u otras causas de enfermedad hepática[28]. Esta similitud clínica con la cirrosis es una de las razones por las que la HTPI suele ser erróneamente diagnosticada de cirrosis criptogénica[27, 38 36]. Es, por tanto, de vital importancia establecer características específicas que permitan diferenciar la HTPI de otras entidades que cursan con hipertensión portal, como la cirrosis, o incluso mejor, la identificación de un marcador diagnóstico positivo y no invasivo de HTPI.

La etiología de la HTPI no se conoce. Se han descrito diferentes teorías que implican trastornos en la respuesta inmune, infecciones, trastornos protrombóticos, agentes con capacidad tóxica sobre la célula endotelial sinusoidal hepática o factores genéticos en la etiopatogenia de esta enfermedad[25, 66]. Las alteraciones histológicas hepáticas de los pacientes con HTPI son similares a las presentes en los pulmones de pacientes con hipertensión pulmonar arterial (HAP)[26, 69, 71]. Las mutaciones en el gen del receptor de la proteína morfogenética ósea 2 (BMPR2) se han asociado con el desarrollo de HAP (idiopática y familiar)[75]. Sin embargo hasta la fecha no se ha evaluado la posible implicación de esta alteración genética en el desarrollo de la HTPI.

El SBC es otra de las causas raras de HTPNC. El SBC está ocasionado por la obstrucción al flujo venoso hepático y causa de una elevada morbi-mortalidad[6-8]. El conocimiento actual sobre pronóstico de pacientes con SBC se basa fundamentalmente en estudios antiguos, retrospectivos y dada la baja incidencia de la enfermedad, incluyen un escaso número de pacientes que además han sido incluidos en un largo periodo de

tiempo, en el que el manejo terapéutico ha sido muy heterogéneo. Esto conlleva que existan pocos datos sobre el pronóstico actual a largo plazo de estos pacientes y los factores asociados con él, lo que tendría un gran impacto en el tipo de tratamiento a realizar. Recientemente, y como consecuencia, de una iniciativa multicéntrica en la que participaron 9 países europeos se pudieron reclutar y seguir un número importante de pacientes incidentes con SBC[11]. No obstante, el seguimiento de los pacientes era corto y no se pudo evaluar el pronóstico a largo plazo ni desarrollar modelos pronósticos.

Justificación y objetivos específicos:

Los trabajos de investigación de la presente tesis están orientados a ampliar el conocimiento de la etiopatogenia de la HTPI y mejorar su diagnóstico (mediante estudios hemodinámicos, de elastografía de transición y marcadores metabolómicos). Así mismo, también pretende ampliar el conocimiento sobre el pronóstico a largo plazo de pacientes con SBC.

A continuación se detalla la justificación, hipótesis y objetivos de cada uno de los subestudios que componen esta tesis.

Estudio 1. Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension

Los pacientes con sospecha de HTPI deben someterse a procedimientos invasivos y de riesgo, como una biopsia hepática, con el fin de excluir la presencia de cirrosis u otras enfermedades que causan de hipertensión portal. Además, los hallazgos de la biopsia hepática no son patognomónicos por lo que su diagnóstico se hace por exclusión de otras entidades. Por todo ello, la falta de una prueba diagnóstica positiva específica hace que el diagnóstico de la HTPI sea un desafío[27, 38]. Son necesarias por lo tanto, nuevas herramientas diagnósticas y la identificación de un patrón clínico que facilite el diagnóstico de la HTPI.

El GPVH y la mediciones de rigidez hepática mediante ET (Fibroscan ®) son técnicas ampliamente empleadas en la evaluación de pacientes con enfermedad hepática crónica y de pacientes con hipertensión portal[2, 45, 92]. En pacientes con cirrosis hepática, el GPVH es la técnica estándar oro para evaluar la hipertensión portal [2, 92]. Del mismo modo, la ET

permite evaluar el grado de fibrosis y estimar la severidad de la hipertensión portal[45, 93]. Sin embargo, los datos de las mediciones de GPVH, y más aún de ET, en la HTPI son muy escasos. El posible impacto de estas herramientas en el diagnóstico diferencial de la HTPI con otras causas de hipertensión portal no se ha investigado hasta ahora.

Con estas premisas la *hipótesis* del presente estudio fue que los pacientes con HTPI tienen un patrón hemodinámico y de ET característico y que permite su diagnóstico diferencial con otras causas de hipertensión portal.

Por tanto, el *objetivo* del estudio fue evaluar el papel de la hemodinámica hepática y la elastografía de transición en el diagnóstico diferencial de la HTPI, en particular en su diferenciación con la hipertensión portal de origen cirrótico.

Estudio 2. Metabolomics discloses potential biomarkers for the non-invasive diagnosis of idiopathic portal hypertension.

Como mencionamos en el punto anterior es de vital importancia identificar nuevas técnicas que permitan el diagnóstico de la HTPI. Dado que los pacientes deben someterse a pruebas invasivas en su proceso diagnóstico sería de vital importancia identificar un marcador diagnóstico no invasivo de la HTPI.

La metabolómica es una tecnología de alto rendimiento reciente que permite medir simultáneamente miles de metabolitos en diversas muestras como fluidos biológicos (suero, plasma, orina, líquido cefelorraquídeo, etc) o tejidos en un corto período de tiempo. Esta tecnología es capaz de analizar semi-cuantitativamente una amplia gama de especies moleculares, tales como lípidos, ácidos biliares y aminoácidos. La metabolómica tiene múltiples aplicaciones en la investigación clínica y básica. Además en los últimos años se han publicado varios estudios sobre posibles aplicaciones de la metabolómica en el estudio de diversas enfermedades hepáticas: discriminación entre diferentes formas o gravedad de las enfermedades del hígado[46, 47], diferenciación de la cirrosis compensada de la descompensada[48], o como una herramienta prometedora para el diagnóstico de encefalopatía hepática mínima en la cirrosis[49]. Además, un estudio reciente ha identificado un perfil metabólico en suero capaz de distinguir de forma no invasiva la esteatohepatitis no alcohólica de pacientes con esteatosis simple [50, 51].

En base a los antecedentes descritos, la *hipótesis* del presente estudio fue que los pacientes con HTPI presentan un patrón metabolómico específico en plasma. Dicho pa-

trón metabolómico permite el diagnóstico no invasivo de la enfermedad y su diagnóstico diferencial con otras causas de hipertensión portal como la cirrosis.

Por tanto, el *objetivo* específico de este estudio es determinar el papel de los perfiles metabolómicos en el diagnóstico no invasivo de los pacientes con HTPI.

Estudio 3. Bone morphogenetic protein receptor 2 in patients with idiopathic portal hypertension.

Los pacientes con HTPI presentan lesiones histológicas en el hígado similares a las observadas en los pulmones de pacientes con hipertensión pulmonar arterial (HAP)[26, 69, 71] : ambas entidades causan una proliferación de la capa íntima con fibrosis e hipertrofia muscular de la capa media de vasos que lleva a una oclusión de la luz y a una mayor resistencia al flujo [69-71]. Además, se ha observado agregación familiar en ambas enfermedades. Las mutaciones del gen BMPR2 constituyen uno de los mayores factores de riesgo para la HAP: representan hasta el 25% de las HAPI y hasta el 80% de las formas familiares de HAP [72-75]. El gen BMPR2 codifica un receptor de membrana celular del TGF-β [94]. Todas las mutaciones conocidas en la actualidad causan una pérdida de la función del receptor. Esto ocasiona un aumento en la actividad TGF-β que promueve la fibrogénesis, la hiperplasia de la capa íntima y el crecimiento del músculo liso [76, 77]. Hasta la fecha no se ha explorado la prevalencia de las mutaciones del gen BMPR2 en la HTPI y si éstas pueden tener un papel en la etiopatogenia de la misma.

La *hipótesis* del estudio fue que la presencia de mutaciones del gen de la BMPR2 es un factor de riesgo para el desarrollo de la enfermedad.

Por tanto el *objetivo* del estudio fue analizar la prevalencia de las mutaciones del gen de la BMPR2 en la HTPI y valorar su implicación en la etiopatogenia de la enfermedad.

Estudio 4. Good long-term outcome of Budd-Chiari syndrome with a step-wise management.

El SBC es una causa rara de HTPNC provocada por la obstrucción al flujo venoso hepático que causa una elevada mortalidad [6-8]. Sin embargo dada la baja incidencia de la enfermedad, la mayoría de los estudios publicados hasta la actualidad son retrospectivos, incluyen un escaso número de pacientes y éstos además han sido incluidos en un largo periodo de tiempo, por lo que el manejo terapéutico es muy heterogéneo. Un estudio reciente,

prospectivo, multicéntrico ha permitido reclutar y seguir un número importante de pacientes con SBC incidentes diagnosticados en un corto periodo de tiempo (2 años)[11]. No obstante, el seguimiento de los pacientes era corto (seguimiento mediano de 17 meses) y no se pudo evaluar el pronóstico a largo plazo ni desarrollar modelos pronósticos. Por lo tanto existen pocos datos sobre el pronóstico a largo plazo de estos pacientes y los factores asociados con él. Esto dificulta el identificar pacientes con mal pronóstico que podrían beneficiarse de un tratamiento más invasivo y por tanto mejorar su supervivencia.

La *hipótesis* del presente estudio fue que el conocer el pronóstico a largo plazo de pacientes con SBC permite identificar variables clínicas significativamente asociadas a mal pronóstico, lo que facilita una mejor estratificación de los pacientes y la aplicación de terapias más invasivas en pacientes de alto riesgo.

El *objetivo* del presente estudio es estudiar la supervivencia a largo plazo de los pacientes con SBC, y seguidamente determinar los factores pronósticos y predictivos de respuesta a largo plazo a los diferentes tratamientos disponibles.

Aspectos éticos

Los estudios clínicos se han desarrollado siguiendo los principios expresados en la Declaración de Helsinki. Se ha obtenido la autorización del comité de ética del Hospital Clínic para todos los subestudios que conforman esta tesis (números de registros: 2009/4479 para los subestudios de la HTPI y 2003/1442 para el estudio sobre el Síndrome de Budd-Chiari).

Los pacientes han firmado un consentimiento informado en el momento de la extracción de la muestra de sangre que permite su uso con fines de investigación en proyectos que hayan sido aprobados por el Comité de Ética. Las muestras biológicas han sido almacenadas a -80ºC en el Biobanc del Hospital Clinic-IDIBAPS, en Barcelona (www.clinicbiobanc.org) hasta su uso en los proyectos descritos. El Biobanc del Hospital Clinic-IDIBAPS garantiza el cumplimiento de la normativa sobre actividades con las muestras biológicas, con conformidad con la nueva Ley de Investigación Biomédica y la estricta legislación en vigor referente a la protección de datos.

ESTUDIO 1

Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension

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Liver, pancreas and biliary tract

Role of hepatic vein catheterisation and transient elastography in the diagnosis o idiopathic portal hypertension

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ABSTRACT

Background: Idiopathic portal hypertension is a rare cause of portal hypertension, frequently misdiag nosed as cryptogenic cirrhosis. This study evaluates specific findings at hepatic vein catheterisation c liver stiffness in idiopathic portal hypertension.
Methods: 39 cases of idiopathic portal hypertension patients were retrospectively reviewed. Hepatic vei catheterisation and liver stiffness measurements were compared to matched patients with cirrhosis an portal hypertension, and non-cirrhotic portal vein thrombosis, included as controls.
Results: Hepatic vein-to-vein communications were found in 49% idiopathic portal hypertension patient precluding adequate hepatic venous pressure gradient measurements in 12. In the remaining 2 patients, mean hepatic venous pressure gradient (HVPG) was 7.1 ± 3.1 mmHg. Only 5 patients ha HVPG ≥ 10 mmHg. HVPG was markedly lower than in cirrhosis (17 ± 3 mmHg, $p < 0.001$). Mean liver stifl ness in idiopathic portal hypertension was 8.4 ± 3.3 kPa; significantly higher than in non-cirrhotic porta vein thrombosis (6.4 ± 2.2 kPa, $p = 0.009$), but lower than in cirrhosis (40.9 ± 20.5 kPa, $p = 0.005$). Only idiopathic portal hypertension patients had liver stiffness >13.6 kPa.
Conclusions: Patients with idiopathic portal hypertension frequently have hepatic vein-to-vein commu nications and, despite unequivocal signs of portal hypertension, HVPG and liver stiffness values muc lower than the cut-off for clinical significant portal hypertension in cirrhosis. These findings oblige t formally rule-out idiopathic portal hypertension in the presence of signs of portal hypertension.

1. Introduction

Idiopathic portal hypertension (IPH) is a rare cause of intrahepatic presinusoidal portal hypertension (PH) of unknown aetiology [1–3], characterised by the absence of portal vein thrombosis (PVT), a wide spectrum of non-specific changes at liver histology, and the absence of cirrhosis or of other specific liver diseases [4,5].

IPH may cause PH related complications, such as variceal bleeding or ascites [1,6]. The lack of specific tests to diagnose IPH makes excluding other causes of PH mandatory. As a consequence IPH is frequently misdiagnosed as cryptogenic cirrhosis. In addition, the finding of minimal changes at liver biopsy may be interpreted as sampling error. On the other hand, since PVT is a frequent event in the natural history of IPH [2,7], patients first studied once PVT has developed could be erroneously identified as idiopathic non cirrhotic PVT.

Hepatic venous pressure gradient (HVPG) and liver stiffnes (LS) measurements by transient elastography (TE; Fibroscan®) ar increasingly used in the evaluation of patients with chronic live disease and PH [8–10]. In patients with cirrhosis HVPG is the gol standard for evaluating PH [8]. Similarly, LS allows to evaluate th degree of fibrosis and to estimate the severity of PH. However, dat on HVPG measurements in IPH are scarce, particularly in easter forms and never considering the possible influence of the prope occlusion of the hepatic vein. Similarly, there is only one study c LS performed in a small group of patients of human immunode ficiency virus (HIV)-related IPH [3]. The possible impact of thes tools in the differential diagnosis of IPH from other causes of PI has not been investigated so far.

The aim of our study was to characterise hepatic haemodynam ics and LS in a large series of patients with IPH. In addition, we aime at evaluating whether these findings may be of help to diagnose o suspect the presence of IPH.

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2. Methods

2.1. Patients

All consecutive patients with unequivocal IPH referred to our unit from December 1989 to October 2010 were eligible for inclusion in the study. Since 1989, hepatic vein catheterisation with transjugular liver biopsy is included per protocol in the routine work-up of patients with non-cirrhotic PH. Since 2006, TE is included in the routine evaluation of incidental patients and of those previously diagnosed but in active follow-up.

Diagnosis of IPH was based on the following previously reported criteria [10]: presence of unequivocal signs of PH (gastroesophageal varices, ascites, and/or splenomegaly); absence of cirrhosis, advanced fibrosis or other causes of chronic liver diseases by appropriate serological, biochemical tests and liver biopsy, performed in all patients; absence of thrombosis of hepatic or portal vein at imaging studies performed at diagnosis.

Hepatic biopsies were performed by the transjugular or percutaneous route and were re-evaluated for the purpose of the study by one experienced pathologist (RM). Hepatic biopsies for diagnosis of IPH were transjugular in 24 cases and percutaneous in 15. Six patients had more than 1 biopsy. Only specimens containing ≥6 complete portal tracts were considered suitable for diagnosis [11]. Those patients with liver biopsies including <6 portal tracts were not included in the study because the diagnosis of IPH was not definite. Briefly, the specimens were formalin-fixed, embedded in paraffin and cut at 4 μm sections. Haematoxylin–eosin, Masson's trichrome and reticulin stainings were performed. Patients with HIV infection were not included in the study if liver biopsy showed other findings different from those described in patients with IPH. Based on the previously described work-up, only patients with unequivocal diagnosis of IPH were finally enrolled and all clinical, haemodynamic and elastography data were retrospectively collected from clinical records.

A group of patients with chronic non-cirrhotic non-tumoral portal vein thrombosis (NCPVT) and a group of patients with cirrhosis were retrospectively selected from our database at the hepatic haemodynamic laboratory. Consecutive patients matched by gender, presence of varices, Child-Pugh score and use of non-selective beta-blockers (NSBB), seen during the same period of time, were selected and included as controls. NCPVT was diagnosed according to criteria previously described [12]. All patients had a liver biopsy showing normal histological appearance or minimal alterations (mild steatosis (>10%) in 4, focal sinusoidal dilatation in 2 and non-specific minimal lobular inflammatory infiltrate in 2). NCPVT was selected as control group because it is a cause of pre-hepatic PH and has normal or slightly normal liver histology.

Patients with cirrhosis included were diagnosed by liver biopsy. In these patients thrombosis of the hepatic veins or of the portal venous axis was excluded by US-Doppler.

Patients with Budd–Chiari syndrome, transjugular intrahepatic porto-systemic shunt (TIPS) or surgical shunts were excluded. Epidemiological, clinical, laboratory, haemodynamic and TE data were collected and recorded in a predesigned case report form. The protocol was reviewed and approved by the ethical committee at our institution.

2.2. Haemodynamic study

Briefly, after an overnight fast and under local anaesthesia and mild conscious sedation, an 8F venous catheter introducer was placed in the right internal jugular vein using the Seldinger technique. Under fluoroscopy control, a 7F balloon-tipped catheter (Edwards Laboratory, Los Angeles, CA, USA) was guided into the main right or medium hepatic vein to measure occluded (wedged: WHVP) and free (FHVP) hepatic venous pressures as previously described [8]. Hepatic vein-to-vein communications (HVVC) were explored by the injection of iodine contrast medium while the balloon was occluding the hepatic vein. Images were recorded at high speed to obtain hepatic venography. Portal pressure gradient was estimated as the difference between WHVP and FHVP (or HVPG) [8]. After HVPG measurements, in those patients evaluated after year 2006, cardiopulmonary pressures and cardiac output were measured by thermal dilution (Swan-Ganz cathether; Edwards Laboratory, Los Angeles, CA, USA). Hyperdynamic circulation was defined as an elevated cardiac index (CI > 4.0 l/min/m^2) with low vascular systemic resistances (SVR < 900 dyne s cm^{-5}) and pulmonary hypertension as mean PAP > 25 mmHg with PVR > 240 dyne s cm^{-5}.

2.3. Transient elastography study

Transient elastography was performed using the FibroScan® equipment (Echosens, Paris, France). Measurements of LS were performed in fasting conditions on the right lobe of the liver by two highly experienced staff nurses. Ten successful measurements were performed on each patient. Only liver stiffness measurements with a success rate of at least 60% and an interquartile range lower than 30% were considered reliable. Results are expressed in kilopascal (kPa), and median values are representative of liver stiffness.

2.4. Statistical analysis

Data handling and analysis were performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL). Quantitative variables are expressed as mean ± SD, and median and range, and qualitative variables as absolute and relative frequencies. Data were tested for normality using the Kolmogorov–Smirnoff test. Categorical variables were compared using the chi square test. Continuous variables were compared with *t*-Student test or one-way ANOVA followed by preplanned contrast analysis to compare IPH with NCPVT or cirrhosis when necessary. Correlation was performed using Pearson's coefficient. Significance was established at $p < 0.05$.

3. Results

3.1. Characteristics of patients

Sixty-one patients were diagnosed of IPH at our unit during the period of time of the study. Twenty-two patients were excluded (9 due to insufficient clinical data, 8 due to PVT at the haemodynamic study, 3 due to non-consent for the haemodynamic study and 2 due to a previous surgical shunt). Moreover, patients with inadequate liver biopsy were not included in the study. Therefore, finally 39 patients with unequivocal IPH were included. Clinical characteristics of patients are summarised in Table 1. At liver biopsies obliterative portal venopathy was observed in 15% of patients, mild regenerative nodular hyperplasia in 43% and minor/mild sinusoidal dilatation in 51% of patients.

All patients had signs of PH. Thirty six patients (92%) have gastroesophageal varices. The 3 patients without varices had marked splenomegaly and thrombocytopenia and one had also ascites. Eleven patients had ascites that was easily controlled and in 4 patients ascites overlap with a variceal bleeding episode. Nine patients (23%) had HIV infection treated with highly active antiretroviral therapy (viral load < 50 copies/ml), none had viral hepatitis C infection, and 3 patients (8%) had past viral hepatitis B infection.

Clinical characteristics of patients with NCPVT are summarised in Table 1. The aetiology was a haematological disease and/or procoagulant disorder in 16 (41%), a local factor in 9 (23%) and idiopathic in 14 (36%). All patients had unequivocal signs of PH

Table 1
Main clinical characteristics of the patients included in the study.

Variables	IPH $n = 39$	NCPVT $n = 39$	Cirrhosis $n = 39$
Age[a] (years)	41 ± 19[##]	48 ± 14	60 ± 11
Gender[b] (male)	27 (70%)	26 (66%)	27 (70%)
Signs of portal hypertension at study[b]			
Varices	36 (92%)	39 (100%)	36 (92%)
Variceal bleeding	7 (18%)	9 (23%)	2 (5%)
Ascites	11 (28%)	5 (13%)	6 (15%)
Patients receiving NSBB	10/39 (26%)	14/39 (36%)	10/39 (26%)
Laboratory data			
Bilirubin (mg/dl)	1.3 ± 1.1	1.1 ± 1.2	1.4 ± 0.9
Albumin (g/L)	39 ± 4.6[#]	40 ± 4.3	37 ± 4.8
Creatinine (mg/dl)	0.96 ± 0.65	0.91 ± 0.28	0.87 ± 0.28
Platelet count (×10^9/L)	107 ± 87.89[*]	192 ± 119.10	100 ± 42.01
Platelet count < 150 × 10^9/L (%)	80%[*]	44%	82%
Child-Pugh score[a]	5.7 ± 1.04	5.5 ± 0.9	5.8 ± 1.1
Child-Pugh class[b]	A 29 (74%)	A 33 (85%)	A 29 (74%)
	B 10 (26%)	B 6 (15%)	B 10 (26%)

Categorical variables were compared using the chi square test. Continuous variables were compared with *t*-Student test or one-way ANOVA followed by preplanned contrast analysis to compare IPH with NCPVT or cirrhosis when necessary. Abbreviations: IPH, idiopathic portal hypertension; NCPVT, non-cirrhotic non-tumoral portal vein thrombosis; NSBB, non-selective beta-blockers.

[a] Mean ± SD.
[b] *n* (%).
[*] Significance was as follow: for IPH vs NCPVT, $p \leq 0.001$.
[#] Significance was as follow: for IPH vs cirrhosis, $p \leq 0.05$.
[##] Significance was as follow: for IPH vs cirrhosis, $p \leq 0.001$.

In NCPVT patients, platelet count was significantly higher than in patients with IPH ($p = 0.001$). This difference was no longer present if 15 patients with NCPVT and myeloproliferative neoplasia (MPN) were excluded from the analysis. Only one patient had past viral hepatitis C infection (undetectable HCV RNA), but liver biopsy showed no fibrosis and HVPG was normal.

The main characteristics of patients with cirrhosis and PH are summarised in Table 1. Aetiology was alcohol in 13 (33%), hepatitis C in 20 (51%), alcohol and hepatitis C in 3 (8%), NASH in 1 and cryptogenic in 2. All biopsies were performed in a stable situation of the disease and alcoholic hepatitis was ruled out in patients with alcoholic cirrhosis. As expected, patients with IPH were significantly younger to patients with cirrhosis.

3.2. Hepatic haemodynamics

Hepatic vein-to-vein communications (HVVC) were present at hepatic venography in 19 patients with IPH (49%) (Fig. 1). These communications prevented to obtain a proper WHVP in 12 of these cases despite trying different veins and positions, while a proper occlusion of the hepatic vein could be achieved in the remaining 7 patients by advancing the catheter to a more distal position of the vein not showing communications. Thus, adequate WHVP measurements were obtained in 27/39 patients with a mean HVPG of 7.0 ± 3.0 mmHg (Table 2 and Fig. 2A). HVPG was normal (≤5 mmHg) in 6 patients, slightly increased (5–10 mmHg) in 16, and ≥10 mmHg in the remaining 5 patients. In the 12 patients with inappropriate occlusion of the hepatic vein, WHVP was slightly lower, although not reaching statistical significance ($p = 0.10$) than that of patients with proper hepatic vein occlusion (Table 2).

In 4 patients with IPH (mean HVPG = 6 ± 2 mmHg) direct portal pressure measurements were performed by transhepatic portal vein puncture and showed a mean porto-cava gradient of 15.5 ± 4.5 mmHg, demonstrating a marked presinusoidal component of portal hypertension.

There were no differences in HVPG when patients were stratified for ascites ($p = 0.356$; only 1 patient with ascites had HVPG ≥ 10 mmHg) or previous variceal bleeding ($p = 0.418$; only 1 patient had HVPG ≥ 10 mmHg). Also there were no significant correlations between HVPG and serum bilirubin, serum albumin, Child-Pugh or MELD scores (data not shown). In addition there were no significant differences in HVPG according to HIV infection status (8.2 ± 3.2 mmHg in HIV-positive vs 6.8 ± 3.1 mmHg in HIV-negative; $p = 0.329$) and hepatic vein pressures were similar if HIV-positive patients were excluded from the analysis (data not shown).

HVVC were present in 12/39 patients with NCPVT (31%), similar than in IPH ($p = 0.16$). These communications prevented to obtain a proper WHVP in 6 cases. Thus, an adequate wedged measurement was finally obtained in 33 NCPVT patients showing a mean HVPG of 3.5 ± 2.0 mmHg. HVPG was significantly higher ($p < 0.001$) in IPH patients than in those with NCPVT (Table 2 and Fig. 2A). None of the patients with cirrhosis and PH of this series had HVVC. Their mean HVPG was 17.0 ± 3.0 mmHg, significantly higher than that observed in IPH or NCPVT patients (Table 2 and Fig. 2A). A

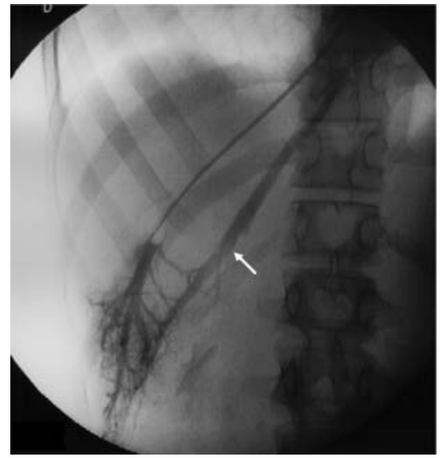

Fig. 1. Hepatic vein venography performed during transjugular hepatic vein catheterisation of a patient with idiopathic portal hypertension, showing a large hepatic vein to vein communication (HVVC) (arrow).

Table 2
Hepatic haemodynamic and transient elastography measurements in patients included in the study.

Variables	IPH		NCPVT		Cirrhosis	
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*
WHVP (mmHg)	14.0 ± 3.0**,##	27	11 ± 3.0	33	25.5 ± 4.5	39
WHVP♦ (mmHg)	11.5 ± 2.5	12	9.0 ± 3.5	6	–	–
FHVP (mmHg)	6.5 ± 2.0#	27	7.5 ± 3.0	33	8.5 ± 4.0	39
HVPG (mmHg)	7.0 ± 3.0**,##	27	3.5 ± 2.0	33	17.0 ± 3.0	39
LS (kPa)	8.4 ± 3.3*,##	30	6.4 ± 2.2	24	40.9 ± 20.5	39
LS (kPa)†	7.8 ± 3.6##	20	6.4 ± 2.2	24	40.9 ± 20.5	39

Results are expressed in mean ± SD. Categorical variables were compared using the chi square test. Continuous variables were compared with *t*-Student test or one-wa ANOVA followed by preplanned contrast analysis to compare IPH with NCPVT or cirrhosis when necessary. Abbreviations: IPH, idiopathic portal hypertension; NCPVT, non cirrhotic non-tumoral portal vein thrombosis; WHVP, wedged hepatic vein pressure in patients with adequate occlusion of the hepatic vein; WHVP♦, wedged hepatic vei pressure in patients with inadequate occlusion of the hepatic vein; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient in patients with adequat WHVP; LS, liver stiffness; LS (kPa)†, liver stiffness performed the same day of HVPG measurements.

* Significance was as follow: for IPH vs NCPVT, $p \leq 0.05$.
** Significance was as follow: for IPH vs NCPVT, $p \leq 0.001$.
\# Significance was as follow: for IPH vs cirrhosis, $p \leq 0.05$.
\## Significance was as follow: for IPH vs cirrhosis, $p \leq 0.001$.

expected, all patients had HVPG > 10 mmHg. Similar results were found removing IPH patients with VIH infection from de analysis (data not shown).

3.3. Systemic haemodynamics

Patients with IPH had mean systemic and pulmonary haemodynamics within normal range (Table 3). Indeed only 6 patients showed mild hyperdynamic circulation. Interestingly, no significant differences in systemic and pulmonary haemodynamics or in the number of patients with hyperdynamic circulation were observed between patients with IPH, NCPVT or cirrhosis matched by liver function (Table 3). Similar results were observed when only patients not receiving NSBB were evaluated (data not shown).

One patient with IPH had mild pulmonary hypertension, but none in the cohorts of patients with NCPVT or with cirrhosis.

3.4. Transient elastography

In 5 patients with IPH LS determinations were not reliable because of ascites ($n = 3$) and obesity/overweight ($n = 2$). In 4 additional patients no LS was performed because of death ($n = 2$), lost to follow-up ($n = 1$) or OLT ($n = 1$) before 2006. In the 30 patients with successful determinations, despite the presence of clear signs of PH, mean LS was 8.4 ± 3.3 kPa. In 14 patients (46.6%) LS was <7.8 kPa, the defined threshold for significant fibrosis [13]. Only 2 patients presented LS values >13.6 kPa (16.3 and 18.5 kPa), minimal threshold for clinical significant PH (CSPH) in cirrhosi [14]. Also no significant differences in LS measurements wer found between patients with and without HIV infection (7.3 ± 1.(vs 8.9 ± 3.7 kPa; $p = 0.200$) and LS measurements were similar i HIV-positive patients were excluded from the analysis (data no shown).

In 20 patients (66%), LS measurements were performed the sam day of the haemodynamic study while in 10 (33%) was performe a median of 38 months after (3–185). Values of LS were not sig nificantly different between both groups (7.8 ± 3.6 vs 9.7 ± 42 kPa $p = 0.14$). Nine patients had ≥2 LS measurements during follow up, with a median time between first and last measurement o 38 months (2–59). No significant changes in LS were observed ove time (9.0 ± 1.9 vs 9.6 ± 2.5 kPa, $p = 0.27$). Therefore, all first 30 L measurements were considered for comparison with HVPG mea surements. Among IPH patients, there was no correlation betwee LS and HVPG ($p = 0.365$) or between LS and previous varicea bleeding ($p = 0.79$). Similar results were found considering onl LS measurements performed the same day of HVPG ($p = 0.25$ an $p = 0.79$, respectively) (Table 2).

LS measurements were available in 24 (62%) NCPVT patients In 7 patients no successful determinations were achieved becaus of ascites ($n = 3$) and obesity/overweight ($n = 4$). In the remain ing 8 patients LS was not performed because of death ($n = 1$) o lost to follow-up ($n = 7$) before 2006. Mean LS was 6.4 ± 2.2 kPa Patients with NCPVT had significantly lower LS than patients witl IPH (Table 2 and Fig. 2B). As expected, LS was markedly increase

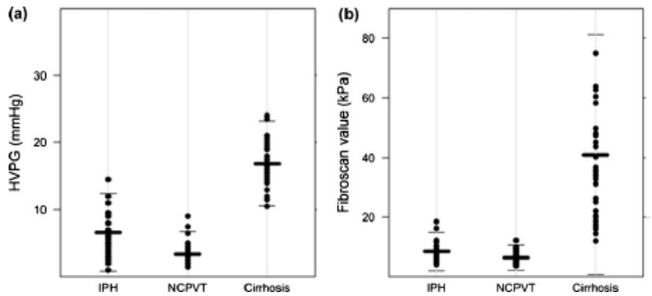

Fig. 2. Individual and mean values of hepatic vein pressure gradient HVPG (A) and of liver stiffness (LS) (B) in patients with idiopathic portal hypertension (IPH), non-cirrhoti portal vein thrombosis (NCPVT) and cirrhosis.

Table 3
Systemic and cardiopulmonary haemodynamic of patients included in the study.

Variables	IPH *n* = 27	NCPVT*n* = 34	Cirrhosis*n* = 39	Normal value
MAP (mmHg)[a]	86 ± 11	88 ± 13	89 ± 11	80–95
HR (bpm)[a]	69 ± 11	72 ± 14	73 ± 13	60–95
CO (L min^{-1})	6.8 ± 1.9	6.95 ± 2.1	6.8 ± 1.7	4.4–8.3
CI (L min^{-1} m^{-2})	3.9 ± 1.0	3.9 ± 1.2	3.8 ± 0.7	2.5–4.0
RAP (mmHg)	4.3 ± 2.5	5.4 ± 2.5	5.3 ± 2.3	2–10
PAP (mmHg)	13.4 ± 4.6	15.3 ± 5.6	14.9 ± 4.2	7–19
PCP (mmHg)	9.2 ± 6.9	9.4 ± 4.4	8.5 ± 3.5	8–12
SVR (dyne s cm^{-5})	1013 ± 313	1017 ± 296	1034 ± 246	900–1440
PVR (dyne s cm^{-5})	72 ± 60	73 ± 37	79 ± 25	11–99
SVRI (dyne s cm^{-5} m^{-2})	1730 ± 502	1802 ± 495	1831 ± 360	1.600–2580

Results are expressed in mean ± SD or *n* and percentage. Categorical variables were compared using the chi square test. Continuous variables were compared with *t*-Studen test or one-way ANOVA followed by preplanned contrast analysis to compare IPH with NCPVT or cirrhosis when necessary. *No significant differences in the parameters evaluate were observed between IPH* vs *NCPVT or IPH* vs *cirrhosis*. Abbreviations: IPH, idiopathic portal hypertension; NCPVT, non-cirrhotic non-tumoral portal vein thrombosis; MAI mean arterial pressure; HR, heart rate; CO, cardiac output; CI, cardiac index; RAP, right atrial pressure; PAP, pulmonary arterial pressure; PCP, pulmonary capillary pressure SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; SVRI, SVR index.

[a] MAP and HR measurements were available for 38 patients with IPH and 39 patients with NCPVT and 39 patients with cirrhosis.

in patients with cirrhosis, with a mean value of 40.9 ± 20.5 kPa, markedly higher than in IPH patients ($p < 0.001$) (Table 2 and Fig. 2B). Similar results were found removing IPH patients with VIH infection from de analysis (data not shown).

4. Discussion

In Western countries liver cirrhosis is the most common cause of PH. However PH can be present in the absence of cirrhosis, commonly named "non-cirrhotic portal hypertension". IPH is a rare cause of non-cirrhotic intrahepatic presinusoidal PH of unknown aetiology [1–3], characterised by the absence of either PVT, cirrhosis or other specific liver diseases [4,5]. The lack of a specific positive test makes that the diagnosis of IPH represents a challenge, even in experienced liver units [1,6].

Measurements of HVPG by hepatic vein catheterisation, and of LS by Fibroscan®, are increasingly used in the evaluation of patients with chronic liver diseases and PH [8,9]. To the best of our knowledge, this is the first study providing complete hepatic and systemic haemodynamic evaluation and LS measurements in a large series of patients with unequivocal IPH. Moreover, these data were compared to those obtained in matched patients with NCPVT and with liver cirrhosis. Age was significantly different, a fact not unexpected because patients with IPH and NCPVT are usually younger than patients with cirrhosis [15].

Our study confirms that the presence of HVVC is a common finding at venography in IPH patients [15,16]. HVVC were found in 49% of our cases, similar to the 45% prevalence reported by Okuda et al. [15] albeit lower to the 100% prevalence reported by Futagawa et al. [16] (probably due to a selection bias). As confirmed by the current study, HVVC are also frequent in long-standing NCPVT, which diminish the relevance of this finding with regards to distinguishing IPH complicated by PVT from PVT alone. On the other hand, the absence of HVVC does not rule out IPH, as shown by the fact that half of our IPH patients do not have these communications.

Finally, HVVC have been also described in some patients with cirrhosis, but with a lower prevalence than that observed IPH patients, ranging 1.3–13% [16–19]. In fact, HVVC were not observed in our cohort of patients with cirrhosis. As a consequence, the finding of HVVC in the evaluation of patients with PH and suspected cirrhosis should raise the suspicion of IPH. However, and because our patients with cirrhosis had rather good liver function to match with patients with IPH and NCPVT, we cannot discard that patients with more severe liver dysfunction may have higher degree of HVVC. In any case, the concomitant use of HVPG measurements will help to easily differentiate both diseases. Indeed, in patients with cirrhosis with sinusoidal PH, the presence of HVV(induces an underestimation of the real value of portal pressure but WHVP is still found markedly elevated [19]. By contrast, i patients with IPH, WHVP underestimates the real portal pressur because of the predominant presinusoidal component of the PI in IPH [1,3,20]. Indeed, the mean difference in portal pressure an WHVP in patients with IPH in whom direct portal pressure mea surements were performed was of 8.5 mmHg. However, only sligh differences in WHVP are observed in patients with IPH with or with out HVVC (Table 2). Direct portal pressure undoubtedly reflects rea portal pressure in these patients due to the presinusoidal compo nent of this disease. However this technique has been abandone because it is an invasive and risky procedure and we are convince that despite some limitations, HVPG measurements although no assessing portal pressure provide useful information in the diagno sis of IPH.

In 5 patients with IPH (19%), the HVPG was ≥10 mmHg, th minimal threshold for developing complications of PH in cirrhosi [8]. As increased HVPG implies an increased sinusoidal resistanc which is usually associated with fibrosis and structural damage [8] it could be that these patients represent more advanced IPH. How ever, although the numbers were small no significant difference in clinical parameters were observed between these 5 patients an the remaining patients with IPH.

The majority of our patients (81.5%) had a normal or slightl elevated HVPG, but below the previously described cut-off for clin ically significant PH in cirrhosis. In our opinion this negative findin can be very useful in the evaluation of a patient with clinical sign of PH, because the finding of an HVPG < 10 mmHg strongly argu against the diagnosis of cirrhosis, and should increase the suspicio of IPH, a rare hepatic vascular disorder.

LS measurements allow to evaluate the degree of fibrosis an to estimate the severity of PH in chronic liver diseases [9,21], bu have not been studied systematically in IPH. Our results confirn and extend our preliminary observation in a small group of HI infected patients with IPH [3] confirming that LS is not particularl elevated in IPH. Mean LS was much lower than the described cut off values for diagnosing cirrhosis, presence of varices or clinicall significant PH [13,14,22]. Indeed, in only 2 patients with IPH LS wa within the so-called "grey zone" (between 13.6 and 21 kPa; neithe discarding nor confirming clinically significant PH in cirrhosis) an no patient had a LS >21 kPa. Also, our results are in accordance t LS performed in patients with nodular regenerative hyperplasia although not all patients had portal hypertension [23]. Thus, L may be a very helpful diagnostic tool by ruling out cirrhosis in given patient with unequivocal signs of PH, thus complementin

the information provided by HVPG, and increasing the suspicion of IPH. LS was significantly higher in IPH patients compared to NCPVT patients, suggesting a moderate degree of structural abnormalities in IPH, but this is speculative. There was a marked overlap in LS values, and therefore LS is of not help in differentiating both diseases. Whether increased LS in IPH patients is associated with a more severe alteration of liver architecture or a poorer prognosis should be evaluated in prospective studies of greater patient numbers. A possible limitation of our study is that in one third of patients LS and HVPG measurements were not performed at the same time. However, no significant differences in LS values were observed between patients with IPH having their measurements performed at the same time or months after HVPG measurements. Similarly, in those patients with IPH and several LS measurements, no significant change in LS over time was observed. Thus, we believe that the fact that some patients have LS measurements performed sometime after HVPG measurements does not change the meaning of our findings.

Evaluation of systemic haemodynamics disclosed that patients with IPH have systemic and pulmonary haemodynamics similar to patients with NCPVT and to patients with cirrhosis and PH. These findings are in accordance with previous reports [24,25]. It is important to mention that patients were matched by liver function evaluated by Child-Pugh score and that most patients were Child class A. Thus, it is not possible to distinguish IPH, NCPVT or cirrhosis from the systemic haemodynamic pattern.

In conclusion, patients with IPH with unequivocal signs of PH frequently have HVVC. IPH is further characterised by moderate elevations of HVPG and of LS values, but below the published cut-off for clinically significant PH in cirrhosis. Therefore, the finding of HVVC or of unexpectedly low LS values in a patient with clear-cut signs of PH should raise a strong suspicion of IPH, and lead to further investigations, including liver biopsy for definitive diagnosis.

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Conflict of interest statement
None.

List of abbreviations

CI, cardiac index; CO, cardiac output; CRF, case report form; CSPH, clinical significant portal hypertension; FHVP, free hepatic vein pressure; HAART, highly active antiretroviral treatment; HR, heart rate; HVPG, hepatic venous pressure gradient; HVVC, hepatic vein to vein communications; IPH, Idiopathic portal hypertension; kPa, kilopascals; LS, liver stiffness; MAP, mean arterial pressure; NCPVT, non-cirrhotic non-tumoral portal vein thrombosis; NSBB, nonselective beta blockers; PAP, pulmonary arterial pressure; PCP, pulmonary capillary pressure; PP, portal pressure; PVR, pulmonary vascular resistances; PVT, portal vein thrombosis; RAP, right atrial pressure; SVR, systemic vascular resistance; SVRI, SVR index; WHVP, wedged hepatic vein pressure.

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ESTUDIO 2

Metabolomics discloses potential biomarkers for the non-invasive diagnosis of idiopathic portal hypertension

SEIJO S, LOZANO JJ, ALONSO C, REVERTER E, MIQUEL R, ABRALDES JG, MARTINEZ-CHANTAR ML, GARCIA-CRIADO A, BERZIGOTTI A, CASTRO A, MATO JM, BOSCH J, GARCIA-PAGAN JC

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Metabolomics Discloses Potential Biomarkers for the Noninvasive Diagnosis of Idiopathic Portal Hypertension

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OBJECTIVES: **Idiopathic portal hypertension (IPH) is a rare cause of portal hypertension that lacks a specific diagnostic test. Requiring ruling-out other causes of portal hypertension it is frequently misdiagnosed. This study evaluates whether using high-throughput techniques there is a metabolomic profile allowing a noninvasive diagnosis of IPH.**

METHODS: **Thirty-three IPH patients were included. Matched patients with cirrhosis (CH) and healthy volunteers (HV) were included as controls. Metabolomic analysis of plasma samples was performed using UPLC-time-of-flight-mass spectrometry. We computed Student's *P*-values, corrected by multiple comparison and VIP score (Variable Importance in the Projection). The metabolites were selected with an adjusted Benjamini Hochberg *P* value <0.05. We use markers with a greater VIP score, to build partial least squares projection to latent structures regression with discriminant analysis (PLS-DA) representative models to discriminate IPH from CH and from HV. The performance of the PLS-DA model was evaluated using R^2 and Q^2 parameter. An additional internal cross-validation was done.**

RESULTS: **PLS-DA analysis showed a clear separation of IPH from CH with a model involving 28 metabolites (Q^2=0.67, area under the curve (AUC)=0.99) and a clear separation of IPH from healthy subjects with a model including 31 metabolites (Q^2=0.75, AUC=0.98). After cross–validation, both models showed high rates of sensitivity (94.8 and 97.5), specificity (89.1 and 89.7), and AUC (0.98 and 0.98), reinforcing the strength of our findings.**

CONCLUSIONS: **A metabolomic profile clearly differentiating patients with IPH from CH and healthy subjects has been identified using subsets of 28 and 31 metabolites, respectively. Therefore, metabolomic analysis appears to be a valuable tool for the noninvasive diagnosis of IPH.**

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INTRODUCTION

Idiopathic portal hypertension (IPH) is a rare cause of intrahepatic presinusoidal portal hypertension of unknown etiology (1–3) that may cause portal hypertension-related complications, such as variceal bleeding or ascites in the absence of cirrhosis (CH) or other causes of liver diseases (4,5). Such clinical similarity with CH is one of the reasons why IPH is frequently misdiagnosed as cryptogenic CH (1,6). However, in patients with IPH the incidence of hepatopulmonary syndrome and pulmonary hypertension (1,7) and hepatocellular carcinoma (8,9) is much lower and survival much better than in patients with CH (10,11). Therefore, it is relevant to perform an accurate diagnosis of IPH in patients with portal hypertension. This makes mandatory that patients with a suspicion of IPH undergo invasive and risky procedures, such as a liver biopsy, to exclude other causes of portal hypertension. In addition, findings at liver biopsy are not pathognomonic, but in most cases only compatible with the diagnosis of IPH. In addition, the finding of minimal or nonspecific changes at liver biopsy in patients with portal hypertension may be interpreted as sampling error and the patient being misdiagnosed as cryptogenic CH.

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In summary, the lack of a specific positive test makes the diagnosis of IPH a challenge even in experienced liver units (1,6). Hence new diagnostic tools for IPH are needed.

Metabolomics is a growing high-throughput technology that allows measuring simultaneously thousands of metabolites from a variety of complex samples (biological fluids or tissue extracts) in a short-time period. In the last few years, there were noteworthy advances in metabolomic technology. Liquid chromatography (LC) coupled to mass spectrometry (MS) technology is able to semiquantitatively analyze a wide range of molecular species, such as phospholipids and sphingolipids acids, bile acids, and amino acids. Therefore, Metabolomics has applications in both clinical and basic research, such as disease differentiation, clustering different subgroups of a disease, hypothesis generation, drug development or drug–response, or toxicity. In the last few years, several applications for metabolomics in liver diseases have been reported, such as the potential to discriminate among different forms or severity of liver diseases (12,13), to differentiate compensated from decompensated CH (14), or as a promising tool for the diagnosis of minimal hepatic encephalopathy in CH (15). In addition, a recent study from our institutions identified a serum metabolic profile that was able to noninvasively distinguish nonalcoholic steatohepatitis from patients with simple steatosis (16,17).

The aim of our study was to discover a noninvasive metabolomic profile in plasma allowing differentiating IPH from healthy individuals or patients with CH.

METHODS

Patients

Diagnosis of IPH was based on the following previously reported criteria:(18) (i) presence of unequivocal signs of portal hypertension (gastroesophageal varices, ascites, and/or splenomegaly); (ii) absence of CH, advanced fibrosis, or other causes of chronic liver diseases by appropriate serological, biochemical tests and by liver biopsy (performed in all patients); and (iii) absence of thrombosis of the hepatic veins or of the portal vein at imaging studies performed at diagnosis. Liver biopsies for diagnosis of IPH were performed by transjugular or percutaneous route and were evaluated by one experienced pathologist (RM) for the purpose of the study. Briefly, hematoxylin–eosin, Masson's trichrome, and reticulin stainings were examined. Only specimens containing 6 complete portal tracts were considered suitable for diagnosis (19). Absence of bridging fibrosis or CH was the main features to exclude. In addition, liver biopsies consistent with a clinical diagnosis of IPH must not show other significant pathological abnormalities (steatosis, inflammation, granulomas, and so on) (20). Those patients with liver biopsies including <6 portal tracts were not included in the study, because the diagnosis of IPH was not definite. Patients with HIV infection were not included in the study if liver biopsy showed other findings different from those described in patients with IPH. Only patients with unequivocal diagnosis of IPH were finally included.

A group of healthy volunteers (HV) and a group of patients with CH and portal hypertension were included as controls. Patients with CH were retrospectively selected from the hepatic hemodynamic laboratory patient database. Diagnosis of CH was based on liver biopsy and/or clinical data and imaging techniques. patients with CH, portal hypertension was defined as an hepatic venous pressure gradient ≥10 mm Hg and/or presence of gastroesophageal varices or ascites. Thrombosis of the hepatic veins of the portal vein was excluded by US-Doppler. Patients with C were matched by gender, signs of portal hypertension and li function, and HVs were matched by age and gender.

Exclusion criteria were: refuse consent to participate; age < years, presence of hepatocellular carcinoma, hepatic vein thrombosis, portal vein thrombosis, liver transplant, and a liver biop with <6 complete portal tracts.

Blood sample details

Since 2003, all consecutive patients with incident or prevalent noncirrhotic portal hypertension (including IPH) seen at our u are asked permission for obtaining a blood sample for resear purposes. In addition, we have collected a blood sample fr a large cohort of patients with CH referred for hepatic venc pressure measurements for evaluation of portal hypertensi and from a large cohort of HVs. Written informed consent v obtained from all subjects.

Peripheral blood was collected into a citrate-containing tu (0.129 M, 3.8%, Vacutainer System, Becton Dickinson, San Jo CA). The samples are centrifuged and aliquots of the platelet-p plasma are frozen at −80 °C until assayed. Ninety-nine plas samples (33 IPH, 33 CH, and 33 HVs) were included in the stu

Ethical statement

The protocol was reviewed and approved by the ethical comm tee at our institution. All subjects gave written informed cons to participate.

Experimental procedures

A global metabolite profiling UPLC-MS methodology was us where all endogenous metabolite-related features, characteriz by mass-to-charge ratio *m/z*, and retention time *Rt*, are includ in a subsequent multivariate analysis procedure used to stu metabolic differences between the different group of samp (21–24). Sample preparation and LC/MS analysis was perform as described in detail previously (16,17).

Chemicals

High-performance liquid chromatography MS-grade solve were purchased from Sigma-Aldrich (St Louis, MO). Referer metabolite standard compounds were obtained from Sign Aldrich, Avanti Polar Lipids (Alabaster, AL), and Larodan F Chemicals (Malmö, Sweden).

LC-MS system

Samples were analyzed using an Acquity UPLC System (Wate Milford, MA) coupled to the time-of-flight (TOF)-MS L Premier (Waters). Chromatography was performed on 1 mm i.d.×100 mm Acquity 1.7 µm C8 BEH column (Wate

maintaining the column at 40 °C and applying a 10-min linear gradient. The mobile phase, at a flow rate of 140 µl/min, consisted of 100% solvent A (0.05% formic acid) for 1 min followed by an incremental increase of solvent B (acetonitrile containing 0.05% formic acid) up to 50% over a further minute, increasing to 100% B over the next 5 min before returning to the initial composition. The volume of sample injected onto the column was 1 µl.

The mass spectrometer was operated with an electrospray (ESI) source held at 150 °C. The nebulization gas was set to 600 liters/h at a temperature of 350 °C. The cone gas was set to 10 liters/h with the capillary and cone voltages set to 3,200 and 30 V, and 2,800 and 50 V in positive and negative modes, respectively. The data acquisition rate was set to 0.2 s, with a 0.01 s interscan delay. The mass range, 50–1,000 *m/z*, was calibrated with cluster ions of sodium formate, using leucine enkephaline as an internal reference compound for instrumental drift correction. A test mixture of standard compounds (Acetaminophen, Sulfaguanidine, Sulfadimethoxine, Val-Tyr-Val, Terfenadine, Leucine-Enkephaline, Reserpine and Erythromicyns—all 5 nM in water) was analyzed before and after the entire set of randomized, duplicated sample injections to examine the retention time stability (generally <6 s variation, injection-to-injection), mass accuracy (generally <3 p.p.m. for *m/z* 400–1,000, and <1.2 mDa for *m/z* 50–400), and sensitivity of the LC/MS system throughout the course of the run.

Data processing

All data were processed using the MarkerLynx application manager for MassLynx 4.1. software (Waters). The LC/MS data are peak-detected and noise-reduced in both the LC and MS domains such that only true analytical peaks are further processed by the software (e.g., noise spikes are rejected). A list of intensities (chromatographic peak areas) of the peaks detected is then generated for the first sample, using the *Rt* and *m/z* data pairs as the identifier for each peak. This process is repeated for each LC/MS run and the data from each LC/MS analysis in the batch are then sorted such that the correct peak intensity data for each *Rt-m/z* pair are aligned in the final data table. The ion intensities for each peak detected are then normalized, within each sample, to the sum of the peak intensities in that sample. Normalization between assays was performed by considering the injections of the reference serum samples injected and following the Linear Regression (within-batch) procedure describe by van der Kloet *et al.* (25). The resulting normalized peak intensities form a single matrix with *Rt–m/z* pairs for each file in the data set.

All processed data were mean centered and pareto scaled (26) during multivariate data analysis.

Pairwise univariate data analysis was performed in IPH vs. CH samples and IPH vs. HVs, to eliminate biomarkers that do not discriminate between groups.

For clinical variables, data handling and analysis were performed using the PASW Statistics 18 program (SPSS, Chicago, IL). Quantitative variables are expressed as mean±s.d., and median and range, and qualitative variables as absolute and relative frequencies. Categorical variables were compared using the χ^2-test. Continuous variables were compared with *t*-Student's test or one-way analysis of variance followed by pre-planned contrast analysis to compare IPH with HV or CH when necessary. Significance was established at $P<0.05$.

Multivariate data analysis

First, for each comparison (IPH vs. CH and IPH vs. HV) variables with missing values were not considered. Resulting peak lists (1,449 IPH–CH and 1,552 IPH–HV). Second, we compute Student's *P* values, corrected by multiple comparison and VIP score. VIP score, the Variable Importance in the Projection, estimates the importance of each variable in the projection used in a Partial Least Squares (PLS) model. A variable with a VIP score close to or greater than 1 can be considered important in a given model. Variables with VIP scores significantly less than 1 are less important and might be good candidates for exclusion from the model. This process ended with a set of 202 (IPH–CH) and 57 (IPH–HV) metabolomic-selected markers with an adjusted Benjamini Hochberg *P* value <0.05 (27). We use the markers with a greater VIP (VIP threshold: 2.2 and 2.1) to build Partial Least Squares Projection to Latent Structures regression with Discriminant Analysis (PLS-DA) representative models to discriminate IPH from CH and IPH from HV. The performance of the PLS-DA model was evaluated using the R^2 and Q^2 parameter. R^2 provides an indication of how much of the variation within the data set can be explained by the model (goodness of fit). Computed Q^2 parameter describes the predictive ability of model (goodness of prediction) under sevenfold cross-validation. Values between 0.7 and 1.0, as close to 1, for both R^2 and Q^2 indicate a very good model with an excellent predictive power.

A additional validation strategy was done in the proposed PLS-DA models for class discrimination and class membership prediction, data for the subjects from different subgroups were randomly divided into the training (~2/3 of all subjects in a given subgroup) and test (~1/3 of the subjects) sets. Test sets were excluded from model construction. Following construction of PLS-DA models using training sets, the models were then used to predict class membership of the subjects in the testing sets. This procedure was repeated one-hundred times, different subjects in training and testing sets were included and a new PLS-DA model was constructed each time. We compute the corresponding random sampling cross-validated AUC measures (area under the curve, sensibility, and specificity) as mean and standard deviation in both sets: training (2/3) and testing (1/3). For PLS-DA computations we used the mixOmics R-packages (28).

Finally, heatmaps were created to represent the selected models. A hierarchical clustering algorithm was performed on both variables and samples. Rows and columns are reordered according to hierarchical clustering method that identified both groups.

RESULTS

Patients

Clinical characteristics of patients are summarized in **Table 1**. All patients had signs of portal hypertension. The five patients without varices had marked splenomegaly and thrombopenia.

Table 1. Main clinical characteristics of the patients included in the study

Variables	IPH, *n*=33	Cirrhosis, *n*=33	Healthy volunteers, *n*=33
Age at time of blood sample[a] (years)	42±16**	59±8	39±10
Gender (male), *n* (%)	21 (64)	27 (82)	19 (58)
Signs of portal hypertension, n (%)			
Varices	28 (85)	25 (76)	—
Variceal bleeding	13 (39)	8 (24)	—
Ascites	10 (30)	11 (33)	—
Hepatic encephalopathy	0	2 (6)	—
Laboratory data[a]			
Hematocrit (%)	39±5.6#	39±6.4	41±3.2
Platelet count (x10^9/l)	114±92##	99±36.9	265±49.5
Creatinine (mg/dl)	0.9±0.2	0.86±0.3	0.89±0.2
AST (UI/l)	36±15*.##	67±44	19±4.7
ALT (UI/l)	41±29*.#	66±49	20±10.2
Albumin (g/l)	41±5.3**	37±3.9	43±3.1
Bilirubin (mg/dl)	1.3±1.2#	1.1±0.4	0.7±0.3
Prothrombin ratio (%)	78±13##	79±11	93±7.9
Child-Pugh class, n (%)			
A	27 (82)	27 (82)	—
B	6 (18)	6 (18)	—
C	0	0	—

IPH, idiopathic portal hypertension; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a]Mean±s.d.

Significance was as follows: for IPH vs. cirrhosis *$P \leq 0.05$, **$P \leq 0.001$; for IPH vs. healthy volunteers' #$P \leq 0.05$, ##$P \leq 0.001$.

Eight patients with IPH had ascites in seven of them only detected at imaging studies. In all cases, ascites was easily controlled, and in three patients ascites overlapped with a variceal bleeding episode.

Nine patients (27%) had HIV infection controlled with HAART (viral load < 50 copies/ml). Hepatic biopsies were percutaneous in 11 (33%) and transjugular in 22 cases (67%).

The main characteristics of patients with CH and HVs are summarized in **Table 1**. Etiology of CH was alcohol in 15 (46%), hepatitis C in 12 (36%), alcohol and hepatitis C in 4 (12%), and nonalcoholic steatohepatitis in 2 (6%). As expected, patients with IPH were significantly younger than patients with CH.(29)

Metabolites analysis: PLS-DA

A supervised analysis with PLS-DA was performed. PLS-DA can maximize the difference among both groups and help in the screening of metabolites that are responsible for class separation by removing systematic variations unrelated to pathological status.

On the basis of PLS-DA models, patients with IPH and patients with CH were discriminated with a model with a R^2 of 0.77, a Q^2 of 0.67, and a VIP threshold of 2.2 with an AUC of 0.99 (**Figure 1a**). In this model, 28 metabolites were found to carry the class separation. A heatmap representing the hierarchical clustering of both group of patients (IPH vs. CH) with the subset of 28 metabolites that are included in the model showed a good separation of both group of patients (**Figure 1b**). Interestingly, as shown in the figure, no differences in metabolomic profile were observed among IPH patients based on their HIV status.

For cross-validation analysis, subjects in each group were randomly divided into training and testing sets (~2/3 and ~1/3 of all subjects in a given subgroup, respectively) and it was carried out hundred times with different subjects included in the testing set each time. Our 28-metabolite model allows to accurately diagnose IPH patients with a median probability of 98.4%. On the other hand, cirrhotic patients have a median probability of 7.6% to be IPH in the training set. These results were confirmed in the testing set (97.9 and 9.4%; **Figure 1c**). Mean (±s.e.) sensitivity, specificity, and AUC of the model at cross-validation were 94.8±3.2, 89.1±4.2, and 0.98±1.2, respectively, in the training set and 89.5±9.7, 86.0±12.1, and 0.95±4.9, respectively in the testing set.

PLS-DA analysis also showed a clear separation of patients with IPH from HVs with a model including 31 metabolites with a R^2 of 0.82, a Q^2 of 0.75, and a VIP threshold of 2.1 with an AUC of 0.98 (**Figure 2a**). Also a heatmap performed with both populations (IPH and healthy individuals) with the subset of 31 metabolites included in the analysis shows a clear clustering of both groups of patients (**Figure 2b**). After cross-validation, our 31-metabolite model allows to accurately diagnose IPH patients with a median probability of 99.5%. On the other hand, using this model HVs were misdiagnosed as IPH in only 7.7% cases in the training set. These results were confirmed in the testing set (99.2 and 9.5%; **Figure 2c**). Mean (±s.e.) sensitivity, specificity, and AUC of the model of this approach were found to be 97.5±2.6, 89.7±5.4, and 0.98±0.2, respectively, in the training set and 95.6±6.5, 79.3±12.4, and 0.93±0.65, respectively, in the testing set.

DISCUSSION

IPH is frequently an unrecognized disease or misdiagnosed as CH, even in experienced centers, and its diagnosis currently requires a comprehensive exclusion of common causes of portal hypertension (1,6). Hence novel and easier diagnostic tools for IPH are mandatory.

To the best of our knowledge, this is the first study of plasma global metabolic profiling in patients with IPH. The PLS-DA models show a clear differentiation of IPH and cirrhotic patients based on a subset of 28 metabolites, with an excellent predictive power (based on R^2 and Q^2 values) with an AUC of 0.99. Also when compared with healthy controls, the plasma samples from patients with IPH show clear differences in the metabolic profile with a subset of 31 metabolites disclosing an AUC of 0.98. These results support the hypothesis that metabolomic signatures of plasma samples could be useful to discriminate IPH from cirrhotic patients and from healthy controls. The cross-validation showed an excellent performance of both models with a good sensibility, specificity,

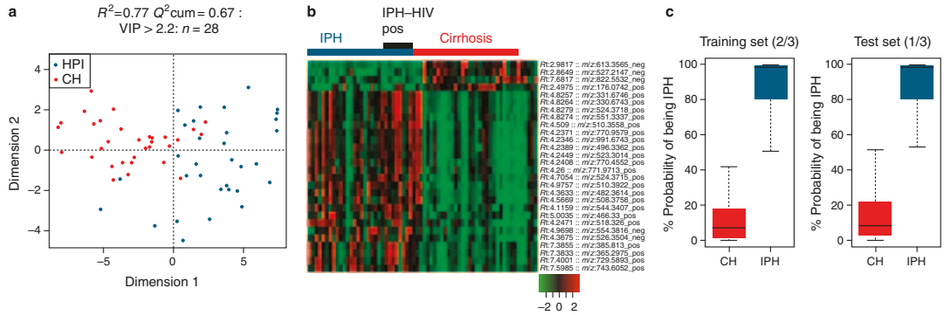

Figure 1. Metabolomic signature of idiopathic portal hypertension and cirrhosis. (**a**) Partial least square discriminant analysis (PLS-DA) based on the metabolite profile data of patients with idiopathic portal hypertension (IPH) and patients with cirrhosis (CH). The PLS-DA score plot (**a**) discriminate both groups with a model including 28 metabolites with a R^2 of 0.77, a Q^2 of 0.67 at VIP threshold of 2.1. (**b**) Heatmap representation of clustering of the discriminating metabolites across the two groups of patients (IPH and CH). Sample classes are indicated by the colored bars (IPH in blue, CH in red, IPH patients HIV positive in blue and black). Columns represent individual samples and rows refer to distinct metabolites. Shades of red represent elevation of a metabolite, and shades of green represent decrease of a metabolite relative to the median metabolite levels. HIV positive and HIV negative could not be differentiated by the metabolic profiling. (**c**) Probabilistic sensitivity analysis (from the explained cross-validation process) of the proposed models to discriminate patients with IPH from CH. Boxplots represent the distribution of the cross-validated probability for each group of being a patient with IPH.

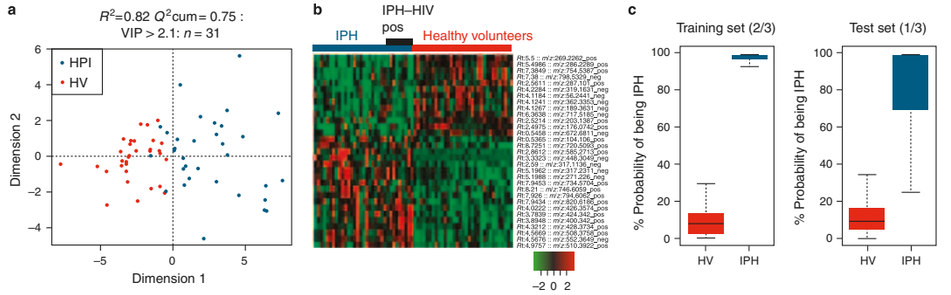

Figure 2. Metabolomic signature of idiopathic portal hypertension and healthy volunteers. (**a**) Partial least square discriminant analysis (PLS-DA) based on the metabolite profile data of patients with idiopathic portal hypertension (IPH) and patients with cirrhosis (CH). The PLS-DA score plot (**Figure 1a**) discriminate both groups with a model including 28 metabolites with a R^2 of 0.82, a Q^2 of 0.75 at VIP threshold of 2.1. (**b**) Heatmap representation of clustering of the discriminating metabolites across the two group of patients (IPH and healthy volunteers (HV). Sample classes are indicated by the colored bars (IPH in blue, HV in red, IPH patients HIV positive in blue and black). Columns represent individual samples and rows refer to distinct metabolites. Shades of red represent elevation of a metabolite, and shades of green represent decrease of a metabolite relative to the median metabolite levels. HIV positive and HIV negative could not be differentiated by the metabolic profiling. (**c**) Probabilistic sensitivity analysis (from the explained cross-validation process) of the proposed models to discriminate patients with IPH from HV. Boxplots represent the distribution of the cross-validated probability for each group of being a patient with IPH.

and AUC in the training and testing sets. These results supported the strength of the selected models for the accurate diagnosis of IPH.

IPH is considered a clinical entity that may include several different disorders of unknown etiology, finally leading to the development of presinusoidal intrahepatic portal hypertension (2,18,30,31). In that regard, HIV-associated IPH has been recently identified as one of such differential disorders (3,18,32,33) and although the exact mechanism is unclear, it is thought to be the consequence of liver injury caused by HIV itself or more likely by antiretroviral drugs (i.e., didanosine) (3,34–36). Interestingly, the subanalysis of the metabolic profile of our patients with IPH was unable to cluster patients in different subgroups of IPH patients in the hierarchical clustering algorithm. Thus, although further studies need to be done in larger population of patients with IPH, the result of our study strongly suggests that the metabolic profile is reflecting noninvasive diagnostic markers of a clinical syndrome rather than its etiology.

The possible influence of the drugs that patients are taking on the metabolic profile may be a concern. However, it is important to

mention that the subgroup of patients with IPH–HIV positive (all of them under HAART treatment) had a metabolic profile similar to IPH–HIV-negative patients (without HAART treatment). Similarly, patients with IPH and CH have the same prevalence of varices, so the prevalence of β-blockers treatment was almost the same in both groups. It is understandable that some of the detected metabolites may reflect some of the drugs that patients are taking. However, it seems that the statistical approach used for the analysis did not select any of them for the final metabolic profile.

In this proof of concept, phase 2 diagnostic study (37), we decided to include only patients with unequivocal diagnosis of IPH. For this reason, we decide not to include patients with portal vein thrombosis as a control group, because portal vein thrombosis can develop on a patient with normal liver but also in patients with underlying liver disease (including also IPH and that may be previously unrecognized). The relatively low number of patients with IPH enrolled could be considered a limitation of the study; however, as IPH is a rare condition, a sample over 30 patients could be considered adequate. Indeed, with this number of patients, we were able to find a metabolic profile that successfully and accurately clusters patients with IPH from those with CH or from healthy individuals, with an extremely high statistical significance. Another potential drawback of the study is the lack of an external validation set. However, it must be emphasized that an internal cross-validation was performed. In addition, this is a pilot study that opens a new line of investigation. In our opinion, such external validation studies will be more appropriate at a later step, when the specific metabolites included in the models could be identified with new technologies. The finding of such a group of metabolites encourages pursuing in such identification that could lead to future simple-to-use kits that will help to diagnose IPH. In addition, the fact that the metabolic profile discriminates patients with IPH from those with CH makes unlikely that the differences in metabolic profile were related to differences in liver function or portosystemic shunting. This opens the interesting possibility that the identification of these specific metabolites may disclose some keys for a better understanding of the pathogenesis of IPH.

In conclusion, metabolic profiling was found to be a suitable platform for the noninvasive diagnosis of IPH. The results from this study disclose a subset of putative biomarkers of IPH. Hence, patients with IPH could be identified based on their metabolic profile, obviating the need for invasive investigations and facilitating the correct diagnosis of this uncommon disease.

CONFLICT OF INTEREST

Guarantor of the article: Juan Carlos Garcia-Pagan, MD, PhD.

Specific author contributions: S.S. and J.C.G.-P. designed the study. S.S., E.R., R.M., A.G.-C., and A.B. collected the data. J.J.L., C.A., A.C., J.G.A., S.S., and J.C.G.-P. performed the statistical analysis. S.S., J.G.A., J.J.L., and J.C.G.-P. wrote the paper. J.M.M., M.L.M.-C., J.B., and J.C.G.-P. supervised the manuscript preparation. All the authors read and approved the final version of the paper.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Idiopathic portal hypertension (IPH) is a rare cause of portal hypertension of unknown etiology.
- ✓ The diagnosis of IPH currently requires an extensive diagnostic work out to exclude other causes of portal hypertension. Indeed, IPH is usually misdiagnosed as cryptogenic cirrhosis.
- ✓ Hence novel tools for the positive and noninvasive diagnosis of IPH are mandatory.

WHAT IS NEW HERE

- ✓ It is possible to identify a metabolic profile that is able to clearly differentiate patients with IPH from patients with cirrhosis and portal hypertension and from healthy individuals.
- ✓ Metabolomic analysis could be an important tool for the noninvasive diagnosis of IPH.

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ESTUDIO 3

Bone morphogenetic protein receptor 2 in patients with idiopathic portal hypertension

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Bone morphogenetic protein receptor 2 in patients with idiopathic portal hypertension

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Abstract

In idiopathic portal hypertension (IPH) typical vascular lesions are present in the branches of the portal vein or in the perisinusoidal area of the liver. Similar histological alterations have been reported in the pulmonary vasculature of patients with idiopathic pulmonary artery hypertension (IPAH). As IPAH is associated with mutations of the bone morphogenetic protein receptor 2 (*BMPR2*) gene, the aim of this study was to investigate whether this association might also be found in patients with IPH. Twenty-three samples belonging to 21 unrelated caucasian patients with IPH followed in the hepatic haemodynamic laboratory of the Hospital Clinic in Barcelona were included in the study. All patients were studied for the entire open reading frame and splice site of the *BMPR2* gene by direct sequencing and multiple ligation probe amplification (MLPA) in order to detect large deletions/duplications. None of the 23 patients had pulmonary artery hypertension. Four patients presented one single nucleotide polymorphism (SNP) in intron 5, four patients had a SNP in exon 12 and a SNP in exon 1 was found in two cases. Two patients had both intron 5 and exon 12 polymorphisms. All SNPs were previously described. Except for these three SNPs, neither mutations nor rearrangements have been identified in the *BMPR2* gene in this population. We did not detect mutations or rearrangements in the coding region of the *BMPR2* gene in our patients with IPH. These findings suggest that, in contrast to IPAH, mutations in *BMPR2* are not involved in the pathogenesis of IPH.

Keywords: hepatoportal sclerosis • non-cirrhotic portal hypertension • bone morphogenetic proteins • pulmonary artery hypertension • HIV • *BMPR2* gene

Introduction

Idiopathic portal hypertension is a progressively debilitating and life-threatening disease of unknown etiology characterized by the absence of cirrhosis or portal vein obstruction [1].

Typical lesions are generally vascular and are present in the portal vein, its branches or in the perisinusoidal area of the liver. Essentially, there is a marked sub-endothelial thickening of the large and medium-sized branches of the portal vein, with obliteration of small portal venules, microthrombi incorporated into the vessel wall and preisinusoidal fibrosis [2, 3].

The mechanisms causing these lesions remain largely unknown. Prothrombotic disorders are considered important causal features [4, 5], but also infections [6], trace metals and chemicals [7] and immunological factors [8, 9] have been proposed. Furthermore, genetic mutations may play a role in the pathogenesis of IPH [10, 11]. Familial aggregation has been described, raising the question about the existence of one or more genes at the origin of this disorder [11].

Interestingly, the pathological alterations observed in the smallest vessels of the lung of patients with IPAH, *i.e.* intimal

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proliferation with perivascular fibrosis and muscular hypertrophy of the media [12] are very similar to those found in the liver in IPH [5]. In addition, some patients with IPH also present clinical features of IPAH [13].

Bone morphogenetic protein receptor 2 (*BMPR2*) gene, which encodes a membrane receptor of the transforming growth factor beta (TGF-β) superfamily, has been mapped on chromosome 2q33 (locus PPH1) and the sequence of this 4-kb gene is composed by 13 exons and encodes a 1038–amino acid protein [14]. Mutations in *BMPR2* account for 7–25% of the IPAH forms and for up to 80% of the familial forms of pulmonary arterial hypertension [15–17]. Large rearrangements account for 12% in familial forms of pulmonary arterial hypertension and 5% in sporadic cases [18]. In a Spanish study, these proportions resulted significantly lower (11% and 25%, respectively) [19]. This difference could be attributable to population heterogeneity or to a clinical selection or failure to detect mutations by the technology used (single-strand conformation polymorphism, SSCP, analysis).

A total of 144 distinct mutations in the *BMPR2* gene have been so far described in 210 patients with pulmonary arterial hypertension [20]. Approximately 70% of the *BMPR2* mutations underlying pulmonary artery hypertension are predicted to lead to premature truncation of the *BMPR2* transcript and are likely to be lost by the process of nonsense-mediated decay. All the currently known mutations cause a loss of receptor function.

Recent studies suggest that *BMPR2*-related IPAH is due to the failure of BMPR2 opposing a competing TGF-β signalling function, whose activation causes an increase in the TGF-β activity that has been shown to promote fibrogenesis, intimal hyperplasia and smooth-muscle growth [21, 22]. This hypothesis implies that the fundamental mechanism of *BMPR2*-related pulmonary artery hypertension is an imbalance of growth signalling caused by a reduction in the braking function of BMPR2.

As the pathophysiology of IPH remains largely unknown and IPH and IPAH share similar histopathological vascular lesions, we hypothesized that mutations in the *BMPR2* gene may be found in patients with IPH. Consequently, the aim of this study was to assess the prevalence of mutations in *BMPR2* in patients with IPH.

Materials and methods

Patients

Diagnosis of IPH was based on the following criteria: (1) presence of unequivocal signs of portal hypertension (gastroesophageal varices, ascites, splenomegaly and/or presence of portosystemic collaterals), (2) absence of cirrhosis or advanced fibrosis or of other additional causes of chronic liver diseases causing portal hypertension, at liver biopsy (performed in all patients), (3) absence of hepatic or portal vein thrombosis at imaging studies performed at diagnosis, (4) absence of toxic exposure to arsenic, vinyl chloride or copper sulphate (clinical history). All liver biopsy specimens were re-evaluated for the purpose of the study by an experienced pathologist (M.B.). These criteria were selected based on two reference papers from Japan and Europe [23, 24].

Patients with IPH followed-up at the Liver Unit of our Hospital, who have given written informed consent to obtain a blood sample for genetic studies, were considered eligible for the study. To avoid genetic noise related to inherited genetic traits and to have a homogeneous ancestry population we selected only caucasian patients for the study. Because of the exploratory nature of this study and the elevated costs, the study was limited to the initial consecutive 23 patients.

The protocol was approved by the Institutional Review Board of Hospital Clinic in Barcelona. Clinical, epidemiological, laboratory and imaging features were recorded in a pre-designed case report form.

BMPR2 gene molecular studies

Aliquots of whole blood were stored at −80°C. DNA extraction from whole blood was performed using an automatic MagnaPure system (Roche Diagnostics, Madrid, Spain) according to the manufacturer instructions.

BMPR2 exons and their associated boundary regions were amplified by PCR with previously reported primers [15, 19]. PCR products were sequenced using the Big-Dye Terminator Chemistry Kit v3.1, run on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analysed using ABI PRISM GeneMapper software version 3.0. All sequences were compared to NCBI RefSeq NM_001204.6. The standard nomenclature recommended by HGVS (www.hgvs.org/mutnomen) was used to number nucleotides and name mutations.

Frequencies of *BMPR2* SNPs were compared to those previously described in the HapMap database (http://hapmap.ncbi.nlm.nih.gov/index.html.en).

Multiple ligation probe amplification (MLPA) reaction

Multiple ligation probe amplification analysis was performed with Salsa P093-B2 HHT (MRC-Holland, Amsterdam, the Netherlands) as described previously by Madrigal *et al.* [25]. Samples were loaded onto an ABI3130 Genetic Analyzer and results were visualized using the Gene Mapper program and analysed with the SEQUENCE Pilot-module MLPA® program (JSI Medical Systems GmbH, Kippenheim, Germany).

Quantitative polymerase chain reaction (qPCR)

DNA copy number of two *BMPR2* genes was determined by qPCR using SYBR Green on an ABI PRISM 7300 Real-Time PCR System (Applied Biosystems). Primers were designed for exons 4, 9, 11 and 12 (4F-CAGCCTTTCTAAAGGGCAGTC, 4R-CCAAAGCATAAGGCAACTATC; 9F-AGAATATGCTACGTTCTCTC, 9R-CCTGGGAAGAGGTCTGTACATC; 11F-CAGGCAGTGAGGTCACTCAA; 11R-TGATAGATGCCACACCCCTTA; 12F-GTGTGCCAAAAATTGGTCCT; 12R-TTGTGCTTGCTGTCGTTCAT). Each reaction was performed in triplicate. Amounts of DNA in each amplification were determined by comparing the results to a standard curve produced by real-time PCR of serial dilutions (*e.g.* undiluted, 1:4, 1:16 and 1:64) of a known amount of DNA.

RNA extraction and cDNA synthesis

Total RNA was extracted from peripheral blood using the PAXGene Blood RNA Kit following their basic protocol (QIAGEN, Germany). RNA concentration and purity were measured with a spectrophotometer (NanoDrop™ Spectrophotometer ND-100; Thermo Fisher Scientific, Madrid, Spain). Finally, cDNA was synthesized with 200 ng of the extracted RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions.

Real-time quantitative PCR (RT-qPCR) and data analysis

Real-time quantitative PCR experiments were carried out in an ABI7300 Real-Time PCR System (Applied Biosystems). No cDNA was added to negative control reactions. Beta-glucoronidase (GUS-β) was used as a housekeeping gene RT-qPCR and analysis was performed as previously described [26].

mRNA expression analysis

PCR was performed using cDNA to amplified exons 4, 11 and 12 of *BMPR2* gene. Primers used were the ones previously described at the quantitative real-time PCR method.

Results

Study population

Clinical, demographic characteristics and laboratory findings of the 23 patients with IPH (21 unrelated) included in the study are shown in Table 1. Five patients belonged to three unrelated families (two brothers, a father and a son both included in the study and a woman whose sister was not included in the study). Three patients presented features associated with immunological diseases. The first one was diagnosed with rheumatoid arthritis, the second suffered from anti-phospholipid syndrome and the third one was a patient with positive anti-transglutaminase antibodies, but with a normal duodenal biopsy.

Table 1 Clinical, demographic characteristics and laboratory findings of the 23 patients with IPH included in the study

	Median (range) or *n* (%)
Number of patients	23
Age at diagnosis of IPH	28 (9–86)
Gender (male) (%)	14 (61%)
HCV infection	0 (0%)
HBV infection	1 (4%)*
HIV infection	4 (17%)
Signs of portal hypertension	
Presence of oesophageal or gastric varices	23 (100%)
Ascites	5 (22%)
Splenomegaly	17 (74%)
Laboratory data†	
Leucocytes (G/L)	4.7 (1.2–8.9)
Haemoglobin (g/L)	128.8 (100–167)
Platelet count (G/L)	112 (27–308)
Prothrombin time (%)	75 (40–100)
Creatinin (mg/dl)	0.87 (0.60–1.28)
AST (IU/l)	39 (17–95)
ALT (IU/l)	38 (12–95)
GGT (IU/l)	81 (7–423)
Total bilirubin (mg/dl)	1.45 (0.40–7.8)
Sodium (mEq/l)	140 (135–145)
Albumin (g/l)	42 (32–51)

IPH: idiopathic portal hypertension. *HBV past infection. †Data at the time of blood draw for genetic analysis.

Mutation analysis of the BMPR2 gene

We did not detect any causative mutations in the *BMPR2* gene in the 21 unrelated patients with IPH included in the study. However, in 12 patients SNPs were detected. Four patients presented one SNP in intron 5 (rs7575056), four patients had a SNP in exon 12 (rs1061157) and two patients had a SNP in exon 1 (5′-UTR-301G>A). Two additional patients had both polymorphisms at intron 5 and exon 12. No other SNPs were identified.

All SNPs that we found have been previously described in the HapMap database (http://hapmap.ncbi.nlm.nih.gov/index.html.en). The prevalence of these SNPs in our patients was similar to the prevalence described in the healthy caucasian population.

Multiple ligation probe amplification

Screening of *BMPR2* by using the MLPA technique revealed five patients with possible duplications of *BMPR2* gene: two patients presented a duplication of exon 4, one patient of exon 9 and two other patients of exons 11 and 12.

Quantitative PCR only confirmed three of these duplications in two patients (exon 4) and one patient (exons 11 and 12). However, these duplications do not alter the normal expression of the gene, regarding RNA studies.

Discussion

Mutations in *BMPR2* gene have been involved in the pathogenesis of IPAH [27]. Based on the observation that IPAH and IPH share common vascular alterations, our aim was to investigate whether IPH also present mutations in the BMPR2 gene.

In contrast to our initial hypothesis, in this study we did not find any possible causative mutations in the *BMPR2* gene in patients with IPH. In addition to this finding, several other results that have emerged from this study, *i.e.* the confirmation of known SNPs and DNA duplications, as well as an unchanged mRNA expression, clearly argue against a possible role of *BMPR2* in the pathogenesis of IPH. Structural alterations such as deletions or duplications affecting the *BMPR2* gene have been described as a cause of familial pulmonary hypertension. In our samples, however, although at DNA level we have found some duplications, we could not confirm the alteration at mRNA and cDNA levels.

Previous studies in IPAH included small number of patients (less than 50) and the prevalence of *BMPR2* mutations was up to 26% [28]. One of the largest series included 126 patients with IPAH and the prevalence was 21% [18]. Also an increased occurrence of BMPR2 duplications has been reported in IPAH [29].

With the lack of evidence of any *BMPR2* mutation in this study and bearing in mind the low prevalence of IPH in western countries [5, 30], the number of patients in this study should be considered sufficient to reasonably believe that mutations in this gene may not be involved in the pathogenesis of IPH. Similarly, studies in familial PAH including a highly variable number of patients showed a prevalence of *BMPR2* mutations of up to 82% [31]. Consequently, more than one of our patients should have had the *BMPR2* mutation, reinforcing the finding that *BMPR2* gene mutations are not involved in the pathogenesis of familial forms of IPH.

Our study, however, showed that in 12 patients (52%) with IPH three different SNPs were detectable. The three SNPs identified in our patients, in intron 5, exon 12 and exon 1, have been previously described in a normal population with the same prevalence as in the population of our study. In addition, the possibility that *BMPR2* mutations occur in a subpopulation of patients with IPH also remains potentially open, because the sample size in this study (21 unrelated patients) was relatively small. However, due to the low prevalence of this disease in western countries [5, 30], the number of patients in this study should be considered sufficient to reasonably exclude this hypothesis.

It is currently recognized that up to 30% of hereditary and 80% of idiopathic cases of pulmonary artery hypertension do not have mutations identified in *BMPR2* despite comprehensive testing. Hence, it is likely that mutations at one or more other loci contribute to the pathogenesis of IPAH, as demonstrated by the association of IPAH with hereditary haemorrhagic telangiectasia and mutations in activin-like kinase type 1 and endoglin [32]. Similarly, familial or IPH may be associated with mutations in genes other than *BMPR2*.

An association of IPH with immunological abnormalities has been described [8, 9]. In this study we identified three patients in whom an autoimmune disease was known. However, due to an important diagnostic heterogeneity, we recognize that it is not possible to draw any conclusions from this study in terms of relationship between autoimmunity and the development of IPH.

Idiopathic portal hypertension has also been associated with HIV infection [33] and recent data by Caldwell *et al.* [34] indicate that HIV may repress BMPR2 transcription in macrophages. In the present study we focused on possible genetic alterations in the *BMPR2* gene and we did not assess the level of expression of BMPR2. Considering this methodological difference, whether the expression of BMPR2 was decreased in HIV patients in this study remains unknown. This intriguing hypothesis deserves further investigation.

In conclusion, the data of this study suggest that, in contrast to IPAH, IPH is highly unlikely that mutations in the *BMPR2* gene were involved with pathogenesis of IPH. Idiopathic portal hypertension may be related to other mutations in the TGF-β superfamily and further investigation is needed to understand the pathogenesis of this condition.

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Contributors: Study concept and design: J.C.G.P. and A.D.G. Acquisition of data: A.D.G., S.S., M.M., M.I.A. and M.B. Analysis and interpretation of data: A.D.G., S.S., M.M., M.I.A., J.G.A., J.B. and J.C.G.P. Drafting of the manuscript: A.D.G. and S.S. Critical revision of the manuscript for important intellectual content and statistical analysis: A.D.G., S.S., J.G.A. and J.C.G. Obtained funding: J.B. and J.C.G.P. Study supervision: J.C.G.P.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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ESTUDIO 4

Good long-term outcome of Budd-Chiari syndrome with a step-wise management

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Good Long-Term Outcome of Budd-Chiari Syndrome With a Step-wise Management

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Budd-Chiari syndrome (BCS) is a rare, life-threatening disease caused by obstruction of hepatic venous outflow. The aim of the study was to assess long-term outcome and identify prognostic factors in BCS patients managed by a step-wise approach using anticoagulation, angioplasty/thrombolysis, transjugular intrahepatic portosystemic shunting (TIPS), and orthotopic liver transplantation (OLT). We reviewed long-term data on 157 patients previously included by the European Network for Vascular Disorders of the Liver, a multicenter prospective study of newly diagnosed BCS patients in nine European countries. Patients were followed for a median of 50 months (range, 0.1-74.0). During the study, 88 patients (56%) received at least one invasive intervention (22 patients angioplasty/thrombolysis, 62 TIPS, and 20 OLT) and 36 (22.9%) died. Most interventions and/or deaths occurred in the first 2 years after diagnosis. The Rotterdam score was excellent in predicting intervention-free survival, and no other variable could significantly improve its prognostic ability. Moreover, BCS-TIPS prognostic index (PI) score (based on international normalized ratio, bilirubin, and age) was strongly associated with survival and had a discriminative capacity, which was superior to the Rotterdam score. *Conclusions:* The current study confirms, in a large cohort of patients with BCS recruited over a short period, that a step-wise treatment approach provides good long-term survival. In addition, the study validates the Rotterdam score for predicting intervention-free survival and the BCS-TIPS PI score for predicting survival. (HEPATOLOGY 2013;57:1962-1968)

Budd-Chiari syndrome (BCS) is an uncommon, life-threatening disorder arising as a consequence of obstruction to hepatic venous outflow regardless of its causal mechanism or level of obstruction. This obstruction, usually caused by thrombosis, can occur from the small hepatic venules up to the entrance of the inferior vena cava into the right atrium.[1,2] In the vast majority of cases, it is possible to identify at least one inherited or acquired prothrombotic risk factor as the underlying cause of thrombosis. Therapeutic options include pharmacological management with anticoagulants and diuretics as well as invasive procedures, such as thrombolysis, percutaneous transluminal angioplasty (PTA), transjugular intrahepatic portosystemic shunting (TIPS), surgical portosystemic shunting, and orthotopic liver transplantation (OLT).[1,3] As a consequence of these therapies, especially anticoagulation, TIPS, and OLT, the prognosis

Abbreviations: AUC, area under the curve; BCS, Budd-Chiari syndrome; BCIS score, BCS-intervention-free survival prognostic score; BCS-TIPS PI, BCS-TIPS prognostic index; CI, confidence interval; CRF, clinical record form; EN-Vie, European Network for Vascular Disorders of the Liver; GI, gastrointestinal; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; OLT, orthotopic liver transplantation; PH, portal hypertension; PTA, percutaneous transluminal angioplasty; TIPS, transjugular intrahepatic portosystemic shunting.

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of these patients has markedly improved over recent decades.[4-7]

However, because of the low incidence of the disease,[4,8] studies showing improvement in prognosis were mostly retrospective.[6,7,9-11] In fact, only one prospective study exists, albeit with a short follow-up (median, 17 months).[4] Hence, there are scarce data on the current long-term prognosis of BCS. Given that most patients included in the prospective cohort[4] are being actively followed in their original centers, we have been able to evaluate the long-term prognosis of patients with BCS.

Patients and Methods

Study Design and Data Acquisition. The current study involves extended follow-up of the prospective European Network for Vascular Disorders of the Liver (EN-Vie) study that included 163 consecutive incidental patients with BCS diagnosed between October 2003 and October 2005 in academic and large regional hospitals in nine European countries.[4] To standardize patient management, all participating centers had received guidelines with instructions on diagnostics tests and general indications for invasive procedures, such as TIPS, portosystemic shunting, and OLT, that were previously agreed upon by the EN-Vie steering committee. Further details on the study design of this original study can be found elsewhere.[4]

For the purpose of the present study, all previous participating centers were contacted again and agreed to participate in the extended follow-up study. Data were collected on a new, specifically designed CRF (clinical record form) where significant clinical events—defined as clinical deterioration (any new hospital admission or any clinical event), new liver-related imaging study, or any BCS-related intervention—were recorded from the end of the previous study (May 2006) until death or the end of the current study (June 2009). One investigator per country reviewed all CRFs before its inclusion in the database.

Patients were considered lost to follow-up if the last visit preceded the 9 months before study closure. All national and, if necessary, local ethics committees approved the study, and all patients provided written informed consent to participate.

Prognostic Scores. Child-Pugh and Model for End-Stage Liver Disease (MELD) scores were calculated using the data at diagnosis of BCS, as previously reported.[12,13] The Rotterdam score was previously published to predict survival and is defined as follows: 1.27 × encephalopathy + 1.04 × ascites + 0.72 × prothrombin time + 0.004 × bilirubin (where ascites was scored as present "1" or absent "0"). The 5-year survival rate was 89% (95% confidence interval [CI]: 79-99) for class I, 74% (95% CI: 65-83) for class II, and 42% (95% CI: 28-56) for class III.[9] The BCS-TIPS prognostic index score (TIPS-BCS PI score) was developed to predict OLT free survival in patients that received TIPS and is defined as follows: age (years) × 0.08 + bilirubin (mg/dL) × 0.16 + international normalized ratio (INR) × 0.63. The cutoff of 7 points had a sensitivity of 58%, a specificity of 99%, a positive predictive value of 88%, and a negative predictive value of 96% for death or OLT 1 year after TIPS.[6]

Statistical Analyses. Results are expressed as N (proportions) for categorical variables and as medians (range) for continuous variables. Actuarial transplantation-free and intervention-free survival rates were calculated by using Kaplan-Meier's method. Uni- and multivariable Cox's regression analysis was used to explore the association between different variables and prognosis. New prognostic scores were constructed by combining (in a linear equation) those variables independently associated with the event multiplied by their regression coefficients. To add potential advantages to these models, we did not include subjective parameters (e.g., presence or absence of hepatic encephalopathy; HE) or INR in patients that may have initiated anticoagulation that were integrated in the previously described scores. Statistical significance was defined as a P value less than 0.05. All statistical analyses were conducted with the PASW Statistics 18 program (SPSS, Inc., Chicago, IL).

Results

Study Population. All 163 patients included in the previous EN-Vie study were eligible, and all centers, except one, that took part in the first study agreed to participate. Finally, 157 patients were included in the current study (Belgium, N = 5; France, N = 35; Germany, N = 14; Great Britain, N = 29; Italy, N = 18; Spain, N = 33; Switzerland, N = 4; The Netherlands, N = 19).

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Table 1. Baseline Characteristics of the 157 Patients Included in the Study

Characteristic	n (%) or Median and Range
Gender: male	67 (42.7)
Age at diagnosis	37 (16-83)
Symptoms at diagnosis:	
Ascites	128 (81.5)
Edema of lower limbs	43 (27.4)
Abdominal pain	97 (61.8)
Esophageal varices	45/73 (patients with EGD)
Hepatic encephalopathy	14 (8.9)
Hepatorenal syndrome	11 (7)
GI bleeding	8 (5.1)
Laboratory at diagnosis	
ALT (U/L)	60 (12-10,011)
AST (U/L)	52 (10-5,122)
Albumin (g/L)	34 (17-55)
Creatinine (umol/L)	79.6 (36-589)
Bilirubin (umol/L)	31 (4-325)
Prothrombin time (quick time %)	62 (7-100)
INR	1.4 (1.0-10.9)
Child-Pugh score	8 (5-13)
Rotterdam score	1.25 (0.02-3.57)
Rotterdam class (I/ II/ III)*	43(27)/76(48)/35(22)

Abbreviation: EGD, esophageal gastroduodenoscopy.
*In 3 patients, the Rotterdam score was not possible to be calculated.

Overall median follow-up of these 157 patients was 50 months (range, 0.1-74.0). Twenty-six patients (17%) were lost to follow-up after a median time of 25 months (range, 0.3-61.0). The remaining 131 patients were followed until death (n = 36; 23%; median time to death: 10 months [range, 0.1-41.0]) or study closure (n = 95; 61%; median follow-up: 57 months [range, 43-74]).

Baseline Characteristics. Table 1 describes the baseline characteristics. Median age at diagnosis of BSC was 37 years (range, 16-83), and 90 patients (57.3%) were female.

Etiologic Factors. Supporting Table 1 describes the etiology for the total study population. With reference to the original EN-Vie study, we found additional causal factors in 12 patients: myeloproliferative neoplasms in 7; celiac disease in 2; and antiphospholipid syndrome, factor V Leiden mutation, and hyperhomocysteinemia in 1 each.

Management. One hundred and thirty-nine patients (88.5%) received long-term anticoagulation. Twenty-eight bleeding complications occurred in 24 patients (17%) during the study. Main causes of bleeding were portal hypertension (PH) related (n = 14; 2 died), intracranial hemorrhage (n = 3; 1 died), and abdominal wall bleeding (n = 2), genital bleeding (n = 2), bronchial bleeding (n = 1), and peptic ulcer (n = 1; all alive). Figure 1 shows the flowchart of treatments received by patients.

Angioplasty/Thrombolysis. Twenty-two patients underwent angioplasty (n = 13), thrombolysis (n = 7), or both (n = 2) as first invasive treatment. In 6 of these 22 patients, a vascular stent was placed at the time of angioplasty. After this initial intervention, 14 patients (64%) required further treatment with either

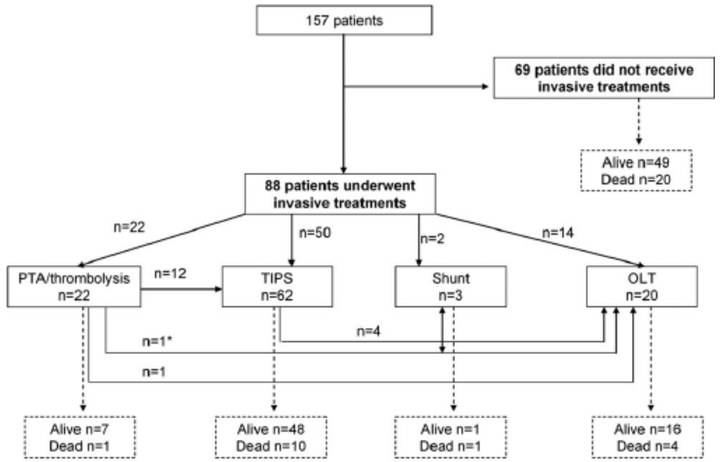

Fig. 1. Invasive treatments applied to patients included in the study.

TIPS (N = 12) or OLT (N = 2) after a median time of 1.5 months (range, 0.2-19.0) (Fig. 1). The remaining 8 patients were only treated with angioplasty/thrombolysis (in 2 patients more than once). Seven of them are alive and free of ascites with a median follow-up of 47 months (range, 32-61), but 1 died 6 months later as a result of liver failure.

TIPS. Sixty-two patients underwent TIPS (39.5%). Main indications were refractory ascites (69%), liver failure (13%), and variceal bleeding (7%). Four of these (6.45%) had rescue OLT a median of 1.8 months after TIPS (range, 0.03-13.0) for the following reasons: HE (n = 1); fulminant liver failure (N = 1); and TIPS thrombosis with refractory ascites (N = 2). Three of these four patients died a median of 35 months after OLT (range, 7-45) as a result of liver failure (N = 2) and extrahepatic malignancy (N = 1). Of the remaining 58 patients, 10 (17%) died within 5.8 months (range, 0.2-39) and 48 (83%) were alive after a median follow-up of 51 months (range, 0.3-69.0).

Thus, overall, 13 patients died, 9 of them resulting from a liver-related cause. One, 3-, and 5-year actuarial survival and OLT-free survival of patients treated with TIPS was 88%, 83%, and 72% and 85%, 78%, and 72%, respectively (Fig. 2). Similar results were found if deaths clearly unrelated to liver disease were removed from the analysis or considering the date of TIPS as time zero (data not shown).

Median time from diagnosis to TIPS was 1 month (range, 0-38). Indeed, 50% of TIPS were placed in the first month, 60% in the first 3 months after diagnosis, 73% in the first 6 months, and 84% in the first 12 months. Patients who underwent TIPS in the first month had more-severe liver disease at diagnosis, as shown by a worse Rotterdam score (1.54 ± 0.59 versus 1.18 ± 0.77; $P = 0.017$) and Child-Pugh score (9.3 ± 1.7 versus 7.8 ± 1.9; $P < 0.000$). However, no differences in overall survival or OLT-free survival were observed in patients with TIPS performed before or after the first month after diagnosis. Similar results were observed when comparing patients receiving TIPS before or later than 3 or 6 months from diagnosis (data not shown).

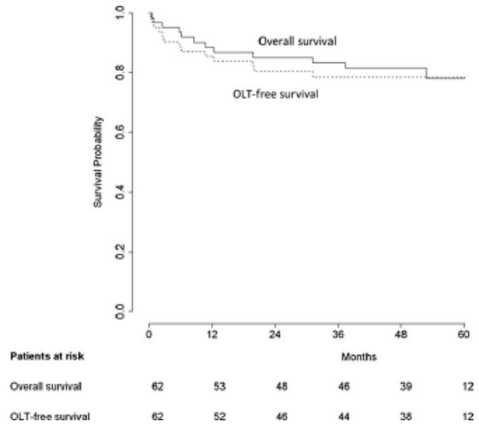

Fig. 2. Overall survival and OLT-free survival in patients treated by TIPS (n = 62).

On univariable analysis, only age and BCS-TIPS PI score (either as continuous or categorical variable [≥7 points])[6] were significantly associated with survival or OLT-free survival (Supporting Tables 2 and 3). At multivariable analysis, only BCS-TIPS PI score was shown to be independently associated with survival and OLT-free survival. Because BCS-TIPS PI score was obtained at diagnosis, we performed a sensitivity analysis including only the 45 patients receiving TIPS in the first 6 months after diagnosis, obtaining similar results. No additional variables could improve the predictive ability of BCS-TIPS PI score in multivariable or classification and regression tree models (data not shown).

Portosystemic Shunting. Three patients underwent a side-to-side portocaval shunt (2%), in 2 after an attempt at TIPS was unsuccessful. One patient developed shunt thrombosis and died soon thereafter, and another patient underwent OLT 9.8 months after shunt placement as a result of refractory ascites, despite shunt patency, and is alive at the end of follow-up. The third patient was alive and free of ascites at the end of follow-up.

OLT. Twenty patients received OLT (12.7%) a median of 2.3 months (range, 0-24) after BCS diagnosis. Sixty percent and 85% of OLT were performed in the first 6 and 12 months after diagnosis, respectively. Main indications for OLT were liver failure (40%), refractory ascites (35%), and variceal bleeding (10%). One, 3-, and 5-year actuarial survival after OLT was 95%, 89%, and 78%, respectively.

In 15 patients, OLT was the first-line proposed treatment (n = 14) or after angioplasty failure (n = 1). These 15 patients had more-frequent HE ($P = 0.006$) as well as higher Rotterdam score ($P = 0.004$) and class ($P = 0.002$) at diagnosis than the 62 patients receiving TIPS (n = 50 as first-line treatment and n = 12 after initial angioplasty failure) (Supporting Table 4). Despite this, no significant differences in survival were observed among groups (Supporting Fig. 1).

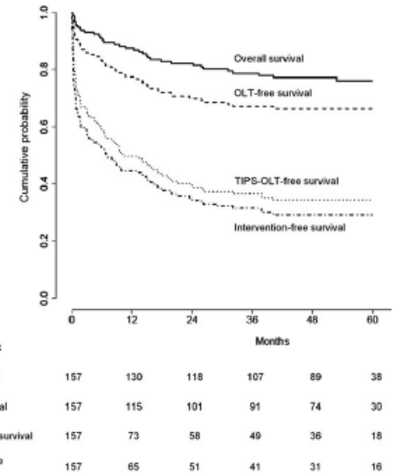

Fig. 3. Cumulative probability of overall, OLT-free, TIPS-OLT-free, and intervention-free survival. Each curve represents, in the entire cohort of patients, the cumulative probability of being free of an event, defined as follows. The upper curve shows overall survival. The second curve shows the rate of development of death or transplantation (OLT-free survival). The third curve refers to a composite event defined as TIPS or death or transplantation (survival free of TIPS and OLT). In the lower curve, the endpoint is any invasive intervention or death (survival free of intervention). Overall survival rates at 1, 3, and 5 years were 88% (95% CI: 83-93), 79% (95% CI: 72-86), and 74% (95% CI: 66-82), respectively. Respective OLT-free survival rates were 77% (95% CI: 71-84), 67% (95% CI: 59-75), and 64% (95% CI: 56-73). Respective TIPS-OLT-free survival rates were 50% (95% CI: 43-59), 37% (95% CI: 30-45), and 34% (95% CI: 28-43). Intervention-free survival rates were 45% (95% CI: 37-53) at 1 year, 31% (95% CI: 25-40) at 3 years, and 29% (95% CI: 23-37) at 5 years.

Similar results were found when comparing TIPS or OLT as first-line intervention after excluding those patients with previous angioplasty/thrombolysis (50 TIPS versus 14 OLT; $P = 0.29$).

Long-Term Post-Therapeutic Prognosis of BCS. Figure 3 shows the cumulative overall, OLT-free, TIPS-OLT–free and (any) intervention-free survival.

Intervention-Free Survival. Sixty-nine patients did not undergo any invasive intervention during the study. Twenty died after a median time of 11 months (range, 0.10-40.0), only 2 as a result of non-liver-related death. The remaining 49 were alive after a median of 55 months (range, 0.7-69.0).

Uni- and multivariable analysis for intervention-free survival is detailed in Supporting Table 5. The Rotterdam score had an excellent prognostic value, and no further variable could significantly improve its prognostic ability. This validates the Rotterdam score as a useful prognostic tool in this post-therapeutic series of BCS. Supporting Fig. 2 shows survival curves for Rotterdam class I, II, and III.

Because the Rotterdam score includes the INR, which could not be calculated in a substantial number of patients (already on oral anticoagulants), we performed a multivariable analysis without including scores or INR. Baseline ascites, bilirubin, and creatinine were independently associated with intervention or death (BCS-intervention-free survival prognostic score [BCIS score]: ascites [yes = 1, no = 0]*1.675 + ln creatinine [umol/L]*0.613 + ln bilirubin [umol/L]*0.440). This data-driven new score showed an adequate discrimination (area under the curve [AUC] = 0.819), but it did not outperform the Rotterdam score (AUC, 0.821)[9] (Supporting Fig. 3). The probability of intervention-free survival among different intervals of the BCIS score is shown in Supporting Table 7.

Mortality. Thirty-six patients (23%) died during the study. Median time to death was 10 months (range, 0.1-41.0). Main causes of death are reported in Table 2. Factors associated with mortality are shown in Supporting Table 6. The BCS-TIPS PI score was strongly associated with the risk of death, so that no other variable could improve its predictive capacity. Supporting Table 8 shows survival among different ranges of BCS-TIPS PI scores. Because this score includes the INR, we performed a multivariable analysis excluding all scores and INR. Age, bilirubin, and creatinine were independently associated with survival [BCSurvival score: age/10*0.370 + ln creatinine [umol/L]*0.809 + ln bilirubin [umol/L]*0.496). The discriminative capacity was comparable to that of the BCS-TIPS PI score and better than the Rotterdam score (Supporting Fig. 4).

Discussion

BCS is a rare, life-threatening disorder caused by obstruction of hepatic venous outflow. Until recently, most evidence regarding BCS was generated in small retrospective studies of patients diagnosed over long periods and managed using heterogeneous

Table 2. Causes of Death

Related or Probably Related Liver Deaths (n = 30)	Non-Liver-Related Deaths (n = 6)
Liver failure (n = 12)	Extrahepatic malignancy (n = 1);
Multiorgan failure (n = 4)	Complication/progression of hematological disease (n = 4);
GI bleeding (n = 2)	Intracraneal hemorrhage (n = 1)
Sepsis (n = 4)	
Hepatobiliary malignancy (n = 2)	
Unknown (n = 6)	

strategies.[7,9,14] However, an international initiative, funded by the Fifth Framework Program of the European Commission, entitled the EN-Vie, was able to prospectively gather a large multicenter cohort of consecutive patients with BCS diagnosed and treated following homogeneous criteria.[4] Previous retrospective studies evaluating prognosis in BCS showed that fatal events occur throughout the first 5 years after diagnosis.[7,9,10,14] Therefore, it is feasible that prognostic predictors were underestimated in the initial EN-Vie study where the median follow-up was short (only 17 months) and the number of events relatively low.[4] Indeed, in the present extended EN-Vie study, surviving patients were followed up more than 3 additional years, and during this additional period, 8 patients received TIPS, 2 OLT, and 7 died. Thus, the present study was able to evaluate long-term outcome of BCS patients (median follow-up of almost 5 years, with a minimum of 43 months).

Our updated data confirm that, in Western countries, a step-wise therapeutic strategy confers good long-term survival in patients with BCSurvival score. Most of our patients (88.5%) received long-term anticoagulation. Interestingly enough, the rate of bleeding complications in patients receiving anticoagulation was lower than that previously reported.[15] This is most likely the result of more adequate prevention of PH complications as well as careful management of anticoagulation during invasive procedures.[15]

Only 22 patients (14%) underwent angioplasty/thrombolysis as primary invasive therapy, and only 8 of them did not require further intervention, such as TIPS, surgical shunt, and/or OLT. It seems that angioplasty/stenting, although an attractive, minimally invasive technique with the potential of restoring physiological sinusoidal flow, has low applicability in the treatment of our BCS patients. These results contrast with a recent retrospective study from China showing a great applicability and efficacy of angioplasty/stenting in a large cohort of patients with BCS.[16] In our opinion, these differences could be most likely explained by different pathogenic mechanisms of hepatic venous outflow obstruction,[8] because hepatic vein stenoses are less frequent in the Western world than in Eastern countries. Therefore, angioplasty/stenting remains a potentially valuable treatment of the BCS subtype with short-length stenosis and investigation of the patients' suitability for this approach is mandatory, because the benefits are potentially significant.

Strikingly, no additional patient received a surgical shunt during the extended follow-up period, and thus only 3 patients (2%) received this therapeutic modality. TIPS has emerged as the preferred derivative treatment in Europe. The fact that two recent small retrospective studies from North America have shown excellent outcomes of BCS patients after surgical shunts does, in our opinion, not change the trend in current practice to prefer less-invasive over more-invasive procedures.[17,18] Moreover, we would like to emphasize that previous multicenter retrospective studies were unable to demonstrate a solid survival advantage in BCS patients treated with surgical shunts.[7,19-22] The low number of patients treated with surgical shunting in our data set precludes shedding more light on this issue.

Sixty-two patients required TIPS as rescue therapy after failures of medical or minimally invasive treatments (angioplasty/stenting/thrombolysis). Overall survival and OLT-free survival was comparable to that observed in a previous retrospective multicenter European study including 124 BCS patients treated with TIPS.[6] These results confirm that TIPS is an effective, safe rescue therapy in patients with BCS. Interestingly, although most TIPS were placed during the first year after diagnosis, the timing was not uniform, ranging from 0 to 38 months. One of the major concerns in the management of patients with BCS is whether delaying the use of a rescue TIPS could influence outcome. Our data showed a good outcome after TIPS, regardless of whether the procedure was performed soon after diagnosis or later during follow-up. This outcome, which requires further confirmation, suggests that the approach of close clinical surveillance while reserving TIPS for those patients who progress or fail to respond to medical treatment does not have a deleterious effect on outcome. Furthermore, the current study validates our previously reported BCS-TIPS PI score >7[6] as the only independent factor associated with poor survival and OLT-free survival after TIPS. Whether the initial use of OLT in these patients with a high BCS-TIPS PI score may improve outcome needs to be proved. Comparing the subgroup of patients that received TIPS to those with OLT as first invasive therapy, we found that both groups had similar long-term outcome, despite the OLT subgroup of patients having had worse hepatic disease at presentation. Unfortunately, our current data do not allow us to asses the potential role of OLT as an initial procedure in these sickest patients.

Fifty-six percent of our patients underwent an invasive therapeutic procedure, most of them within the first year after diagnosis. In contrast with the population from which the Rotterdam score was defined,[9] TIPS and OLT have been more widely used.

Nevertheless, our study validates the use of the Rotterdam score for predicting the need of invasive intervention and death in this more-recent, prospectively studied cohort of BCS patients.

The new score (BCIS score) has an almost identical discrimination capacity to that obtained with the Rotterdam score, but with some potential advantages, including the exclusion of subjective parameters, such as the presence or absence of HE and INR in patients that may have initiated anticoagulation.[9] We cannot dismiss the influence of more-rapid intervention in the sickest patients, which may have influenced our findings in relation to predicting intervention-free survival.

Another important finding of our study was that the BCS-TIPS PI score showed adequate accuracy in predicting mortality in the overall cohort of patients and better predictive capacity than the Rotterdam score. In addition, in the present study, we have identified a new survival score (BCIS score) that has an almost identical discrimination capacity to that obtained with the BCS-TIPS PI score, but with the potential advantage of not including the INR within its determinants. This may be important, because many patients may already be on anticoagulation when they arrive at referral centers.

In contrast to previous studies, validation of previous scores and identification of new ones has been done in a large cohort of patients, prospectively recruited in a short period of time and managed in a homogeneous step-wise invasive strategy.

In summary, our study validates a therapeutic algorithm aimed at providing a general framework for evidence-based decision making in patients with BCS. In addition, the present study validates the Rotterdam score for predicting intervention-free survival and BCS-TIPS PI score for survival. Furthermore, we report on two new prognostic scores that may help to better inform the choice of treatment strategy in any given BCS patient, but which need to be validated in future prospective multicenter studies.

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Supplementary table 1. Thrombotic risk factors of the 157 patients included in the study.

Risk factor	n (%)
Inherited thrombophilia	
Factor V Leiden	19 (12.1)
Prothrombin gene G20210A mutation	5 (3.2)
Protein C deficiency	5 (3.1)
Protein S deficiency	3 (1.9)
Antithrombin deficiency	4 (2.5)
Acquired thrombophilia	
Antiphospholipid syndrome	37 (24)
Hyperhomocysteinemia	29 (18)
Paroxysmal nocturnal hemoglobinuria	15 (9.6)
Myeloproliferative neoplasms (MPN)	52 (33)
Polycythemia vera	28 (18)
Essential thrombocythemia	12 (7.6)
Idiopathic myelofibrosis	4 (2.5)
Unclassified or occult	8 (5)
Hormonal factors	
Oral contraceptive use	29 (18.5)
Pregnancy within 3 months before diagnosis	6 (3.8)
Systemic diseases and local factors	37 (24)

Supplementary table 2. Univariable and multivariable analysis for predicting survival of baseline characteristics in the 62 patients that underwent TIPS.

	Univariable Cox model		
Variables at baseline	**HR**	**95%CI**	**P value**
Sex (male)	1.608	0.539-4.796	0.393
Age at diagnosis (per 10 years increase)	**1.795**	**1.250-2.579**	**0.001**
Ascites at diagnosis (present vs absent) #	-	-	0.345
HE at diagnosis (present vs absent)	0.991	0.129-7.622	0.993
Hepatomegaly (present vs absent)	0.507	0.163-1.573	0.254
Splenomegaly (present vs absent)	0.577	0.193-1.720	0.323
Leukocytes (per unit increase)	0.023	0.928-1.128	0.657
Platelet count (per unit increase)	0.999	0.996-1.002	0.440
lnALT (per unit increase)	0.747	.437-1.278	0.256
lnAST (per unit increase)	0.978	0.55-1.722	0.985
FALK ULN (times upper limit normal)	1.181	0.869-1.604	0.340
Albumin levels (per unit increase) *	0.955	0.869-1.050	0.340
Bilirubin levels (per mmol/dl increase)	1.007	1.000-1.013	0.096
Creatinine (per 100mmol/dl increase) *	1.469	0.999-2.162	0.108
INR (per unit increase)	0.511	0.095-2.750	0.375
Rotterdam score (per unit increase)	1.424	0.635-3.195	0.412
Rotterdam class at diagnosis # Class II vs class I Class III vs class I	-	-	0.229
Child score (per unit increase)	1.089	0.834-1.421	0.533
Child Class B vs A C vs A	 1.082 1.244	 0.224-5.231 0.227-6.812	0.962
MELD (per unit increase)	1.051	0.986-1.121	0.155
TIPS 1 month (previous vs after 1 month)	1.410	0.472-4.213	0.538
TIPS 3 month (previous vs after 3 months)	1.958	0.599-6.408	0.250
TIPS 6 month (previous vs after 6 months)	0.996	0.305-3.245	0.994
Time to TIPS (per 1 month increase)	0.979	0.909-1.054	0.540
BCS-TIPS PI score (per unit increase) *	**1.722**	**1.259-2.354**	**0.002**
BCS-TIPS PI (≥7 points)	**9.448**	**2.466-36.192**	**0.007**

no deaths were observed in patients without ascites or Rotterdam class I at diagnosis.
* variables selected for multivariate analysis. Age and Bilirubin were not included in the multivariable analysis since they are included in the BCS-TIPS PI score. **Multivariate analysis only showed BCS-TIPS PI score as independent factor for survival in patients with TIPS.**
Abbreviations. HE: hepatic encephalopathy; ALT: alanine aminotransferase; AST: aspartate aminotransferase FALK ULN: phosphatase alkaline upper limit of normal; INR: International normalized ratio; BCS-TIPS PI: BCS-TIPS prognostic index.

Supplementary table 3. Univariable and multivariable analysis for predicting OLT-free survival of baseline characteristics in the 62 patients that underwent TIPS.

	Univariable Cox model		
Variables at baseline	**HR**	**95%CI**	**P value**
Sex (male)	1.750	.0606-1.055	0.298
Age at diagnosis (per 10 years increase)	**1.717**	**1.215-2.424**	**0.002**
Ascites at diagnosis (present vs absent) #	-		0.352
HE at diagnosis (present vs absent)	0.911	0.119-6.968	0.927
Hepatomegaly (present vs absent)	0.636	0.211-1.917	0.432
Splenomegaly (present vs absent)	0.510	0.177-1.472	0.210
Leukocytes (per unit increase)	1.024	0.934-1.124	0.622
Platelet count (per unit increase)	0.999	0.996-1.002	0.453
lnALT (per unit increase)	0.709	0.424-1.188	0.153
lnAST (per unit increase)	0.925	0.537-1.593	0.773
FALK ULN (times upper limit normal)	1.156	0.858-1.557	0.388
Albumin (per unit increase) *	0.961	0.876-1.053	0.391
Bilirubin (per mmol/dl increase)	1.006	1.000-1.012	0.111
Creatinine (per 100 units increase) *	1.438	0.995-2.078	0.109
INR (per unit increase)	0.546	0.116-2.581	0.388
Rotterdam score (per unit increase)	0.470	0.608-2.942	0.486
Rotterdam class at diagnosis Class II vs class I Class III vs class I			0.240
Child score (per unit increase)	1.104	0.854-1.428	0.453
Child Class B vs A C vs A	 1.014 1.425	 0.210-4.891 0.276-7.355	0.833
MELD (per unit increase)	1.053	0.991-1.119	0.123
TIPS 1 month (previous vs after 1 month)	1.557	0.540-4.493	0.409
TIPS 3 month (previous vs after 3 months)	2.071	0.647-6.630	0.200
TIPS 6 month (previous vs after 6 months)	1.089	0.341-3.484	0.885
Time to TIPS (per 1 month increase)	0.974	0.904-1.05	0.458
BCS-TIPS PI score (per unit increase)*	**1.631**	**1.210-2.199**	**0.003**
BCS-TIPS PI (≥7 points)	**7.044**	**1.909-25.987**	**0.015**

no deaths were observed in patients without ascites at diagnosis.
* variables selected for multivariate analysis. Age and and Bilirubin were not included in the multivariable analysis since they are included in the TIPS score. **Multivariate analysis only showed TIPS score as independent factor for OLT-free survival in patients with TIPS.**
Abbreviations. HE: hepatic encephalopathy; ALT: alanine aminotransferase; AST: aspartate aminotransferase FALK ULN: phosphatase alkaline upper limit of normal; INR: International normalized ratio; BCS-TIPS PI: BCS-TIPS prognostic index.

Supplementary table 4. Clinical and biochemical characteristics of patients submitted to OLT or TIPS.
Results are expressed in mean or %.

	TIPS vs OLT (n=62 vs 15)	
Variables at baseline	**Mean or %**	**p**
Gender (male)	45 vs 40	0.779
Age (years)	38 vs 41	0.464
Ascites (present)	95 vs 94	1.000
HE (present)	**8 vs 40**	**0.006**
HRS (present)	8 vs 20	0.182
ALT (U/L)	379 vs 2103	0.105
AST (U/L)	279 vs 1792	0.084
FALK ULN	1.57 vs 1.78	0.607
Creatinine (umol/L)	102 vs 108	0.975
Albumin (g/L)	32 vs 34	0.357
Bilirubin (umol/L)	50 vs 47	0.871
Hematocrit (%)	43 vs 42	0.675
INR	1.5 vs 3.5	0.127
Quick (%)	58 vs 41	0.070
Child score	8.7 vs 9.3	0.304
Child class	A 12 vs 9% B 57 vs 36% C 31 vs 55%	0.321
Rotterdam score	**1.4 vs 1.9**	**0.004**
Rotterdam class	**Class I 13 vs 7%** **Class II 67 vs 27%** **Class III 20 vs 67%**	**0.002**
BCS-TIPS score (points)	4.4 vs 5.4	0.069
BCS-TIPS class	Class I 93 vs 92% Class II 7 vs 8%	1.000

Abbreviations. HE: hepatic encephalopathy; HRS: hepatorenal syndrome; ALT: alanine aminotransferase; AST: aspartate aminotransferase FALK ULN: phosphatase alkaline upper limit of normal; INR: International normalized ratio; BCS-TIPS PI: BCS-TIPS prognostic index.

Supplementary table 5. Univariable and multivariable analysis for baseline factors associated to the probability of invasive intervention (Angioplasty, TIPS, Portosystemic shunting or OLT) or death during the follow up.

	Univariable Cox model			Multivariable Cox Model		
Variables at baseline	**HR**	**95%CI**	**p value**	**HR**	**95%CI**	**p value**
Sex (male)	1.290	0.883-1.885	0.191			
Age at diagnosis (per 10 years increase)	1.059	0.990-1.194	0.349			
Ascites (clin) at diagnosis (present vs absent) *	**6.102**	**2.824-13.185**	**0.000**	**5.321**	**2.122-13.344**	**0.000**
HE at diagnosis (present vs absent) *	**3.836**	**2.151-6.842**	**0.000**			
Hepatomegaly (present vs absent)	1.066	0.705-1.613	0.760			
Splenomegaly (present vs absent)	0.214	0.870-1.863	0.212			
Leukocytes (per unit increase)*	**1.049**	**1.018-1.080**	**0.003**			
Platelet count (per unit increase)*	**1.001**	**1.000-1.002**	**0.046**			
lnALT (per unit increase)*	**1.326**	**1.154-1.524**	**0.000**			
lnAST (per unit increase)*	**1.504**	**1.291-1.752**	**0.000**			
FALK ULN (times upper limit normal)	1.072	0.982-1.169	0.167			
Albumin (per unit increase) *	**0.955**	**0.929-0.982**	**0.001**			
lnBilirubin (per unit increase)	**1.006**	**1.003-1.008**	**0.001**			
Ln Bilirubin (per unit increase)*	**1.985**	**1.578-2.497**	**0.000**	**1.543**	**1.212-1.964**	**0.001**
lnCreatinine (per 100 mmol/dl increase)	**1.666**	**1.356-2.047**	**0.000**			
Ln Creatinine (per unit increase)*	**2.587**	**1.760-3.803**	**0.000**	**1.846**	**1.239-2.749**	**0.005**
INR (per unit increase)	**1.210**	**1.035-1.413**	**0.040**			
Rotterdam score (per unit increase)	**2.455**	**1.886-3.196**	**0.000**			
Rotterdam class at diagnosis # **Class II vs class I** **Class III vs class I**	**5.139** **6.427**	**2.853-9.258** **3.392-12.179**	**0.000**			
Child score (per unit increase)	**1.465**	**1.311-1.637**	**0.000**			
Child Class **B vs A** **C vs A**	**2.578** **8.883**	**1.330-4.997** **4.360-18.098**	**0.000**			
MELD (per unit increase)	**1.076**	**1.051-1.100**	**0.000**			
PI TIPS score (per unit increase)	1.120	1.002-1.252	0.056			
PI TIPS (≥7 points)	0.794	0.412-1.530	0.504			

* variables included in the multivariable analysis.
Abbreviations. HE: hepatic encephalopathy; ALT: alanine aminotransferase; AST: aspartate aminotransferase FALK ULN: phosphatase alkaline upper limit of normal; INR: International normalized ratio; BCS-TIPS PI: BCS-TIPS prognostic index.

Supplementary table 6. Univariable and multivariable analysis for overall survival of the 157 patients included in the study

	Univariable Cox model			Multivariable Cox Model		
Variables at baseline	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Sex (male)*	1.645	0.854-3.167	0.137			
Age at diagnosis (per 10 years increase) *	**1.603**	**1.316-1.953**	**0.000**	**1.448**	**1.174-1.786**	**0.001**
Presence of myeloproliferative neoplasm (present vs absent)	0.996	0.498-1.992	0.990			
Ascites at diagnosis (present vs absent) #*	**2.813**	**0.862-9.176**	**0.048**			
HE at diagnosis (present vs absent)*	1.439	0.509-4.071	0.513			
Hepatomegaly (present vs absent)	1.252	0.604-2.597	0.539			
Splenomegaly (present vs absent)	1.097	0.568-2.118	0.783			
Leukocytes (per unit increase)*	1.048	1.000-1.099	0.062			
Platelet (per unit increase)	1.000	0.998-1.002	0.824			
lnALT (per unit increase)*	1.038	0.829-1.301	0.747			
lnAST (per unit increase)*	1.218	0.986-1.506	0.086			
FALK ULN (times upper limit normal)	1.144	1.009-1.297	0.084			
Albumin (per unit increase) *	**0.951**	**0.906-0.998**	**0.038**			
lnBilirubin (per unit increase)	**1.008**	**1.004-1.012**	**0.002**			
Ln Bilirubin (per unit increase)*	**2.304**	**1.499-3.542**	**0.000**	**1.643**	**1.071-2.518**	**0.023**
lnCreatinine (per 100 mmol/dl increase)	**1.644**	**1.292-2.091**	**0.001**			
Ln Creatinine (per unit increase)*	**2.726**	**1.575-4.720**	**0.002**	**2.246**	**1.206-4.182**	**0.011**
INR (per unit increase)	0.942	0.656-1.353	0.729			
Rotterdam score (per unit increase)	**1.606**	**1.065-2.421**	**0.029**			
Rotterdam class at diagnosis **Class II vs class I** **Class III vs class I**	**3.523** **3.633**	**1.213-10.231** **1.139-11.589**	**0.020**			
Child score (per unit increase)	**1.258**	**1.064-1.487**	**0.008**			
Child Class **B vs A** **C vs A**	**2.022** **3.938**	**0.675-6.055** **1.283-12.091**	**0.029**			
MELD (per unit increase)	**1.045**	**1.014-1.077**	**0.009**			
BCS-TIPS PI score (per unit increase)	**1.436**	**1.223-1.687**	**0.000**			
BCS-TIPS PI (≥7 points)	**5.122**	**2.296-11.430**	**0.001**			

* variables included in the multivariable analysis.
Abbreviations. HE: hepatic encephalopathy; ALT: alanine aminotransferase; AST: aspartate aminotransferase FALK ULN: phosphatase alkaline upper limit of normal; INR: International normalized ratio; BCS-TIPS PI: BCS-TIPS prognostic index.

Supplementary table 7.
Probability of intervention-free survival among different intervals of the BCIS score.

Probability	**BCIS score intervals**		
	Interval I (Up to 5 points)	Interval 2 (5-6 points)	Interval 3 (6-highest)
Alive	78.3%	27.8%	6.8%
Invasive treatment or dead	21.7%	72.2%	93.2%

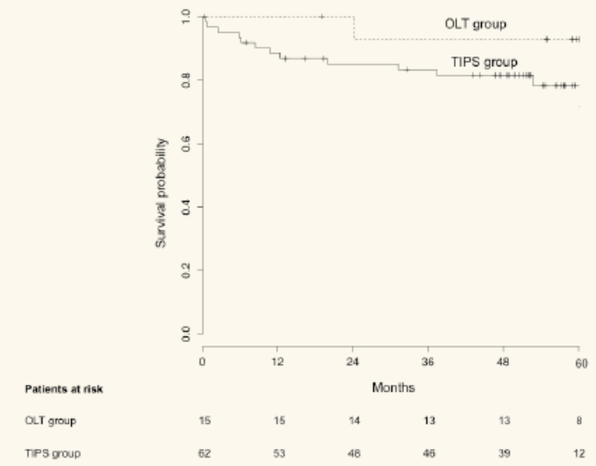
Supplementary table 8.
Probability of survival among different intervals of the BCS-TIPS PI score.

Probability	**BCS-TIPS PI score intervals**		
	Interval I (Up to 5 points)	Interval 2 (5-6 points)	Interval 3 (6-highest)
Alive	88.4%	57.7%	47.8%
Dead	11.6%	42.3%	55.2%

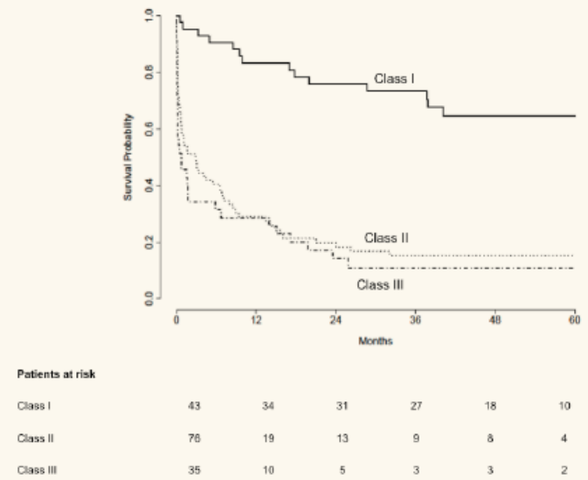
Supplementary table 9.
Probability of survival among different intervals of the BCSurvival score.

Probability	**BCSurvival score intervals**		
	Interval I (Up to 7 points)	Interval 2 (7-8)	Interval 3 (8 highest)
Alive	87.5%	63.3%	42.9%
Dead	12.5%	36.7%	57.1%

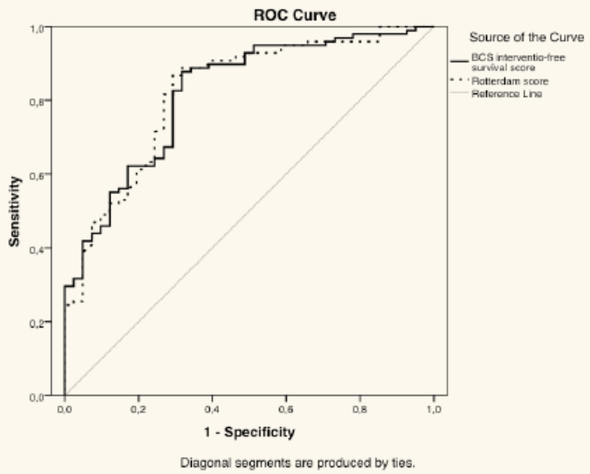
Supplementary figure 1.
Overall mortality in the 77 patients that underwent invasive treatments (62 TIPS vs 15 OLT). Thirteen patients died in the group of TIPS and 1 in the group of OLT; p=0.150.



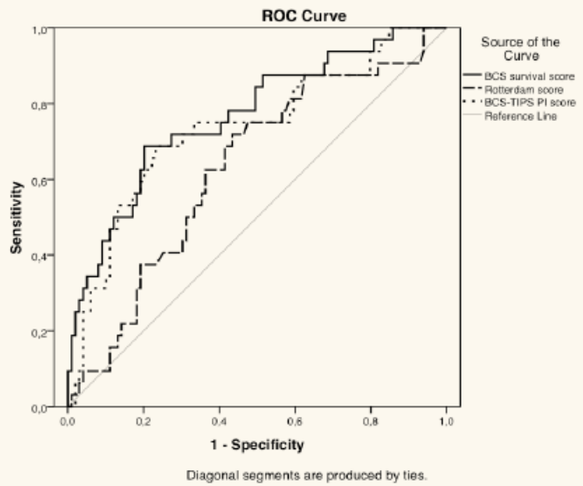
Supplementary figure 2.
Kaplan-Meier curves for intervention free survival rates among Rotterdam score class. This score have a significant impact on intervention-free survival, with a remarkable better rate in patients with class I as compared to those in classes II or III (p<0.000).



Supplementary figure 3. Intervention-free survival.
ROC curves for BCS intervention-free survival score showed a good discrimination capacity (AUC 0.819) but similar to that for the Rotterdam score (AUC 0.821).



Supplementary figure 4. Survival.
ROC curves for survival of the BCS survival score that showed an adequate discrimination capacity based on AUC (AUC 0.767), similar to BCS-TIPS PI score (AUC 0.734) and superior to Rotterdam score (AUC 0.640).



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Resumen de los resultados

RESUMEN DE LOS RESULTADOS

Estudio 1. ***Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension.***

— En nuestra serie, 19 de los 39 (49%) pacientes con HTPI presentaron en la venografía hepática comunicantes venosas entre diferentes venas suprahepáticas o diferentes segmentos de una misma vena suprahepática (CVVH). En 12 de estos casos no pudo lograrse una correcta PSHE.

— En nuestra cohorte de HTPI fue posible obtener una correcta PSHE en 27/39 (69%) de los casos, con un valor medio de GPVH de 7.0±*3.0* mmHg. El GPVH fue normal (≤ 5 mmHg) en 6 pacientes, discretamente elevado (5-10 mmHg) en 16 y ≥10 mmHg en los 5 pacientes restantes.

— En los 12/39 (31%) pacientes restantes no se pudo obtener una correcta PSHE debido a la presencia de CVVH. En estos pacientes la PSHE fue discretamente inferior que en aquellos pacientes en los que se consiguió una correcta presión enclavada, aunque sin alcanzar significación estadística (p=0.10).

— Doce de los 39 (31%) pacientes con TVPNC también presentaban CVVH. Este porcentaje fue inferior del hallado en la HTPI aunque no significativamente (31% vs 49%; p=0.16). Estas CVVH impidieron obtener una adecuada PSHE en 6/39 casos de TVPNC. Por lo tanto, se obtuvo finalmente una correcta PSHE en 33/39 pacientes, que mostraban un GPVH medio de 3.5±2.0 mmHg. GPVH fue significativamente mayor en pacientes HTPI que en pacientes con TVPNC (p<0.001). Ninguno de los pacientes con cirrosis e hipertensión portal de nuestra cohorte del estudio tenía CVVH. El GPVH medio de los pacientes con cirrosis fue de 17.0±3.0 mmHg, significativamente mayor que la observada en pacientes HTPI (p<0.001).

— Los pacientes con HTPI presentaron una hemodinámica sistémica y pulmonar dentro de los límites normales. Sólo 6 pacientes con HTPI mostraron circulación hiperdinámica y uno hipertensión pulmonar. No hubo diferencias significativas en la hemodinámica sistémica y pulmonar o en el número de pacientes con circulación hiperdinámica entre los tres grupos de pacientes: HTPI, TVPNC o cirrosis (matcheados por sexo, función hepática y signos de hipertensión portal).

— Los pacientes con HTPI mostraron un valor medio de ET de 8.4±3.3 kPa. En 53.3% de los pacientes con HTPI la ET fue >7.8 kPa, umbral definido para fibrosis significativa. Sólo 2 de estos pacientes presentaron valores de ET >13.6 kPa (16.3 y 18.5 kPa), el umbral mínimo para CSPH en cirrosis.

— Los pacientes con TVPNC presentaron una ET media de 6.4±2.2 kPa. Los pacientes con TVPNC tenía una ET significativamente más baja que los pacientes con HTPI (p=0.005). Como era de esperar, la ET fue marcadamente mayor en los pacientes con cirrosis que en pacientes HTPI, con un valor medio de 40.9±20.5 kPa (p <0.001).

Estudio 2. ***Metabolomics discloses potential biomarkers for the non-invasive diagnosis of idiopathic portal hypertension.***

— El análisis PLS-DA (*Partial Least Squares Projection to Latent Structures regression with Discriminant Analysis*) permitió una clara separación de los pacientes con HTPI de los pacientes con cirrosis con un modelo que incluye 28 metabolitos con un R^2 de 0.77, un Q^2 de 0.67 y un umbral de VIP >2.2 con una AUC de 0.99. Cabe destacar que no se observaron diferencias en el perfil metabolómico entre los pacientes HTPI en base a la presencia o ausencia de infección por el VIH.

— Se llevó a cabo una validación cruzada interna dividiendo la muestra en la training set (~2/3 de la muestra) y la testing set (~1/3 de la muestra). En la training set nuestro modelo de 28 metabolitos permitió diagnosticar de forma correcta los pacientes con HTPI con una probabilidad mediana de 98.4%. Por otro lado, sólo el 7.6% de los pacientes con cirrosis eran erróneamente diagnosticados de HTPI. Estos resultados fueron confirmados en la testing set (probabilidades medianas de ser diagnosticados de HTPI: 97.9% en el grupo de HTPI y 9.4% en el grupo de cirrosis).

— El análisis PLS-DA también mostró una clara separación de los pacientes con HTPI de los controles sanos con un modelo que incluye 31 metabolitos con un R^2 de 0.82, un Q^2 de 0.71 y un umbral de VIP >2.1 con una AUC de 0.98.

— Tras la validación cruzada interna, nuestro modelo de 31 metabolitos permitió diagnosticar de forma adecuada los pacientes con HTPI con una probabilidad mediana de 99.5%. Por otro lado, utilizando este modelo sólo 7.7% controles sanos fueron mal diagnosticados como HTPI en la training set. Estos resultados fueron confirmados en la testing set (probabilidades medianas de ser diagnosticados de HTPI: 99.2% en el grupo de HTPI y 9.5% en el grupo de controles sanos).

Estudio 3. ***Bone morphogenetic protein receptor 2 in patients with idiopathic portal hypertension.***

— No se detectó ninguna mutación en el gen BMPR2 en los 21 pacientes no relacionados con HTPI incluidos en el estudio.

— Sin embargo, en 12 pacientes se detectaron polimorfismos de un solo nucleótido (SNPs). Cuatro pacientes presentaron un SNP en el intrón 5 (rs7575056), 4 pacientes tuvieron un SNP en el exón 12 (rs1061157) y 2 pacientes tenían un SNP en el exón 1 (5'UTR-301G>A). Otros dos pacientes tenían ambos polimorfismos en el intrón 5 y el exón 12. La prevalencia de estos SNPs en nuestros pacientes fue similar a la prevalencia descrita en la población caucásica sana.

— Mediante análisis de *Multiple Ligation Probe Amplification (MLPA) Reaction* se observó que 5 pacientes podían tener posibles duplicaciones en el gen BMPR2. Sin embargo, la PCR cuantitativa sólo confirmó tres duplicaciones (dos pacientes con la duplicación del exón 4 y uno de los pacientes que albergan la duplicación afectando los exones 11 y 12). Sin embargo, mediante estudios de expresión de ARNm se observó que estas duplicaciones no afectaban la expresión del gen.

Estudio 4. ***Good long-term outcome of Budd-Chiari syndrome with a step-wise management.***

— La mayoría de los pacientes con SBC de nuestra cohorte recibió un tratamiento escalonado: el 88.5% de los pacientes recibieron anticoagulación a largo plazo y el 56% de los pacientes fueron sometidos al menos a un tratamiento invasivo (22 pacientes angioplastia/trombolisis, 62 TIPS, 2 shunt quirúrgico portosistémico y 20 pacientes TH).

— Solo 8 de los de los 22 pacientes que recibieron angioplastia/trombolisis, no realizaron otro tratamiento de rescate. El TIPS fue el tratamiento invasivo más frecuentemente utilizado y en tan sólo 4 pacientes fue necesario realizar un TH de rescate.

— La probabilidad actuarial de morir o recibir un tratamiento invasivo fue de 55%, 69% y 71% al 1, 3 y 5 años respectivamente. El score de Rotterdam fue la variable que mejor predecía la supervivencia libre de intervención en nuestra cohorte de pacientes son SBC. Dado que el score de Rotterdam incluye la encefalopatía hepática y el tiempo de protrombina (lo que dificulta que sea calculado en pacientes tratados con anticoagulantes orales), se realizó un análisis multivariable sin incluir variables subjetivas o influenciadas por la anticoagulación. Se desarrolló un nuevo modelo pronóstico que se asoció de forma independiente con el tratamiento invasivo o la muerte [*BCS-intervention-free survival prognostic score (BCIS score): ascitis (sí=1, no=0)*1.675 + ln creatinina(umol/L)*0.613 + ln bilirrubina(umol/L)*0.440*]. Este nuevo modelo presentó una capacidad de discriminación similar al Rotterdam score (AUC = 0.819 y AUC 0.821; respectivamente).

— Treinta y seis pacientes (22.9%) murieron. La supervivencia al 1, 3 y 5 años fue de 88%, 79% y 74%, respectivamente. El score de BCS-TIPS PI (que incluye la edad, bilirrubina e INR) fue el parámetro basal que mejor predice la supervivencia global de los pacientes con SBC. Dado que este score incluye el INR, se realizó un análisis multivariable excluyendo las variables subjetivas y las influenciadas por la anticoagulación. Se desarrolló un nuevo modelo pronóstico (*BCSurvival score: age/10*0.370 + ln creatinina(umol/L)*0.809 + ln bilirrubina(umol/L)*0.496*), cuya capacidad de discriminación era comparable a la del score de BCS-TIPS PI y superior a la del score de Rotterdam.

— En los pacientes con TIPS, la supervivencia global y la supervivencia libre de TH al 1, 3 y 5 años fue 88%, 83% y 72%, y 85%, 78% y 72%, respectivamente. El análisis multivariado confirmó el score de BCS-TIPS PI como el único factor asociado de forma independiente a la supervivencia global y la supervivencia libre de TH en pacientes con SBC que habían sido sometidos a TIPS.

Discusión de los resultados

DISCUSIÓN DE LOS RESULTADOS

La cirrosis hepática es la principal causa de hipertensión portal en el mundo occidental. Sin embargo, existen otras enfermedades hepáticas que también pueden dar lugar al desarrollo de hipertensión portal y se engloban bajo el término de hipertensión portal no cirrótica (HTPNC)[2, 3]. Dentro de la HTPNC se encuentran los trastornos vasculares hepáticos[6, 7], como el Síndrome de Budd-Chiari (SBC), la trombosis portal no cirrótica (TVPNC) y la hipertensión portal idiopática (HTPI).

El SBC y la HTPI son enfermedades hepáticas raras, lo que ocasiona que el avance en su conocimiento esté obstaculizado por su baja prevalencia. Por ello, los trabajos de investigación de la presente tesis están orientados a ampliar el conocimiento de la etiopatogenia de la HTPI y mejorar su diagnóstico (mediante estudios hemodinámicos, de elastografía de transición y marcadores metabolómicos). Asimismo, también pretende ampliar el conocimiento sobre el pronóstico a largo plazo de pacientes con SBC.

El **primer estudio** de la tesis evalúa el papel del GPVH y de la ET en el diagnóstico de la HTPI en una amplia cohorte de pacientes remitidos a nuestra unidad para la evaluación de la hipertensión portal. Uno de los resultados del presente estudio es la confirmación de que la presencia de CVVH es un hallazgo común en la venografía hepática de pacientes con HTPI[95, 96]. Las CVVH estaban presentes en el 49% de los casos de HTPI, una prevalencia similar al 45% publicada por Okuda[95] aunque inferior al 100% publicada por Futagawa[96] (probablemente debido a un sesgo de selección). Sin embargo, tal y como se deriva de nuestro estudio, las CVVH son también frecuentes en pacientes con TVPNC establecida, lo que disminuye la relevancia de este hallazgo en cuanto a distinguir la HTPI complicada con trombosis portal de la TVPNC. Por otra parte, la ausencia de CVVH no descarta HTPI, como muestra el hecho de que la mitad de nuestros pacientes HTPI no tienen estas comunicantes. Por último, las CVVH también se han descrito en algunos pacientes con cirrosis, pero con una prevalencia inferior a la observada en pacientes con HTPI [14, 96-98]. De hecho, ninguno de los pacientes con cirrosis de nuestra cohorte presentaba CVVH. Debemos mencionar que nuestros pacientes con cirrosis tenían una relativa buena función hepática, por lo que no podemos descartar que pacientes cirróticos con mayor disfunción hepática puedan tener una mayor prevalencia de CVVH. De acuerdo con esto, el hallazgo de

CVVH en la venografía hepática de un paciente con signos clínicos de hipertensión portal y sin trombosis portal debe hacernos considerar la posibilidad de que se trate de un paciente con HTPI.

Este estudio también ha mostrado el impacto de la presencia de CVVH en las mediciones de GPVH. En los pacientes con cirrosis e hipertensión portal sinusoidal, la presencia de CVVH ocasiona una subestimación del valor real de la presión portal (PP), pero la PSHE todavía se encuentra marcadamente elevada[98]. Por el contrario, en pacientes con HTPI, la PSHE subestima la PP real debido al componente presinusoidal de la HTPI[62, 99]: en pacientes con HTPI la diferencia media entre la PP y PSHE es de 8.5 mmHg y las diferencias de la PSHE entre los pacientes con o sin CVVH fueron mínimas. Para determinar la verdadera PP en pacientes con HTPI debería realizarse la medición directa de la misma. Sin embargo, esta técnica ha sido abandonada dado que es un procedimiento invasivo. Creemos que, a pesar de tener algunas limitaciones y de no reflejar la PP real, el GPVH proporciona una información útil en el diagnóstico de la HTPI.

La mayoría de los pacientes con HTPI (81.5%) tenían un GPVH normal o ligeramente elevado, pero por debajo del dintel descrito para CSPH en la cirrosis. En nuestra opinión, este resultado puede ser de gran utilidad en la evaluación de un paciente con signos clínicos de hipertensión portal, ya que el hallazgo de un GPVH <10 mmHg va firmemente en contra del diagnóstico de cirrosis, y debe aumentar la sospecha de HTPI.

Por último el estudio ha evaluado el papel de las mediciones de rigidez hepática mediante ET en la HTPI. El valor medio de ET fue de 8.4±3.3 kPa, muy inferior a los puntos de corte descritos para el diagnóstico de cirrosis, la presencia de varices o CSPH[20, 100, 101]. De hecho, sólo 2 pacientes con HTPI tenían una ET dentro de la considerada "zona gris" (ET entre 13.6 y 21 kPa, no descarta ni confirma CSPH en la cirrosis) y ningún paciente tenía una ET >21 kPa. Por lo tanto, la ET puede ser una herramienta de diagnóstico muy útil para descartar cirrosis en un determinado paciente con signos inequívocos de hipertensión portal, complementando así la información proporcionada por GPVH, y aumentando por tanto la sospecha clínica de HTPI. Por el contrario, aunque la ET fue significativamente mayor en los pacientes con HTPI en comparación con los pacientes con TVPNC, hubo una marcada superposición de valores, por lo que esta técnica no permite diferenciar ambas enfermedades. Una posible limitación de nuestro estudio es que la ET y el GPVH no se realizaron en el mismo momento en un tercio de

los pacientes con HTPI. Sin embargo, no hubo diferencias significativas en los valores de ET entre mediciones realizadas en el mismo momento o meses después de GPVH. Del mismo modo, tampoco se observaron diferencias significativas en la ET a lo largo del tiempo en aquellos pacientes con HTPI que tenían varias mediciones. Por lo tanto, creemos que el hecho de que algunos pacientes tengan mediciones de ET realizadas después de las mediciones de GPVH no cambia el significado de los hallazgos.

No se observaron diferencias en la hemodinámica sistémica y pulmonar entre los 3 grupos de pacientes: HTPI, TVPNC y cirrosis con hipertensión portal. Estos resultados están en concordancia con lo descrito previamente[102, 103]. Es importante mencionar que los pacientes fueron apareados por función hepática (mediante Child-Pugh) y que la mayoría de los pacientes eran Child-Pugh clase A. Lo que explicaría que los pacientes con cirrosis tampoco presentaran circulación hiperdinámica ya que esta se desarrolla en fases más avanzadas de la enfermedad.

En conclusión, los pacientes con HTPI con signos inequívocos de hipertensión portal, tienen frecuentemente CVVH. La HTPI se caracteriza además por elevaciones moderadas del GPVH y de ET, pero por debajo los dinteles descritos para CSPH en la cirrosis. Por lo tanto, el hallazgo de CVVH o de valores de GPVH o ET inesperadamente bajos en un paciente con signos claros de hipertensión portal debe crear una fuerte sospecha de HTPI.

El **segundo estudio** de esta tesis fue diseñado específicamente para evaluar si existe un patrón metabolómico que permita identificar pacientes con HTPI. En nuestro conocimiento, este es el primer estudio de perfiles metabolómicos realizado en muestras de plasma en pacientes con HTPI. El análisis metabolómico mediante modelos PLS-DA permitió identificar un grupo de metabolitos, con un excelente poder predictivo, diferencia los pacientes con HTPI de los pacientes con cirrosis. El análisis metabolómico también permitió una buena diferenciación de los pacientes con HTPI de los controles sanos, mediante un perfil metabólico que incluía 31 metabolitos. Estos resultados apoyan la hipótesis de que las firmas metabolómicas de muestras de plasma podrían ser útiles para discriminar HTPI de pacientes cirróticos y de controles sanos. Mediante técnicas de validación cruzada interna se observó una excelente reproducibilidad de ambos modelos con una buena sensibilidad, especificidad y AUC, tanto en la training como en la testing set, lo que refuerza la solidez de

los modelos seleccionados para el diagnóstico de la HTPI mediante el uso de perfiles metabolómicos de muestras de plasma.

Como hemos mencionado previamente, la HTPI es una enfermedad que puede englobar diferentes trastornos de etiología desconocida y que ocasionan hipertensión portal presinusoidal[9, 25, 26, 43]. Recientemente se ha descrito que la infección por el VIH y/o los tratamientos antiretrovirales (didanosina o estavudina) podrían ser factores de riesgo de HTPI[25, 44, 62, 104-107]. Sin embargo nuestro perfil metabolómico fue incapaz de clasificar los pacientes con HTPI en diferentes subgrupos en base a la infección por el VIH. Por todo ello, nuestro perfil metabólico reflejaría los marcadores plasmáticos resultantes de un síndrome clínico más que la etiología.

Un punto que debe considerarse es la posible influencia del tratamiento concomitante sobre el perfil metabólico. Sin embargo, es importante mencionar que el subgrupo de pacientes con HTPI e infección VIH (todos ellos bajo tratamiento TARGA) tenían un perfil metabólico similar a los HTPI VIH negativos (pacientes sin tratamiento TARGA). Del mismo modo, los pacientes con cirrosis y HTPI tienen la misma prevalencia de varices, así que la prevalencia de tratamiento con fármacos beta-bloqueantes fue similar en ambos grupos. Si bien, algunos de los metabolitos detectados pueden reflejar los fármacos que los pacientes están tomando, creemos que el método estadístico utilizado para el análisis no los ha seleccionado para el perfil metabólico final.

Al ser este un estudio de prueba de concepto, diagnóstico fase 2[108], sólo hemos incluido pacientes con diagnóstico inequívoco de HTPI y por ello no se han incluido pacientes con TVPNC como grupo de control; ya que la trombosis portal se puede desarrollar en un paciente con hígado normal y también en pacientes con enfermedad hepática subyacente (como la HTPI).

Una limitación del estudio es el número relativamente bajo de pacientes con HTPI incluidos, sin embargo, dado que la HTPI es una enfermedad rara, una muestra de más de 30 pacientes podría considerarse adecuada. De hecho, con este número de pacientes, ha sido posible encontrar un perfil metabolómico que permite la diferenciación de forma precisa de pacientes con HTPI de pacientes con cirrosis o controles sanos. Otra limitación es la falta de validación externa con una muestra independiente. Sin embargo, debemos destacar que hemos realizado una validación cruzada interna. En nuestra opinión, los estudios de validación externa serán más apropiados en una etapa posterior, cuando se identifiquen los metabolitos específicos con nuevas tecnologías.

Esto además permitiría el desarrollo de un kit para el diagnóstico no invasivo de la HTPI en la práctica clínica diaria.

En conclusión, el perfil metabolómico puede constituir una herramienta valiosa para el diagnóstico no invasivo de la HTPI. Este estudio abre la puerta a la realización de estudios dirigidos a la identificación de estos metabolitos lo que permitiría que los pacientes con HTPI puedan ser identificados en base a su perfil metabólico plasmático, obviando la necesidad de métodos diagnósticos invasivos y por tanto facilitar el diagnóstico correcto de esta enfermedad poco frecuente.

El **tercer estudio** evalúa la prevalencia de las mutaciones del gen BMPR2, y por tanto, su papel en la etiopatogenia de la HTPI. Nuestra cohorte de pacientes caucásicos con HTPI no presenta ninguna mutación en el gen BMPR2. Este estudio ha mostrado otros resultados como la confirmación de la presencia de SNPs conocidos del gen BMPR2: 12 pacientes (52%) presentaron tres SNPs diferentes. Sin embargo, estos 3 SPNs han sido descritos en una población normal con la misma prevalencia que en nuestro estudio. Otro hallazgo es la presencia de duplicaciones de ADN. Se han descrito deleciones o duplicaciones que afectan al gen BMPR2 como una de las causas de hipertensión pulmonar familiar. Sin embargo en nuestros pacientes, aunque hemos encontrado algunas duplicaciones a nivel de DNA, no se pudo confirmar la alteración en el nivel de ARNm y ADNc. Por todo ellos nuestros resultados descartan un posible papel de la BMPR2 en la patogénesis de la HTPI.

Estudios previos en pacientes con HAPI incluyeron un número pequeño de pacientes ($n < 50$) y la prevalencia de mutaciones BMPR2 era hasta un 26% [109]. Una de las series más grandes incluyó 126 pacientes con HAPI y la prevalencia fue del 21% [110]. Aunque nuestro tamaño muestral es relativamente pequeño, al no haber encontrado ninguna mutación del gen BMPR2, creemos que el número de pacientes de este estudio debe considerarse suficiente para concluir de forma razonable que las mutaciones en este gen no están involucrados en la patogénesis de la HTPI.

Actualmente se reconoce que hasta un 30% de la HAP familiar y 80% de la HAPI no tienen mutaciones en el gen BMPR2. Por lo tanto, es probable que las mutaciones en uno o más loci contribuyan en la patogénesis de la HAPI, como se demuestra por su asociación con la telangiectasia hemorrágica hereditaria y mutaciones en la activin-like kinasa tipo 1 y de la endoglina[111]. De manera similar, la HTPI podría estar asociada con mutaciones en otros genes diferentes al de la BMPR2.

En conclusión, los datos de este estudio sugieren que, en contraste con HAPI, parece muy poco probable que las mutaciones del gen BMPR2 estén involucradas en la patogénesis de la HTPI. La HTPI podría estar relacionada con otras alteraciones genéticas, como mutaciones en la superfamilia del TGF-β y son necesarios más estudios para una mejor comprensión de la patogenia de esta enfermedad.

El **cuarto estudio** muestra que los pacientes con SBC que reciben un tratamiento escalonado tienen un buen pronóstico a largo plazo. La mayoría de nuestros pacientes (88.5%) recibieron anticoagulación a largo plazo. Veintidós pacientes (14%) fueron sometidos a angioplastia / trombolisis como primer tratamiento invasivo, y sólo 8 de ellos no requirieron una intervención posterior. La angioplastia, aunque una técnica atractiva y mínimamente invasiva con potencial de restauración de flujo sinusoidal fisiológico, tuvo una baja aplicabilidad en nuestra cohorte de pacientes. Estos resultados contrastan con un reciente estudio retrospectivo de China que muestra una gran aplicabilidad y eficacia de este tratamiento[112]. En nuestra opinión, estas diferencias podrían estar relacionadas con los diferentes mecanismos de obstrucción del flujo venoso hepático[113]: la estenosis cortas de las venas suprahepáticas y / o de la VCI son más frecuentes en países asiáticos que en países occidentales. Sin embargo, la angioplastia / stent sigue siendo un tratamiento potencialmente valioso en pacientes con SBC con estenosis cortas.

Tan sólo 3 pacientes (2%) recibieron un shunt quirúrgico portosistémico. Esto demuestra que el TIPS se ha convertido en el tratamiento derivativo de elección de estos pacientes en Europa. El hecho de que dos estudios retrospectivos recientes de Norteamérica hayan demostrado excelentes resultados del shunt quirúrgico postosistémico en pacientes con SBC no cambia, en nuestra opinión, la tendencia actual de preferir la técnica menos invasiva sobre las más invasivas[114, 115]. Por otra parte, debemos hacer hincapié en que los estudios multicéntricos retrospectivos previos fueron incapaces de demostrar una mejoría en la supervivencia en los pacientes tratados con shunts quirúrgicos[88, 116-119]. El escaso número de pacientes tratados con esta modalidad terapéutica en nuestro estudio impide proporcionar más información sobre este punto.

Sesenta y dos pacientes requirieron TIPS como tratamiento de rescate tras fracaso del tratamiento médico o de tratamientos mínimamente invasivos (angioplastia / stent / trombolisis). La supervivencia global y la supervivencia libre de TH de este grupo de pacientes tratados con TIPS fue similar a la observada en un estudio retrospectivo

multicéntrico europeo que incluyó 124 pacientes[91]. Estos resultados confirman que el TIPS es un tratamiento de rescate eficaz y seguro en estos pacientes. De forma interesante, a pesar de que la mayoría de los TIPS se colocaron durante el primer año tras el diagnóstico, el tiempo no era uniforme ya que varió desde 0 hasta 38 meses. Una de las preocupaciones principales en el manejo de pacientes con SBC es si el retrasar el uso del TIPS puede tener una influencia en el pronóstico. Nuestros datos muestran un buen pronóstico tras el TIPS, independientemente de si el procedimiento se realiza poco después del diagnóstico o más tarde durante el seguimiento. Este resultado, que requiere confirmación, sugiere que el enfoque de realizar una vigilancia clínica estrecha y reservar el TIPS para aquellos pacientes que no responden al tratamiento médico no tiene un impacto perjudicial sobre el pronóstico. Por otra parte, nuestro estudio valida el score de BCS-TIPS PI [91], como el único factor asociado de forma independiente a la supervivencia global y supervivencia libre de TH tras el TIPS. Sin embargo, nuestro estudio no ha podido responder a la pregunta de si los pacientes con un score BCS-TIPS PI alto podrían beneficiarse de la realización de un TH como primer tratamiento invasivo. Al comparar el subgrupo de pacientes que recibieron TIPS en comparación con aquellos que recibieron TH como primer tratamiento invasivo, observamos que ambos grupos tuvieron un pronóstico similar a pesar de que los pacientes del grupo de TH tenían enfermedad hepática más grave al diagnóstico. Ello hace pensar que quizás estos pacientes más graves deberían ser candidatos iniciales a TH. No obstante, hay que recordar que este estudio no era aleatorizado y sólo uno de los pacientes tratados inicialmente con TH tenía un score BCS-TIPS PI predictivo de mala respuesta al tratamiento con TIPS. Esto sugeriría que estos pacientes podrían haber respondido favorablemente al TIPS a pesar de tener una mayor gravedad. Así pues, nuestros datos no nos permiten evaluar el papel del TH como procedimiento inicial en estos pacientes más graves.

El 56% de los pacientes fueron tratados con un procedimiento invasivo, la mayoría de ellos durante el primer año tras del diagnóstico. En contraste con la población en la que se definió inicialmente el score de Rotterdam como el mejor índice predictivo de supervivencia[90], tanto el TIPS y como el TH son tratamientos de rescate ampliamente utilizados en la actualidad en el SBC. Por ello, no es de extrañar que en nuestro estudio el score de Rotterdam haya sido el mejor parámetro para predecir la necesidad de intervenciones invasivas o muerte en esta cohorte más reciente y prospectiva de pacientes con SBC.

Hemos definido un nuevo score para identificar aquellos pacientes que requerirán un tratamiento invasivo o fallecerán. El nuevo score BCIS tiene una capacidad de discriminación idéntica a la obtenida por el score de Rotterdam pero con algunas potenciales ventajas, tales como la exclusión de los parámetros subjetivos (p.ej. encefalopatía hepática) y el tiempo de protrombina[90]. Dado que este es un estudio de cohortes multicéntrico no podemos descartar la influencia de una intervención más rápida en los pacientes más enfermos que pueden haber influido en nuestros resultados en relación a la predicción de la supervivencia libre de intervención.

Otro hallazgo importante del estudio fue que el score BCS-TIPS PI mostró una buena precisión para predecir la mortalidad global en nuestra cohorte, superior al score de Rotterdam. Además, en el presente estudio se ha identificado un nuevo score para predecir la supervivencia (BCSurvival score) que tiene una capacidad de discriminación idéntica a la obtenida con el score BCS-TIPS PI pero con la ventaja potencial de no incluir INR en sus variables. Esto puede ser importante, ya que muchos pacientes pueden estar ya anticoagulados cuando son remitidos a los centros de referencia.

La validación de los scores de Rotterdam y BCS-TIPS PI, y la identificación de dos nuevos scores se ha hecho, en contraste con estudios previos, en una gran cohorte de pacientes, recogidos de forma prospectiva, durante un corto periodo de tiempo y que han sido manejados de forma homogénea con un tratamiento escalonado.

En resumen, nuestro estudio valida el uso de un tratamiento escalonado en pacientes con SBC. Además, este estudio valida el score de Rotterdam para predecir la supervivencia libre de intervención y el score BCS-TIPS PI para la supervivencia. Asimismo hemos descrito dos nuevos scores que pueden ser de ayuda en la elección de la mejor estrategia terapéutica en un determinado paciente con SBC, pero que necesitan ser validados en futuros estudios multicéntricos prospectivos.

Conclusiones

CONCLUSIONES

Estudio 1. ***Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension.***

— Los pacientes con HTPI presentan frecuentemente comunicantes vena-vena entre las diferentes venas suprahepáticas en la venografía hepática.

— Los pacientes con HTPI, a pesar de mostrar signos inequívocos de hipertensión portal, muestran unos valores de GPVH y del ET inferiores a los dinteles previamente descritos de hipertensión portal clínicamente significativa en la cirrosis.

— Los pacientes con HTPI muestran valores de GPVH y de ET significativamente inferiores a los de los pacientes con cirrosis e hipertensión portal. Por tanto, la presencia de estos hallazgos durante la evaluación de un paciente con hipertensión portal, incrementaría la sospecha clínica de que nos encontramos ante una HTPI.

Estudio 2. ***Metabolomics discloses potential biomarkers for the non-invasive diagnosis of idiopathic portal hypertension.***

— Existe un patrón metabolómico plasmático capaz de diferenciar los pacientes con HTPI de los pacientes con cirrosis y de los controles sanos.

— Existe un patrón metabolómico plasmático que podría permitir el diagnóstico no invasivo de la HTPI.

Estudio 3. ***Bone morphogenetic protein receptor 2 in patients with idiopathic portal hypertension.***

— No se han detectado mutaciones o reordenamientos en la región codificante del gen BMPR2 en pacientes con HTPI.
— Estos resultados sugieren que, a diferencia de en la hipertensión arterial pulmonar, las mutaciones del gen de la BMPR2 no están implicadas en la etiopatogenia de la HTPI.

Estudio 4. ***Good long-term outcome of Budd-Chiari syndrome with a step-wise management.***

— Con el manejo terapéutico escalonado, los pacientes con SBC tienen buena supervivencia a largo plazo (79% a los 3 años, 74% a los 5 años).

— Un 56% de los pacientes recibieron al menos un tratamiento invasivo (como la angioplastia, el TIPS, el shunt quirúrgico portosistémico o el trasplante hepático).

— El TIPS es el tratamiento invasivo más frecuentemente utilizado. La mayoría de las veces es un tratamiento definitivo, y se asocia con una excelente supervivencia a largo plazo.

— El score de Rotterdam permite identificar los pacientes con alto y bajo riesgo de presentar mala evolución (definido como la necesidad de tratamiento invasivo o muerte).

— El score BCS-TIPS PI, es un buen factor predictivo de supervivencia global de los pacientes con SBC y de supervivencia libre de trasplante en pacientes con SBC que han sido sometidos a TIPS.

— Se han identificado dos nuevos scores para predecir la supervivencia y la supervivencia libre de intervención, que deben ser validados en futuros estudios.

Otras publicaciones realizadas durante el periodo de Tesis

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Otras actividades académicas

Otras publicaciones realizadas durante el periodo de tesis

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Otras actividades académicas

Diplomatura de post-grado de Estadística en Ciencias de la Salud (30 créditos), organizado por el Laboratorio de Estadística Aplicada de la Universidad Autónoma de Barcelona. Realizado durante los cursos académicos de 2008-2011.

Máster Universitario en Investigación en Enfermedades Hepáticas (60 créditos), impartido por el Servicio de Hepatología, Hospital Clinic, Barcelona, Universidad de Barcelona. Realizado durante los cursos académicos de 2009-2011.

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