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TESIS DOCTORAL:

RECURRENCIA DE LA INFECCIÓN POR EL VIRUS DE LA HEPATITIS C (VHC) TRAS EL TRASPLANTE HEPÁTICO:FACTORES PREDICTIVOS DE RECIDIVA PRECOZ Y GRAVE.

Presentada por MONTSERRAT GARCÍA RETORTILLO para optar al grado de Doctora en Medicina y Cirugía.

Director: Dr. Xavier Forns Bernhardt

Barcelona, abril de 2005

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1. INFORME DEL DIRECTOR DE TESIS.

Xavier Forns Bernhardt, médico especialista del Servicio de Hepatología del Hospital Clínic de Barcelona y Doctor en Medicina por la Universidad de Barcelona,

CERTIFICA:

Que la tesis doctoral **RECURRENCIA DE LA INFECCIÓN POR EL VIRUS DE LA HEPATITIS C (VHC) TRAS EL TRASPLANTE HEPÁTICO: FACTORES PREDICTIVOS DE RECIDIVA PRECOZ Y GRAVE**, presentada por Montserrat García Retortillo para optar al grado de Doctora en Medicina y Cirugía ha sido realizada bajo mi dirección y cumple todos los requisitos necesarios para ser defendida ante el Tribunal de evaluación correspondiente.

Dr. Xavier Forns Bernhardt
Director de la Tesis

Barcelona, 15 de noviembre de 2004.

2. AGRADECIMIENTOS.

Esta tesis ha sido realizada bajo la tutela del Dr. Forns, sin el cual no hubiese sido posible. Gracias por todo lo que he aprendido y por la energía, rigor y entusiasmo transmitidos día a día.

El Dr. José María Sánchez-Tapias, responsable de la línea de investigación de Hepatitis Víricas, ha hecho posible la formación de un grupo de investigación sólido, productivo y reconocido por la comunidad científica, al cual he tenido la suerte de pertenecer.

Los Dres Rimola y Navasa han sido los culpables de que me dedicara al trasplante hepático y han contribuído con sus consejos a la elaboración de los artículos de la tesis. El Dr. Rimola, además, me "acogió" en su despacho hace unos años. Esto me ha permitido conocer a compañeros estupendos que vinieron de lejos, como María Londoño, Gonzalo Guevara y Laura Cisneros, con los cuales he compartido muy buenos momentos.

El Dr. JM Llovet nos ha asesorado, aconsejado y animado incluso desde la distancia.

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A mis compañeras del laboratorio Anna Feliu y Anna Massaguer les agradezco su simpatía y ayuda fundamentales en la realización de los trabajos que aquí se presentan.

Los cirujanos de la Unidad de Cirugía Hepática y Trasplante me facilitaron los datos técnicos sobre los trasplantes en receptores de donante vivo así como las muestras de los explantes de los pacientes infectados por el VHC.

El personal de enfermería del IMD me ayudó en la recogida de las muestras de suero de los pacientes trasplantados mientras estaban ingresados en el hospital. Alba y Lidia recogieron las muestras de los pacientes ambulatorios. Gracias a todos ellos se ha podido crear una importante seroteca que permitirá la realización de nuevos estudios.

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Mi familia ha sido fundamental para mantener mi homeostasis desde el punto de vista físico y emocional.

Por último, gracias a los pacientes que aceptaron en su día entrar a formar parte de los estudios que forman esta tesis y de otros que hacen posible, en definitiva, que la ciencia siga avanzando y que todo esto tenga sentido.

Para Julia.

3. RELACIÓN DE ARTÍCULOS INCLUIDOS.

La presente tesis se basa en los siguientes artículos publicados:

Artículo1.

Título: "Hepatitis C Virus Kinetics During and Immediately After Liver Transplantation".

Autores: Montserrat García-Retortillo, Xavier Forns, Anna Feliu, Eduardo Moitinho, Josep Costa, Miquel Navasa, Antoni Rimola and Joan Rodes.

Publicación: Hepatology 2002;35:680-687.

Factor de impacto: 9,503

Artículo 2.

Título: "Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation".

Autores: Xavier Forns, Montserrat Garcia-Retortillo, Trinidad Serrano T, Anna Feliu, Francisco Suarez, Manuel de la Mata, Juan Carlos Garcia-Valdecasas, Miquel Navasa, Antoni Rimola and Joan Rodes.

Publicación: J Hepatol 2003;39(3): 389-96.

Factor de impacto: 5,283

Artículo 3.

Título: "Hepatitis C recurrence is more severe after living donor compared to cadaveric liver transplantation".

Autores: Montserrat Garcia-Retortillo, Xavier Forns, Josep Maria Llovet, Miquel Navasa, Anna Feliu , Anna Massaguer, Miquel Bruguera, Josep Fuster, Juan Carlos Garcia-Valdecasas, Antoni Rimola.

Publicación: Hepatology 2004;40:699-707.

Factor de impacto: 9,503

4. INTRODUCCIÓN

4.1.El virus de la hepatitis C. Mecanismos de persistencia en el huésped.

La prevalencia de la infección por el virus de la hepatitis C (VHC) alcanza un 2-3% en nuestra área geográfica. La infección crónica por el VHC es la primera causa de cirrosis y carcinoma hepatocelular y se ha convertido en la primera indicación de trasplante hepático en nuestro medio[1] .

El VHC es un virus de pequeño tamaño recubierto por una membrana lipoproteica. El genoma del VHC está formado por una cadena única de ARN de polaridad positiva que contiene 9600 nucleótidos que codifican para una poliproteína de unos 3000 aminoácidos (Fig 1). La región codificante (ORF, de "Open Reading Frame") se encuentra flanqueada por dos regiones no codificantes (UTR, "Untranslated Region"). La 5' UTR es una región altamente conservada de unos 340 nucleótidos. Dicha región, que adopta una compleja estructura secundaria, contiene una zona de anclaje al ribosoma de la célula del huésped conocida como IRES ("Internal Ribosomal Entry Site") la cual parece jugar un papel clave en la replicación viral. No se sabe con exactitud cuál es la función de la región no codificante 3´ pero también parece ser importante en el proceso de la replicación viral.

En el extremo 5´ se encuentran las regiones que codifican para proteínas estructurales del virus. Así, la proteína del *core* es la que forma la nucleocápside, dentro de la cual se encuentra el ARN viral. El virus cuenta con una envoltura en la que se encuentran dos glucoproteínas virales, E1 y E2, necesarias para formar partículas infectivas. E1 y E2 estarían implicadas en la

unión a los receptores celulares y su posterior fusión, es decir, en la entrada del virus dentro de las células del huésped.

El grupo de las proteínas no estructurales está formada básicamente por proteínas con acción proteasa, helicasa y polimerasa, necesarias para la replicación viral. La NS5A se ha implicado en la modulación de la respuesta antiviral del huésped mediada por el interferón. En concreto se ha observado que el acúmulo de mutaciones en una determinada región de NS5A, la conocida como ISDR ("Interferon sensitivity determining region") se relaciona con una mejor respuesta al tratamiento con interferón en pacientes infectados por el genotipo 1b del VHC [2,3].

Una de las características más destacables del VHC es su extrema heterogeneidad genética debido a los frecuentes errores de la polimerasa viral y a la ausencia de actividad correctora de los mismos. Las proteínas de la envoltura procedentes de diferentes aislados de VHC presentan un alto grado

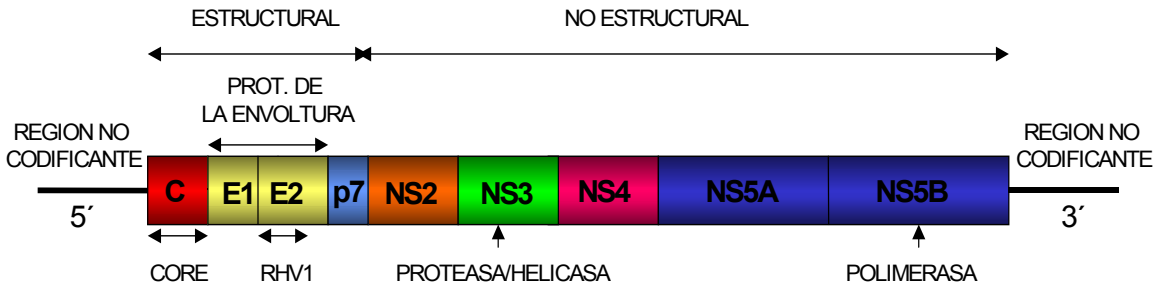

Fig 1. Esquema del genoma del virus de la hepatitis C.

de heterogeneidad genética. El extremo N-terminal de E2 es el que presenta mayor variabilidad por lo que recibe el nombre de región hipervariable 1 (HVR-1). Existen datos que indican que HVR-1 se sitúa en superficie dentro de la estructura de la proteína de la envoltura y que representa un dominio de neutralización [4,5].

La heterogeneidad genética del VHC se describe bajo dos conceptos: los genotipos y las cuasiespecies [6,7]. El genotipo hace referencia a la heterogeneidad genética existente entre los diferentes aislados de VHC en áreas geográficas diversas y refleja la acumulación de mutaciones durante un largo periodo de la evolución del virus. Los análisis filogenéticos han demostrado la presencia de al menos 6 genotipos diferentes los cuales se designan con números arábigos. A su vez, dentro de cada genotipo existen subgrupos con pequeñas diferencias entre sí, conocidos como subtipos, a los que se les asigna una letra (a,b,c). Los diferentes genotipos pueden aparecer en cualquier parte del mundo pero existen diferencias notables en cuanto a su distribución geográfica [8] (Fig 2). Así, los genotipos 1a, 1b, 2a, 2b, 2c y 3a constituyen el 90% de todas las infecciones en toda América, Europa, China, Japón, Australia y Nueva Zelanda. Los genotipos 1b y 1a son los causantes del 40% de todas las infecciones en EEUU y son especialmente prevalentes en el Sur y Este de Europa, así como en China y Japón.

Las cuasiespecies son la traducción de la heterogeneidad genética viral en un mismo individuo. El análisis del virus circulante en un sujeto revela la presencia de múltiples variantes muy similares entre sí pero con algunas diferencias en su secuencia nucleotídica [7].

Como se ha comentado anteriormente, el desarrollo de infección persistente es una de las características más importantes del VHC. En efecto, más del 50% de los individuos que presentan primoinfección desarrollarán una infección crónica [9,10].

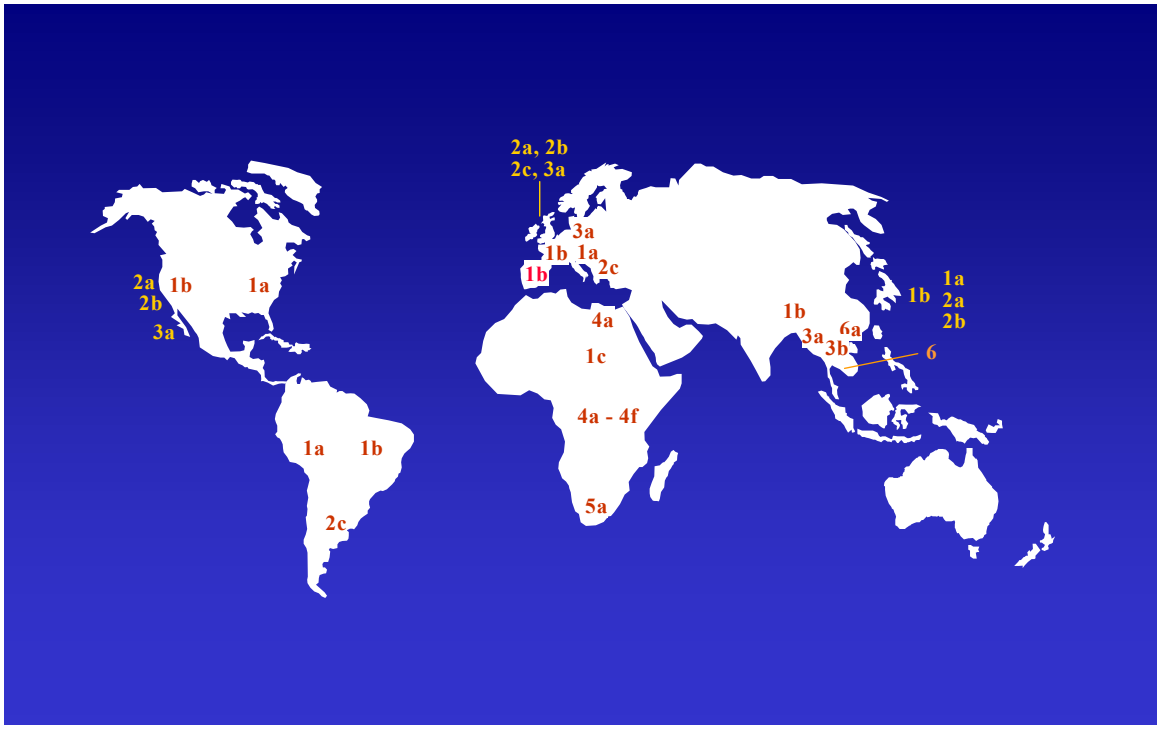

Fig 2. Distribución geográfica de los diferentes genotipos del virus C.

No se conocen con exactitud cuáles son los mecanismos que utiliza el VHC para persistir en el organismo del huésped; la existencia de las cuasiespecies podría ser uno de los factores implicados al facilitar el escape del sistema inmunitario [11,12]. De hecho, el grado de presión inmunológica que ejerce el huésped sobre el VHC parece influir en la evolución de las cuasiespecies y, en general, a mayor presión inmunitaria (humoral y celular) mayor tasa de mutación viral [13]. Sin embargo, el escape inmunitario no parece

ser el único mecanismo implicado en la persistencia y cronificación de la infección por el VHC. En estudios realizados recientemente en chimpancés se ha observado la persistencia de la infección tras la inoculación de ARN viral proveniente de un clon infeccioso de cADN, es decir, con una secuencia única del virus (sin cuasiespecies). A pesar de que los animales de experimentación desarrollan anticuerpos contra las principales proteínas de membrana del virus, apenas se han detectado mutaciones en la HVR-1 y la infección se cronifica en la mayoría de los animales [14-16]. La infección puede cronificarse incluso en ausencia de HVR-1, que como se ha mencionado anteriormente, es la más variable y sometida a mayor presión inmune , por lo que se cree que el escape inmune no debe ser el único mecanismo implicado en la persistencia del virus [17]. Últimamente se ha propuesto que determinadas proteínas del virus (NS5A, E2) podrían interferir con mecanismos antivirales inespecíficos que se activarían en presencia de infección viral facilitando así la persistencia del virus [3,18,20]. Otros virus han desarrollado mecanismos similares (interacción con proteínas del huésped) para persistir en el organismo.

4.2. Historia natural de la infección crónica por el VHC en el sujeto inmunocompetente.

La infección crónica por el VHC es una enfermedad de baja morbi-mortalidad durante las primeras décadas de evolución de la infección. De todos los pacientes infectados por el VHC que evolucionan a la cronificación de la infección, sólo un 20% desarrollará una cirrosis hepática (en unos 15-20 años) [20-22]. En el resto, la infección causa una hepatitis crónica que raramente causa problemas clínicos.

No todos los individuos evolucionan de la misma manera ni con la misma rapidez hacia fases más avanzadas de la hepatopatía. Poynard y colaboradores, describieron diferentes patrones en función de la velocidad de progresión hacia la cirrosis [23]. Se han señalado varios factores que pueden acelerar el curso de progresión de la fibrosis en sujetos infectados por el VHC. La mayoría hacen referencia a características en relación al huésped, como son la edad superior a 40 años en el momento en que se contrajo la infección [26-28], el sexo masculino [9,21,26], el consumo de alcohol y la coinfección con el VHB o el VIH [27]. El papel que desempeñan los factores virales como la carga viral en el momento de la infección y el genotipo es más controvertido. Como ejemplo, existen numerosos estudios que relacionan el genotipo 1 con un mayor riesgo de progresar a la cirrosis hepática [25,28,29], mientras que en otros trabajos no se ha podido demostrar dicha relación [23,30]. Se piensa que la relación entre el genotipo 1 y un mayor riesgo de desarrollo de hepatopatía avanzada se deba, en realidad, a un efecto cohorte. Así, los pacientes más graves son aquellos que se infectaron hace más tiempo (llevan más años de evolución de la infección), cuando la distribución de genotipos en la población era claramente diferente (más proporción de genotipo 1 frente a los no-1). La gravedad histológica de la hepatitis en el momento del diagnóstico, así como niveles elevados de transaminasas también se han relacionado con un peor pronóstico [31-33].

La supervivencia tras el diagnóstico de cirrosis compensada es excelente durante los 10 primeros años tras el diagnóstico (superior al 90%). Sin embargo, ésta empeora de forma significativa una vez se presenta la primera descompensación clínica o se diagnostica un carcinoma hepatocelular (CHC) (50% a los 5 años) [34]. Una vez que se alcanza la fase de cirrosis

hepática, la probabilidad acumulada de aparición de descompensación clínica oscila entre un 10-20% a los 5 años. Las descompensaciones más frecuentes son la aparición de un CHC (alrededor de un 20%) y el desarrollo de ascitis (17%). Los factores que se han involucrado con un mayor riesgo de descompensación son el alcohol, la función hepatocelular en el momento del diagnóstico (niveles de albúmina, tiempo de protrombina, niveles de transaminasas) y la edad [35,36].

El riesgo de desarrollo de un CHC oscila entre un 1 y un 4% anual [34,36,37]. Aproximadamente un tercio de las muertes relacionadas con la hepatopatía por el VHC son secundarias a la aparición de un CHC. De hecho, existe una buen paralelismo entre la supervivencia del paciente cirrótico y la probabilidad de desarrollo de CHC por lo que éste se considera un evento de gran importancia pronóstica en la historia natural de la cirrosis por VHC. Todo ello, junto al aumento de la incidencia de esta complicación que se viene observando en Estados Unidos y en Europa, apoya la necesidad de desarrollar estrategias de diagnóstico precoz y tratamiento para el CHC incluso en aquellos pacientes cirróticos de diagnóstico reciente [37].

El trasplante hepático (TH) constituye la opción terapéutica de elección para aquellos pacientes que han llegado a estadios terminales de su cirrosis (de cualquier etiología) o que han desarrollado un hepatocarcinoma. Dicho procedimiento ha conseguido mejorar la supervivencia de los pacientes cirróticos de forma significativa. En estos momentos, un 60% de todos los pacientes incluidos en la lista de espera para trasplante hepático están infectados por el VHC. Este porcentaje ha ido aumentando de forma progresiva en los últimos años debido, por una parte, a la ausencia de una vacuna protectora contra la infección por el VHC y, por otro, a la falta de

tratamiento antiviral eficaz. Por todo ello, se ha producido un aumento del número de pacientes infectados por el VHC que están en riesgo de progresión a la cirrosis.

4.3.Historia natural de la recurrencia de la hepatitis C post-trasplante hepático.

La recurrencia de la infección por el VHC tras el trasplante hepático se produce de forma universal y precoz [38]. Estudios recientes han demostrado que la hepatopatía por el VHC tras el trasplante progresa más rápidamente que en los sujetos inmunocompetentes de manera que a los 5 años del trasplante un porcentaje significativo de los pacientes (desde un 10 hasta un 28%, dependiendo de las series) han desarrollado cirrosis hepática [39]. Una vez constatada la presencia de cirrosis sobre el injerto hepático, el intervalo de tiempo que transcurre hasta la primera descompensación clínica es también más corto que en los sujetos no trasplantados. Berenguer y colaboradores observaron , a través de un estudio retrospectivo, que este intervalo era tan sólo de 8 meses. El desarrollo de una cirrosis descompensada tras el trasplante hepático marca un punto en el tiempo a partir del cual la expectativa de vida del paciente disminuye de forma dramática (supervivencia al año del 50%) [40]. Como consecuencia de la progresión acelerada de la fibrosis relacionada con el VHC tras el trasplante hepático, la supervivencia a largo plazo de los pacientes trasplantados por esta indicación es significativamente menor que la de los trasplantados por otras etiologías [41] (Fig 3). En los últimos años se está detectando una mayor rapidez en la progresión de la hepatopatía relacionada con el VHC sobre el injerto hepático. El uso de agentes inmunosupresores más potentes y la utilización de órganos procedentes de

donantes de mayor edad se señalan como las principales causas del incremento en la gravedad de la recurrencia de la infección por el VHC [42,43].

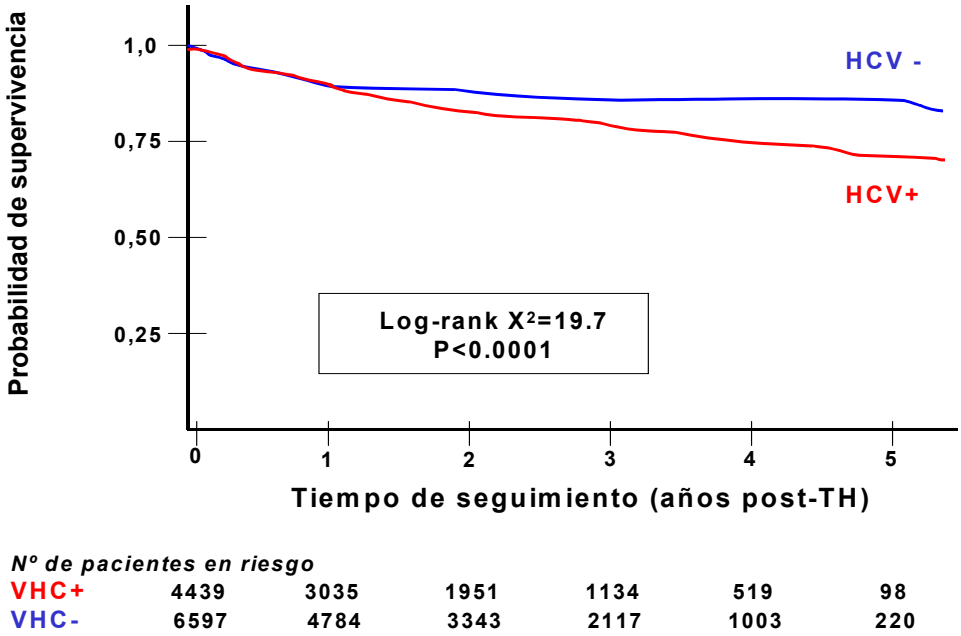

Fig 3. Supervivencia del paciente trasplantado hepático infectado por el VHC respecto a los trasplantados por otras etiologías. De Forman et al.2002.

Todo ello hace que la recurrencia grave de la infección por el VHC, se haya convertido en la primera causa de retrasplante tardío [44]. Sin embargo, la supervivencia tras el retrasplante de los sujetos infectados por el VHC es menor que cuando éste se lleva a cabo por otras indicaciones [45]. La posibilidad de que la evolución de la infección por el VHC tras el retrasplante sea aún más agresiva, unido a la escasez de órganos, han motivado que muchos grupos de trasplante consideren la recurrencia grave y precoz de la infección por el VHC como una contraindicación para el retrasplante [46].

4.4.La escasez de donantes y el aumento de las listas de espera para trasplante.

Los órganos disponibles para ser trasplantados son un recurso escaso cuya distribución exige una selección cuidadosa de los receptores. En los últimos años se ha asistido a un aumento progresivo de las listas de espera para TH debido a la ampliación de las indicaciones. Por contra, este hecho no se ha compensado con un aumento paralelo del número de órganos disponibles (Fig 4) .

La consecuencia de este desequilibrio ha sido el aumento del número de pacientes que fallecen en lista de espera. Para hacer frente a este problema se han propuesto diferentes estrategias. Por un lado, el aumento del número de potenciales donantes, con el objetivo de ampliar el número de órganos disponibles. Entre estas estrategias se incluyen el trasplante de órganos parciales, como el donante vivo o el "split", y el uso de donantes tradicionalmente considerados "marginales", como los donantes con hígados esteatósicos, los donantes añosos, los procedentes de pacientes con polineuropatía amiloidótica familiar o, últimamente, los donantes con anticuerpos contra el VHC positivos. Otra estrategia diseñada para disminuir la mortalidad pretrasplante es la priorización en lista de espera. En algunos países como Estados Unidos se ha instaurado un nuevo sistema de priorización de forma que los pacientes más graves se puedan trasplantar antes, con independencia de cuándo se hayan incluído en lista. Mediante una fórmula que incluye los valores de la creatinina, bilirrubina y tiempo de protrombina de cada paciente es capaz de estimar la supervivencia a tres

meses vista [47,48]. La repercusión de este nuevo sistema de priorización (escala MELD) sobre la supervivencia del injerto y del paciente se verá en los próximos años.

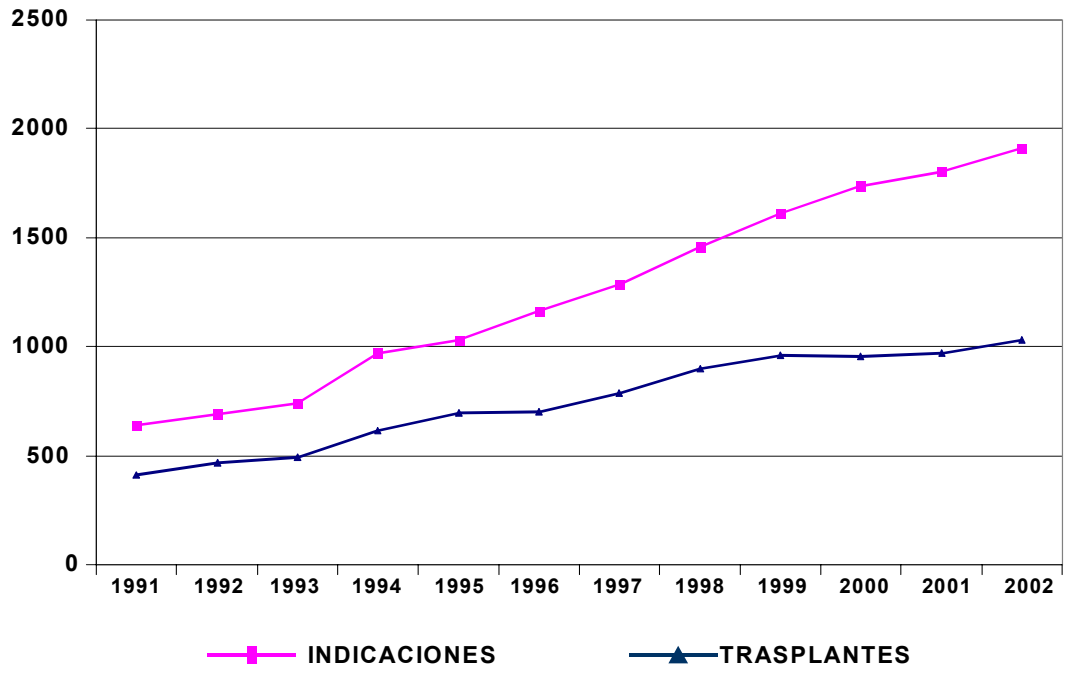

Fig 4.Evolución de la lista de espera para trasplante hepático en España. Datos de la Organización Nacional de Trasplantes (ONT).

4.5. Estrategias profilácticas/terapéuticas para la recurrencia del VHC tras el trasplante hepático.

El tratamiento estándar de la hepatitis C en sujetos inmunocompetentes se basa en la combinación de interferón pegilado (PegIFN) más ribavirina (RBV). Con dicho tratamiento se ha alcanzado la curación o erradicación de la

infección (Respuesta virológica sostenida, RVS) en un 42% para los pacientes con genotipo 1 y en más del 80% para pacientes con genotipos 2 y 3 [49,50]. Sin embargo, en pacientes con genotipo 1 y con un grado de fibrosis avanzado la eficacia es sensiblemente más baja. Los efectos secundarios son frecuentes y obligan a modificar la dosis de antivirales en aproximadamente un 42% de los casos mientras que en un 14% deben suspenderse definitivamente.

Las pautas de tratamiento antiviral en los sujetos trasplantados hepáticos no están bien definidas. No existen indicaciones claras sobre a quién tratar, cuándo y durante cuánto tiempo. Por ello, existen varias estrategias cuya eficacia se está evaluando en la actualidad (Tabla 1).

Tabla 1. Diferentes estrategias profilácticas/terapéuticas para la recurrencia de la infección por el VHC post-TH: ventajas e inconvenientes

	Ventajas	**Inconvenientes**
Tratamiento antiviral pre-TH	-Carga viral baja -Evita tratamiento por largos periodos de tiempo -Eliminación de la fuente principal de viriones (el hígado).	-La RVS es baja en genotipo 1 -Efectos secundarios frecuentes y graves -Mala tolerancia. -Baja aplicabilidad.
Inmunoprofilaxis	-La carga viral alcanza un mínimo durante e inmediatamente después del trasplante. -No efectos secundarios descritos.	-Los datos preliminares sugieren una baja eficacia. -La producción de inmunoglobulinas es problemática.
Tratamiento antiviral post-TH	-Mejor tolerancia teórica que en el periodo pre-TH. -La decisión de tratamiento se puede basar en los resultados de la biopsia hepática.	-Carga viral elevada (inmunosupresión) -RVS baja en genotipo 1 -Tratamiento necesario durante periodos prolongados -La anemia secundaria a la ribavirina es muy frecuente.

4.5.1. Tratamiento de la hepatitis crónica C después del trasplante.

En general, se tiende a iniciar el tratamiento antiviral una vez pasados varios meses tras el trasplante, cuando ya se ha constatado una lesión histológica significativa producida por el VHC. La tolerancia al tratamiento antiviral parece ser mejor después del primer año post-trasplante; además, los niveles de inmunosupresión en ese momento son menores y la carga viral más baja con lo que, teóricamente, el tratamiento sería más eficaz en relación al post-trasplante inmediato. Sin embargo, los resultados obtenidos hasta el momento apuntan a que la RVS sólo se alcanza en alrededor del 20% [51-55]. (Tabla 2). Samuel y colaboradores, llevaron a cabo el único estudio controlado que se había realizado hasta el momento con el fin de establecer la eficacia del tratamiento antiviral en la recurrencia de la infección por el VHC tras el trasplante hepático. Se incluyeron pacientes trasplantados hepáticos con infección crónica por el VHC confirmada por biopsia hepática. El intervalo de tiempo transcurrido desde el trasplante hasta la inclusión en el estudio fue de 54-57 meses después del trasplante. Un total de 52 pacientes fueron aleatorizados para formar parte del grupo de tratamiento (3 MU de IFN estándar, tres veces por semana más ribavirina a dosis entre 800 y 1200mg/día, repartidos en dos dosis) o del grupo control. La respuesta virológica del grupo tratado y del grupo control al final del tratamiento fue del 32% y del 0%, respectivamente. Al final de seguimiento, la respuesta virológica sostenida fue del 21,4% en el grupo tratado frente a un 0% en el control. Los efectos adversos fueron de especial relevancia. Obligaron a suspender el

tratamiento en un 50% de los pacientes. La principal causa de abandono del tratamiento fue la anemia secundaria a la ribavirina [56].

Tabla 2. Tratamiento de la recurrencia de la infección por VHC post-trasplante hepático en pacientes con hepatitis crónica establecida. **RVS**: Respuesta virológica sostenida; **IFN**: interferón; **RBV**: Ribavirina. [a]: Estudio aleatorizado.

Autor	Nº pacientes	Tratamiento	RVS(%)
Bizollon et al. 1997	21	IFN 3MUI, 3v/sem (6m)+RBV 1.2g/d(1 año)	24
Firpi et al. 2002	54	IFN 3 MUI, 3v/sem+RBV 1g/d, 1 año.	30
Lavezzo et al. 2002	57	IFN 3MUI, 3v/sem+RBV 0,8g/d, 6 ó 12 meses	22 /17
Shakil et al. 2002	38	IFN 3MUI, 3v/sem, 1 año+RBV 0,8g/d, 18 meses	7
Samuel et al. 2003[a]	52	IFN 3MUI,3v/sem+RBV 1-1,2 g/d 12 meses vs placebo	21 /0
Samuel et al. 2004	22	PegIFN alfa-2b 1mcg/kg/sem +RBV(7,5mg/kg/día), 12 meses	23
Neumann et al. 2004	25	PegIFN alfa-2b 1,5mcg/Kg/sem+RBV 400-800mg/d, 12 meses	36
Dumortier et al. 2004	20	PegIFN alfa-2b 0,5-1mcg/Kg/sem+RBV(400-1200mg/d), 12 meses.	45

En los últimos años se está evaluando la eficacia del interferón pegilado en este contexto. Los estudios de Neumann y de Dumortier demuestran un porcentaje de respuesta virológica sostenida del 36% y del 45%, respectivamente, tras completar 1 año de tratamiento antiviral combinado. Sin

embargo, estos resultados tan prometedores deben ser confirmados con series más amplias de pacientes [57,58] .

4.5.2.Tratamiento precoz de la recurrencia de la infección por el VHC tras el TH

Aunque el tratamiento antiviral establecido de la recurrencia de la hepatitis C generalmente se inicia varios meses o años tras el trasplante, recientemente se está analizando la eficacia de otras estrategias que implican el inicio precoz del tratamiento. Dentro de estas nuevas estrategias se enmarcan las llamadas "preventivas" que consisten en iniciar el tratamiento antiviral pocas semanas después del trasplante, cuando aún no existe evidencia clínica ni histológica de hepatitis sobre el injerto. Desde un punto de vista teórico, ésta sería una buena opción ya que trataría de erradicar la infección por el VHC antes de que apareciera la lesión histológica. El tratamiento antiviral en la fase aguda de la infección es muy eficaz en sujetos inmunocompetentes [59]. Sin embargo, existen diferencias importantes entre la primoinfección C en sujetos inmunocompetentes y la recurrencia de la infección por el VHC tras el trasplante hepático. Ésta última se produce en sujetos que están sometidos a un tratamiento inmunosupresor de manera que es excepcional la resolución espontánea de la infección (que se ha descrito hasta en un 20-50% de los pacientes inmunocompetentes). Por otro lado, la recurrencia tras el trasplante se produce en sujetos en los que ya existe una respuesta inmune frente al VHC, la cual ha sido insuficiente para erradicar la infección. Además, la posibilidad de desarrollar rechazo celular y la baja tolerancia al tratamiento en esta fase precoz post-trasplante limitan considerablemente la aplicabilidad de esta estrategia. Por último, el tratamiento

llamado “precoz” no se puede considerar una opción preventiva, puesto que, como se sabe, la infección del injerto aparece de forma inmediata tras la reperfusión y la replicación viral se inicia mucho antes de que se altere el perfil hepático o exista evidencia de lesión histológica. Los primeros estudios aleatorizados que utilizaron interferón estándar en monoterapia no consiguieron demostrar un beneficio en cuanto a respuesta viral sostenida en esta fase post-trasplante [60,61]. Posteriormente Mazzaferro et al. compararon la eficacia del tratamiento precoz post-trasplante con interferón en monoterapia versus tratamiento combinado con interferón más ribavirina durante 48 semanas. La RVS fue del 13% y el 33%, respectivamente. La suspensión del tratamiento fue frecuente por recidiva de la hepatitis, por efectos adversos relativos a la toxicidad o por la aparición de rechazo (14%)[62]. Estos resultados, sin embargo, no se han podido confirmar en otros estudios que han empleado una estrategia similar. En el estudio de Terrault y colaboradores, no se detectaron diferencias en cuanto al tratamiento con interferón en monoterapia o en combinación con ribavirina, y la respuesta virológica sostenida fue de sólo 11% y 12%, respectivamente. Tal vez, la ausencia de diferencias entre los dos grupos se deba a la alta incidencia de anemia hemolítica en relación a la ribavirina (sólo 23% de los pacientes del brazo de tratamiento combinado fue capaz de mantener las dosis completas de ribavirina) [63].

4.5.3. <u>Inmunoprofilaxis</u>.

La inmunoprofilaxis ha jugado un papel fundamental en la prevención de la recurrencia de la infección por el VHB tras el trasplante. Sin embargo, no ocurre lo mismo en el caso de la hepatitis C. Esto se debe a varias razones. En primer lugar, existen pocos datos sobre la presencia y la localización de los

epítopos de neutralización del VHC. En segundo lugar, no existen modelos animales pequeños (ratón, conejo...) que permitan desarrollar ensayos de neutralización frente al VHC [64] . Finalmente, la extrema heterogeneidad genética que exhibe el VHC facilita que éste escape al sistema inmune del huésped [65]. Existen algunos indicios sobre la existencia de anticuerpos con capacidad neutralizante. A nivel clínico, Féray describió una menor frecuencia de recurrencia de la hepatitis C entre aquellos pacientes que recibieron gammaglobulina hiperinmune anti-B antes de 1990 (cuando aún no se disponían de métodos de cribaje anti-VHC) en comparación con aquellos que la recibieron después de 1990, lo que sugiere la presencia de anticuerpos neutralizantes del VHC en los preparados antiguos [66]. A nivel experimental, se ha demostrado que la neutralización *in vitro* del VHC es posible a partir de la utilización de un suero hiperinmune contra la región hipervariable 1 de la proteína E2 [13]. Por otro lado, la infusión de globulina hiperinmune anti-C puede retrasar la aparición de hepatitis tal y como demostró Krawczynski en chimpancés que habían sido inoculados experimentalmente con el virus [67]. Desgraciadamente aún no se sabe el papel que puede jugar la inmunoprofilaxis en la prevención de la recurrencia de la infección por el VHC tras el trasplante. Sin embargo parece lógico pensar que ésta debería ser particularmente útil durante la fase peritrasplante, donde sabemos que la carga viral alcanza un mínimo [68].

4.5.4. Nuevos antivirales.

Debido a la eficacia moderada de los antivirales de los que disponemos en este momento y a falta de una inmunoprofilaxis eficaz, en los últimos años se está avanzando en el desarrollo de nuevos fármacos

antivirales. El descubrimiento de la estructura tridimensional de algunas proteínas no estructurales del VHC, así como la construcción de replicones del VHC [69,70] han permitido introducir importantes avances en este área. Los agentes más prometedores por el momento son el inhibidor de la proteasa de NS3 y el inhibidor de la polimerasa de NS5B [71]. Aunque algunos de estos agentes se han ensayado en pacientes infectados con el VHC, los datos sobre la eficacia y seguridad son aún muy limitados. A título de ejemplo, se obtuvieron resultados muy esperanzadores con un inhibidor de la proteasa administrado durante dos días. Todos los pacientes experimentaron una caída significativa en los niveles de carga viral (más de dos logaritmos) que volvió a sus niveles basales tras la interrupción de la administración del fármaco [72].

Los estudios que se están llevando a cabo en estos momentos establecerán la eficacia de los nuevos antivirales, solos o en combinación.

5. JUSTIFICACIÓN DEL TEMA.

La recurrencia de la infección por el VHC tras el trasplante hepático se ha convertido en una prioridad para la mayoría de programas de trasplante en todo el mundo. Para el diseño de estrategias profilácticas/terapéuticas se requiere un mayor conocimiento de los mecanismos implicados en la reinfección del injerto por el VHC. Existen muy pocos datos sobre cómo se produce ésta y cuáles pueden ser los factores implicados en la historia natural de la recurrencia tras el trasplante.

En el primer trabajo que conforma esta tesis se ha descrito la cinética de la recidiva de la infección por el VHC a través del seguimiento de pacientes durante la fase inmediatamente anterior al trasplante, peritrasplante y en la fase precoz post-trasplante. Los estudios de la cinética viral no se han tenido en cuenta hasta ahora para el diseño de estrategias profilácticas/terapéuticas contra la recurrencia de la infección por el VHC. Sin embargo, su estudio en otros campos ha sido de gran importancia para la comprensión de los mecanismos de acción de los agentes antivirales y para entender la emergencia de cepas resistentes y a su vez han ayudado a establecer modelos predictivos del tiempo necesario de tratamiento para erradicar todos los reservorios virales. En la fase inicial de la infección por el VHC algunas variables virológicas, como la carga viral y las cuasiespecies parecen ser datos relevantes para predecir la evolución de dicha infección. Es de prever que, en un futuro cercano, el análisis de datos más sofisticados, como la cinética viral,

se tengan en cuenta para el diseño y la indicación de los diferentes tratamientos antivirales.

En el segundo trabajo de la tesis se investiga la eficacia y la tolerancia de una nueva estrategia para evitar la recurrencia de la hepatitis C tras el trasplante hepático. Dado que la reinfección del injerto se produce de forma inmediata tras la reperfusión (como se demuestra a través del primer estudio de la tesis), una de las opciones sería erradicar el virus antes de que éste pueda iniciar su ciclo vital en el nuevo órgano. Así, se diseñó un estudio en el que se trataron los pacientes infectados por el VHC que se encontraban en lista de espera para trasplante hepático. Hasta la fecha, la cirrosis descompensada constituye una contraindicación para el tratamiento con interferón y ribavirina. Sin embargo, este trabajo ha demostrado que, con una selección y seguimiento adecuados, la terapia antiviral combinada es eficaz en un subgrupo de pacientes y que la respuesta virológica se mantiene después del trasplante a pesar de la inmunosupresión. En estos pacientes, la importancia de la respuesta virológica radica en que es capaz de cambiar la historia natural y por tanto el pronóstico y la supervivencia tanto del paciente como del injerto después del trasplante.

Por último, en el tercer artículo que forma parte de la tesis se ha analizado el curso de la recurrencia de la infección por el VHC tras el trasplante hepático en receptores de donante vivo. El programa de trasplante hepático de donante vivo se inició en nuestro hospital en marzo de 2000. La justificación de dicho programa se basó en el progresivo desequilibrio entre la oferta y la demanda de órganos aptos para el trasplante que había conducido a un aumento de las muertes en lista de espera de alrededor de un 15-20%. Esta

técnica, iniciada hacía años en Japón para el trasplante hepático pediátrico, se generalizó al adulto en los años 90 y poco a poco se ha ido asentando como una opción real y en algunos casos la única opción de trasplante para algunos pacientes con hepatopatía avanzada o hepatocarcinoma (Fig 5). Teóricamente implica ofrecer el mejor órgano (procedente de un donante joven, con tiempo de isquemia corto, sin esteatosis), en el momento adecuado y, por tanto, se asumió desde el principio que los resultados en cuanto a supervivencia serían similares. Así fue en cuanto a resultados a corto plazo según la mayoría de las series publicadas [73-78]. Sin embargo, estudios preliminares, realizados de forma retrospectiva y con un número reducido de pacientes, alertaron sobre una mayor gravedad de la recurrencia de la hepatitis C en los receptores de donante vivo [79,80]. Otros estudios, por el contrario, no han señalado diferencias significativas [81-84]. El hecho de que no existan hasta la fecha estudios prospectivos dirigidos específicamente a analizar este hecho ha constribuído al estado de controversia en este campo. Nuestro estudio es, hasta la fecha, el primer estudio prospectivo realizado en este sentido. El objetivo del mismo fue establecer si la recidiva del VHC en receptores de donante vivo se producía de la misma manera que en el receptor de un órgano cadavérico.

Las características diferenciales del trasplante de donante vivo son básicamente dos. La primera hace referencia al hecho de que se trasplante un órgano parcial. En la mayoría de programas de trasplante hepático de donante vivo en adultos se emplea el lóbulo hepático derecho del donante. Tras implantar el injerto parcial en el receptor se inicia el proceso de regeneración hepática a partir de los hepatocitos y de las células del compartimento oval del

hígado del donante, capaces de diferenciarse, dividirse y dar lugar a hepatocitos maduros [85].

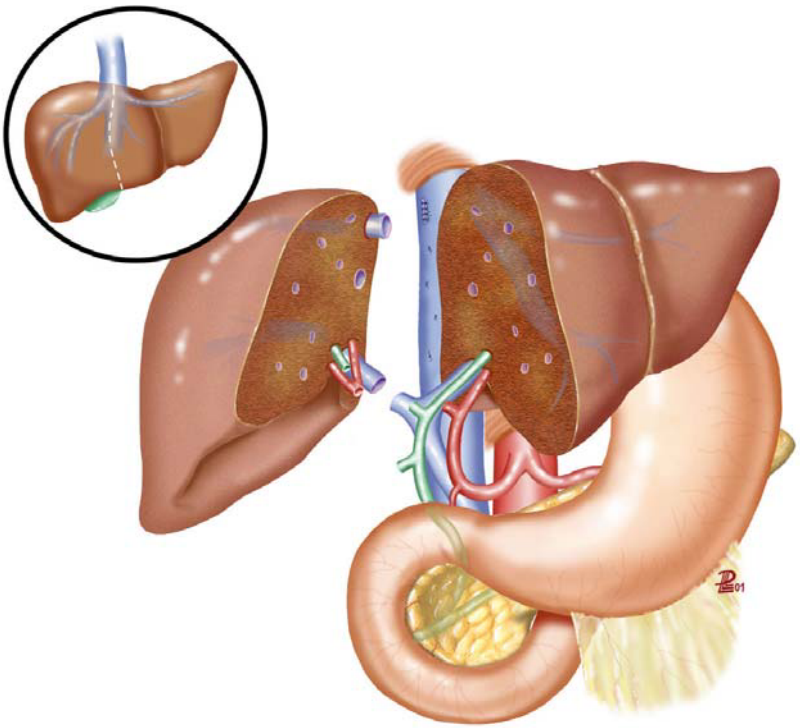

Fig 5.Esquema de la hepatectomía en el donante para el trasplante hepático de donante vivo.

La regeneración hepática se caracteriza por ser un proceso extraordinariamente rápido, de manera que en el primer mes tras el trasplante se alcanza el 65-70% de la masa hepática inicial del hígado antes de la hepatectomía [86,87]. Se desconoce hasta el momento cuál es el impacto del fenómeno de la regeneración hepática sobre la recidiva de la infección por el VHC. En segundo lugar, la técnica quirúrgica del trasplante de donante vivo implica una serie de problemas entre lo que destaca las complicaciones biliares derivadas de fugas o estenosis a nivel de las anastomosis biliares o de la superficie de corte del lóbulo hepático derecho. Dichas complicaciones biliares

se presentan hasta en un 30-70% de los receptores de trasplante de donante vivo comparado con 10-20% en los receptores de órganos procedentes de donantes cadavéricos [88-90.] Muchas de estas complicaciones requieren de exploraciones invasivas o de nuevas intervenciones para su manejo.

Existen pocos datos sobre cómo la colestasis que condicionan puede influir en la gravedad de la recidiva de la infección por el VHC tras el trasplante. Sin embargo, las enfermedades que cursan con colestasis crónica, como la colangitis esclerosante primaria o la cirrosis biliar primaria además de otras entidades que cursan con alteraciones obstructivas de la vía biliar, conducen a la estimulación de la fibrogénesis hepática y , en última instancia, al desarrollo de cirrosis hepática. En nuestro estudio se demuestra que existe una mayor progresión de la fibrosis asociada a la infección por el VHC en este grupo de pacientes y se discute sobre cuáles pueden ser las causas. Las conclusiones de dicho análisis son de gran interés y pueden contribuir a cambiar la pauta de actuación y las indicaciones del trasplante de donante vivo tal y como se ha propuesto recientemente [79].

6. ARTÍCULOS ORIGINALES Y EDITORIALES.

Hepatitis C Virus Kinetics During and Immediately After Liver Transplantation

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The study of hepatitis C virus (HCV) kinetics after liver transplantation (LT) might be important to design strategies to prevent HCV infection of the graft. We analyzed HCV kinetics during and immediately after LT in 20 consecutive patients undergoing LT for HCV-related cirrhosis. HCV RNA was quantified in blood samples obtained at regular intervals before, during, and after transplantation. HCV-RNA concentrations decreased in 18 of 20 patients during the anhepatic phase (mean decay slope −0.92, mean HCV elimination half-life 2.2 hours). We found a significant correlation between the HCV viral load decay and the blood loss during the anhepatic phase, indicating that the observed HCV clearance rates are maximum estimates. In fact, in 1 patient with an unusually long anhepatic phase of 20 hours and with minimum blood loss, the HCV elimination half-life was 10.3 hours. Eight to 24 hours after graft reperfusion a sharp decrease in HCV viral load occurred in 19 patients (mean decay slope −0.34, mean HCV elimination half-life 3.44 hours). HCV RNA became undetectable in only 1 patient. During the following days, HCV-RNA concentrations increased rapidly in 10 patients (mean HCV doubling time 13.8 hours), remained at similar levels in 4, and continued to decrease in 6. The only variable associated with a second-phase viral load decay was the absence of corticosteroids as part of the immunosuppressive regimen. In conclusion, a sharp decrease in HCV viral load occurs during the anhepatic phase and immediately after graft reperfusion, most likely owing to a lack of virion production and hepatic viral clearance. HCV infection of the graft, however, is an extremely dynamic process and viral replication begins a few hours after LT. (HEPATOLOGY 2002;35: 680-687.)

Cirrhosis caused by hepatitis C virus (HCV) infection is the main indication of liver transplantation (LT) in most transplant programs. Infection of the liver graft after transplantation is almost universal and persistent infection leading to chronic hepatitis, cirrhosis, and graft failure is common.[1,2] In our geographic area, 30% of patients undergoing LT for HCV-related liver disease are already cirrhotic 5 years after transplantation.[3] Regretfully, prophylaxis of HCV infection of the graft is not feasible because no specific anti-HCV immune globulin is available. In addition, antiviral treatment in patients on waiting lists for LT appears to be poorly effective and may cause severe adverse effects.[4,5] Currently, treatment of hepatitis C infection after LT seems the most feasible strategy to eradicate HCV.[6-8] Treatment of HCV infection after LT can be initiated before liver damage occurs or once liver disease is already established. Treatment in the early phase of LT seems a reasonable approach because eradication of HCV would prevent liver damage.[9-11] However, there are only a few studies analyzing the efficacy of antiviral therapy in the early posttransplantation period. Interferon monotherapy is not effective in achieving sustained virologic response in this setting, though histologic disease recurrence seems to occur less frequently[10] and the appearance of biochemical hepatitis is delayed.[11] Combination therapy using interferon and ribavirin started as soon as 3 weeks after transplantation appears to be more effective than interferon monotherapy,[9] but controlled studies are necessary to confirm its efficacy.

HCV viral kinetics after LT was not considered to design the therapeutic regimens used in any of the previously mentioned studies.[9-11] Information on HCV kinetics after transplantation is limited, but the data available indicate that HCV RNA decreases dramatically (even disappears) in most patients after transplantation and increases a few days after graft reperfusion.[12] Knowledge of viral kinetics has lead to a better understanding of the mechanisms of drug action, emergence of resistant strains, and development of models to predict the time needed to eradicate all viral reservoirs.[13] In HCV-infected immunocompetent patients the efficacy of antiviral therapy is strongly influenced by virologic vari-

Abbreviations: HCV, hepatitis C virus; LT, liver transplantation; RT-PCR, reverse transcription-polymerase chain reaction; CI, confidence interval.

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ables such as the HCV-infecting genotype and viral load.[14,15] In the near future, more sophisticated data, such as viral kinetics in the early phase of antiviral therapy, will be used to predict treatment efficacy and to decide therapeutic regimens.[16,17]

We have studied HCV kinetics during and immediately after LT in 20 patients undergoing LT for HCV-related cirrhosis. A careful analysis of the changes in HCV-RNA concentration during and after LT has revealed the existence of different phases of HCV kinetics that might facilitate the design of new strategies to prevent HCV recurrence after LT.

Patients and Methods

Patients. Twenty consecutive patients undergoing LT for HCV-related cirrhosis from September 2000 to March 2001 were included in the study. The indication for transplantation was decompensated cirrhosis in 9 patients and hepatocellular carcinoma in the remaining 11 patients. Four of the 20 patients received the right hepatic lobe of a living donor. All patients were HCV-RNA positive in at least 1 determination performed while on waiting list for LT. Serum samples were taken immediately before liver transplantation, at the beginning, and at the end of the anhepatic phase, and at 4, 8, 12, 16, 24, 48, 72, 96, and 120 hours after graft reperfusion. Thereafter, samples were obtained weekly during the first month and at weeks 12 and 24. During the surgical procedure, blood loss and transfusion requirements were recorded for each of the different phases (hepatectomy, anhepatic phase, and reperfusion phase). In hemodynamically stable patients, the Swan-Ganz catheter (Baxter, Irvine, CA), which is usually left the first 48 hours after transplantation, was replaced by a catheter in the hepatic veins. The catheter was placed under radiographic control 8 to 24 hours after graft reperfusion and left for 4 to 5 days; serum samples were taken at the same time-points as stated earlier. A radiograph performed daily was used to verify the correct location of the catheter.

All patients received 0.5 to 1 g of metilprednisolone during the anhepatic phase of LT. Thereafter, patients received 1 of the following immunosuppressive regimens: (1) cyclosporine A or tacrolimus associated with corticosteroids (13 patients); (2) tacrolimus, mofetil mycophenolate, and a monoclonal antibody anti-interleukin-2 receptor (7 patients).

Blood samples taken either from the peripheral circulation or from the hepatic veins were centrifuged within 2 to 3 hours after extraction, aliquoted, and frozen at −80°C. All patients gave their written informed consent before inclusion in the study protocol, which was approved by the Ethics Committee of our Center.

HCV-RNA Detection and Quantification. The concentration of HCV RNA was determined by using a quantitative reverse-transcription polymerase chain reaction (RT-PCR) assay (Cobas Amplicor HCV Monitor 2.0; Roche Diagnostics, Branchburg, NJ), that achieves a sensitivity of approximately 600 IU/mL. The assay was performed according to the manufacturer's instructions. Samples with HCV-RNA concentration exceeding 800,000 IU/mL were diluted to 1/100 and retested. Samples belonging to the same patient were assayed in the same run, except for those that were retested.

Samples testing negative with the commercial assay were retested by a more sensitive in-house RT-PCR assay in 2 independent experiments. Total RNA was extracted from 100 μL of serum with Trizol (GIBCO BRL Life Technologies, Barcelona, Spain), following the manufacturer's instructions. The RNA pellet was resuspended in 10 μL of RNase-free water containing 10 mmol/L dithiothreitol and 5% RNasin (20-40 U/μL) (Promega, Madison, WI). After incubation of the RNA at 65°C for 2 minutes, complementary DNA synthesis was performed with avian myeloblastosis virus reverse transcriptase (AMV; Promega) and the external antisense primer (Table 1). Briefly, 10 μL of RNA were added to a master mix consisting of 2 μL of PCR buffer 10×, 2 μL of dNTP 10 mmol/L, 2 μL of $MgCl_2$ 25 mmol/L, 3 μL of 10 μmol/L external antisense primer, and 1 μL of AMV (total volume 20 μL). After incubation for 1 hour at 42°C, 56.5 μL of water were added and the mixture was incubated at 95°C for 5 minutes. Complementary DNA was then amplified with a nested PCR by amplification of the 5′ untranslated region with nested primer pairs (Table 1).[18] For amplification, a master mix consisting of 8 μL of PCR buffer 10×, 2 μL of 10 μmol/L external antisense primer, 5 μL of 10 μmol/L external sense primer, 8 μL of $MgCl_2$ 25 mmol/L, and 0.5 μL of Taq Expand (Roche Diagnostics, Molecular Biochemicals, Barcelona, Spain) were added. The first round of PCR was performed for 35 cycles with denaturation at 94°C for 1 minute (initial denaturation step for 3 minutes), annealing at 45°C for 2 minutes, and amplification at 72°C for 3 minutes. For the second round of PCR amplification, a 10-μL aliquot of the first PCR was added to a master mix consisting of 9 μL of PCR buffer 10×, 2 μL of dNTP 10 mmol/L, 5 μL of 10 μmol/L internal antisense primer, 5 μL of 10 μmol/L internal sense primer, 9 μL of $MgCl_2$ 25 mmol/L, 0.5 μL of Taq Expand, and 59.5 μL of water. The same cycling conditions were used for the second round of PCR. Two positive controls (HCV-RNA concentration 5 IU/mL and 500 IU/mL, respectively) and 4 negative controls were used in each reaction.

Table 1. Primers Used to Amplify the 5′ Untranslated Region of the HCV Genome

External sense primer	ACT GTC TTC ACG CAG AAA GCG TCT AGC CAT
External antisense primer	CGA GAC CTC CCG GGG CAC TCG CAA GCA CCC
Internal sense primer	ACG CAG AAA GCG TCT AGC CAT GGC GTT AGT
Internal antisense primer	TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG

HCV Genotyping. HCV genotype was determined by restriction fragment length polymorphism analysis of the 5′ untranslated region, as previously described.[19]

Statistical Analysis. Quantitative variables are expressed as mean (95% confidence interval [CI]). Viral load decay slopes during the anhepatic phase and after graft reperfusion were calculated by linear regression by using HCV-RNA concentrations expressed in natural logarithm. The elimination half-life of hepatitis C virions was calculated by using the equation $t_{1/2} = \ln(2)/\text{slope}$. The HCV doubling time in patients with increasing HCV-RNA concentrations were calculated by using the same formula. Comparison between qualitative variables were made by the Fisher's exact test. Linear regression was used to analyze a possible relationship between quantitative variables.

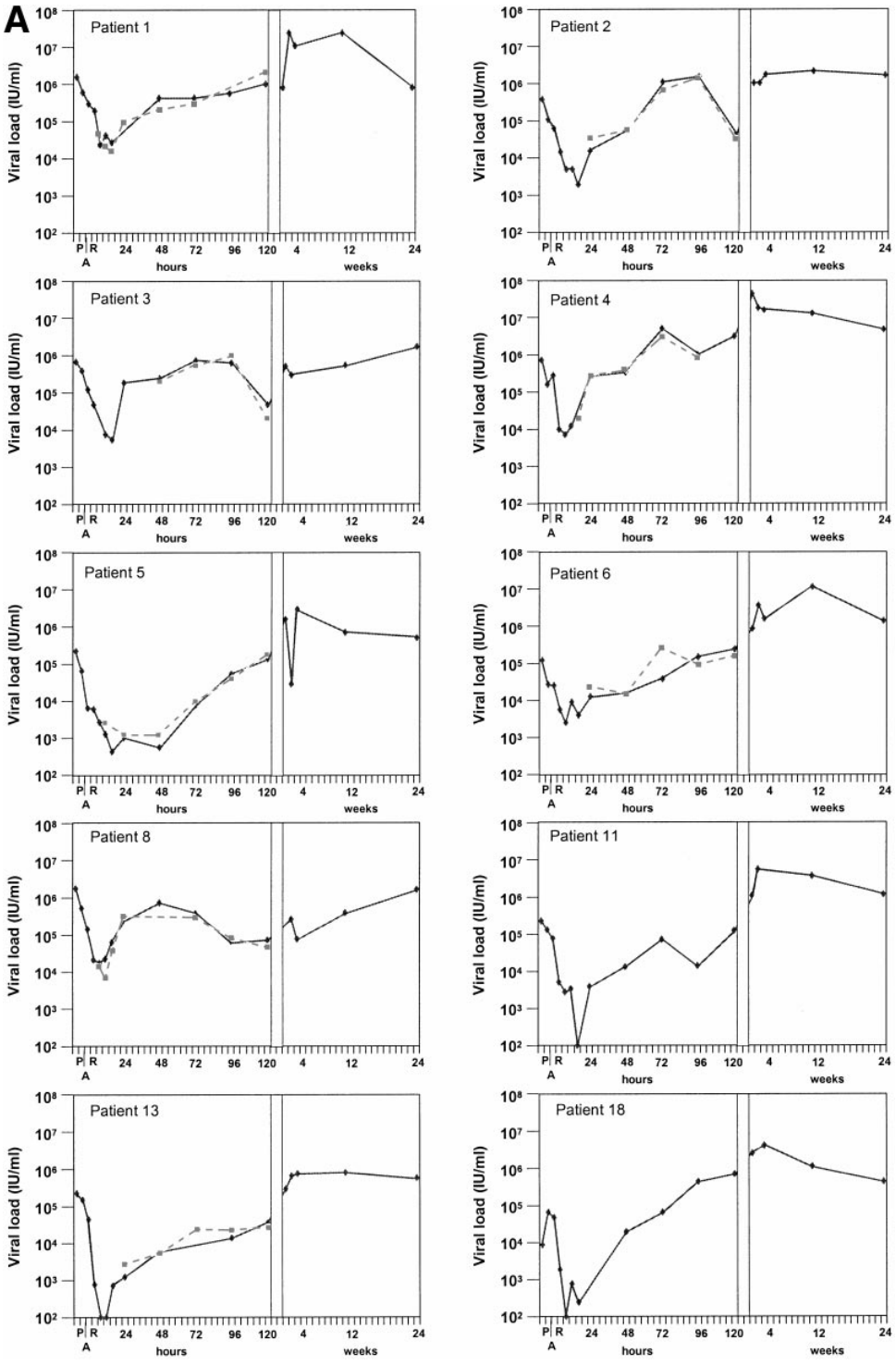

Fig. 1. HCV kinetics during and after liver transplantation. Patients are classified according to the viral kinetics pattern during the first days following graft reperfusion: (A) patients with rapid increase in HCV viral load (~2 $\log_{10}$) or pattern 1.

Results

Viral Kinetics During the Anhepatic Phase. HCV RNA was quantified in serum samples obtained before transplantation and at the beginning and at the end of the anhepatic phase. During the anhepatic phase, HCV-RNA concentration decreased in 18 of 20 patients (mean decrease 0.48 $\log_{10}$ IU/mL, 95% CI 0.29-0.68) and remained practically constant in 2 patients (Figs. 1 and 2). The duration of the anhepatic phase ranged from 45 to 207 minutes. Assuming a first-order elimination kinetics, the mean viral load decay slope was −0.92 (95% CI −0.52 to −1.32) and the mean elimination half-life was 2.2 hours (95% CI 0.65-3.7) (Table 2). We did not find a relationship between the viral load decay (or decay slope) and the pretransplantation viral load or the duration

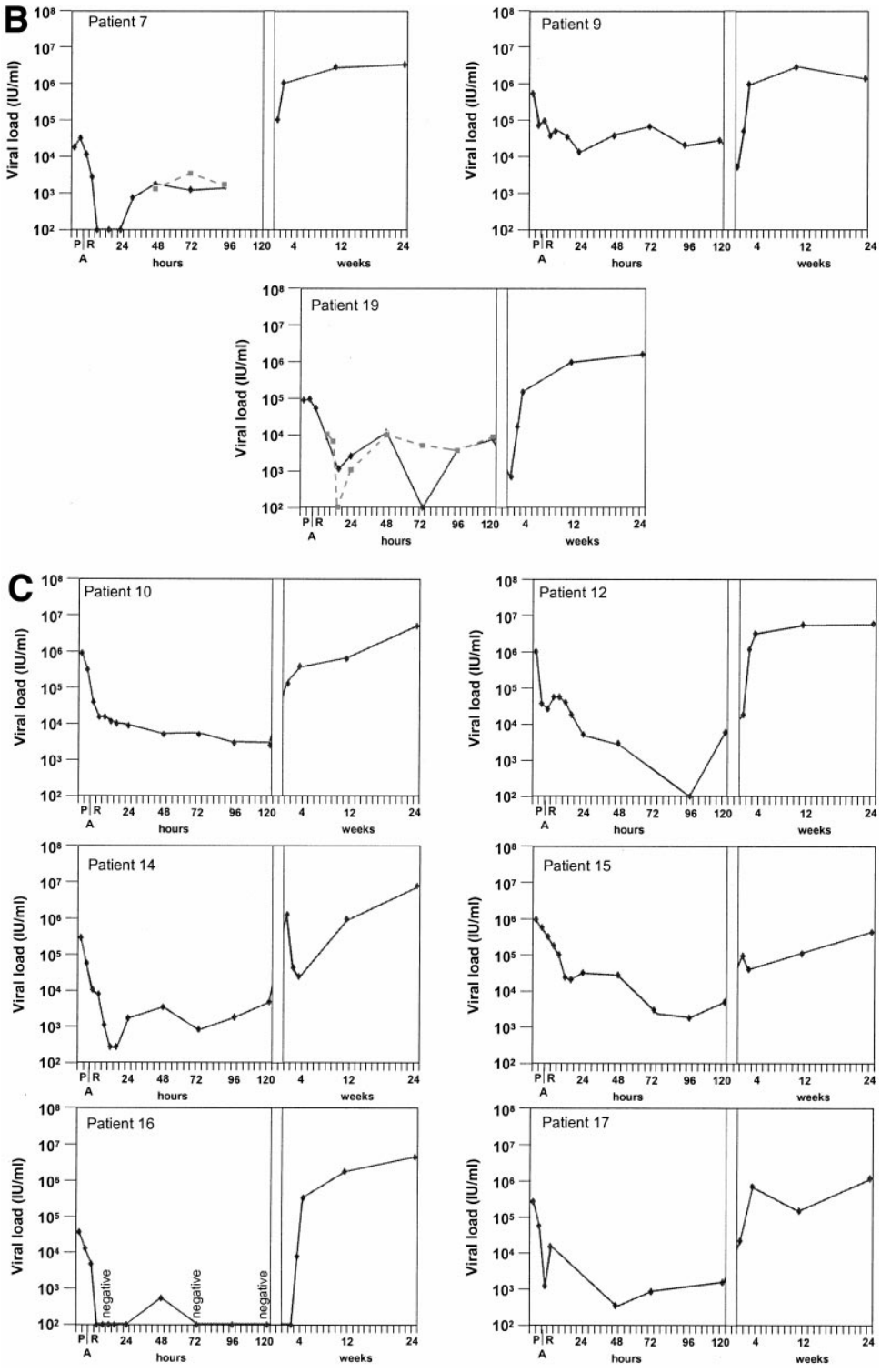

Fig. 1. (Cont'd.) (B) patients with unchanging viral load (or increase in viral load <1 $\log_{10}$) or pattern 2; (C) patients with progressive decrease in HCV RNA or pattern 3. HCV-RNA concentrations are expressed in IU/mL and depicted in the y axis in a logarithmic scale. Time is represented in the x axis, in hours and weeks. Viral load in the systemic circulation is depicted with a **continuous line;** HCV viral load in the hepatic veins is depicted with a **discontinuous line.** Samples that tested negative by a sensitive in-house RT-PCR assay are indicated. P, pretransplantation; A, anhepatic phase; R, reperfusion phase.

of the anhepatic phase. We found, however, a significant correlation between the decrease in viral load and the following variables: the amount of blood loss during the anhepatic phase ($r = 0.78$, $P < .001$), the number of red blood cell concentrates transfused during the anhepatic phase ($r = 0.71$, $P = .001$), and the entire surgical procedure ($r = 0.76$, $P < .001$). A correlation between the viral load decay slope and the amount of blood loss was also evident during the anhepatic phase ($r = 0.6$, $P = .02$). Considering the mean blood loss (2.5 L) and the mean transfusion requirements during surgery (red blood cell 6 U; plasma 1.5 L, plasma expanders 1.8 L), the calculated viral load decay slopes and elimination half-life values should be considered maximum HCV

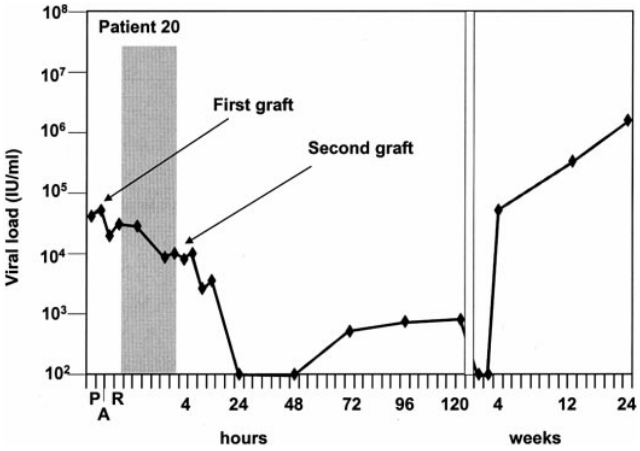

Fig. 2. HCV kinetics in a patient with a prolonged anhepatic phase. After implantation of the first graft the organ increased in size owing to difficult hepatic venous outflow and severe hemodynamic instability forced the surgeons to remove the liver and to perform a portocaval shunt. The patient was put on an urgent waiting list and received a second graft 20 hours later. Implantation of the second graft was uneventful. HCV-RNA concentrations are expressed in IU/mL and depicted in the y axis in a logarithmic scale. Time is represented in hours and weeks in the x axis. The prolonged anhepatic phase is shadowed.

extraction rates. Regarding the 2 patients in whom the viral load did not decrease during the anhepatic phase, we did not find any differences in the analyzed variables in comparison with the remaining patients.

We had the opportunity to study HCV kinetics in a patient with a prolonged anhepatic phase of 20 hours (patient 20). After implantation of the liver, the organ increased in size because of difficult hepatic venous outflow; severe hemodynamic instability forced the surgeons to remove the liver and to perform a portocaval shunt. The patient was put on an urgent waiting list and received a second graft 20 hours later. During this period the patient remained hemodynamically stable, with minimum transfusion requirements (2 U red blood cells and 0.5 L plasma). Serum samples were taken at the beginning, during, and at the end of this prolonged anhepatic phase and HCV-RNA concentrations were determined at each time-point (Fig. 2). In 20 hours, the HCV-RNA concentration decreased 0.58 $\log_{10}$ IU/mL, following a first-order kinetics as deduced by linear regression ($r = 0.95$). The viral load decrease slope was -0.067 and the deduced elimination half-life of hepatitis C virions was 10.3 hours.

Viral Kinetics After Reperfusion of the Graft. HCV RNA was quantified in serum samples taken at 4, 8, 12, 16, and 24 hours after graft reperfusion and daily thereafter until day 5 posttransplantation. A sharp reduction in HCV-RNA concentration occurred after the reperfusion phase in all but 1 patient. HCV viral load reached its lowest level 8 to 24 hours after reperfusion (mean viral load decrease 1.53 $\log_{10}$ IU/mL, 95% CI 1.22-1.85). By the quantitative test, HCV RNA became undetectable after graft reperfusion in 6 patients, 4 of them with pretransplant HCV-RNA concentrations below 10^5 IU/mL (Fig. 1). However, when negative samples were retested by a more sensitive assay, HCV RNA was undetectable in only 1 patient (patient 16) at 3 time-points (Fig. 1). HCV viral load decay after graft reperfusion followed a first-order elimination kinetics, with a mean decay slope of -0.34 (95% CI -0.22 to -0.46). The mean elimination half-life of hepatitis C virions was 3.44 hours (95% CI 2.02-4.86) (Table 2). We did not find a relationship between the viral load decay (or decay slope) and the amount of blood loss or transfusion requirements during the reperfusion phase. In the patient with a pro-

Table 2. Epidemiologic and Virologic Features of 20 Consecutive Patients Undergoing LT for HCV-Related Cirrhosis

Patient Number	Age	Sex	Genotype	Viral Load Before LT*	Elimination $t_{1/2}$ of HCV (hr) Anhepatic/Reperfusion	HCV Kinetics Pattern (HCV Doubling Time in hours)†	Immunosuppressive Regimen‡
1	54	M	1b	1,500,000	3.23/2.21	1 (20.7)	A
2	46	M	1b	373,000	1.40/3.48	1 (8.4)	A
3	55	M	2	667,000	0.59/3.45	1 (34.6)	A
4	59	F	1b	710,000	-/1.53	1 (7.3)	A
5	47	F	1b	221,000	0.22/3.96	1 (11)	A
6	56	M	1b	121,000	10.33/2.44	1 (19)	A
7	31	F	1a	17,700	0.79/1.16	2	A
8	63	F	1b	1,670,000	0.54/2.61	1 (7.4)	B
9	25	M	1a	566,000	-/6.73	2	A
10	65	F	1b	916,000	0.39/12.84	3	B
11	61	M	1a	219,000	1.72/2.03	1 (7)	B
12	62	M	1b	1,016,000	2.10/7.97	3	B
13	59	M	1b	231,000	0.47/0.91	1 (16.1)	A
14	56	M	1b	289,200	0.42/2.14	3	B
15	65	M	1b	966,000	1.62/3.71	3	B
16	43	M	No 1	38,200	0.99/0.71	3	B
17	59	M	1b	280,000	0.39/-	3	A
18	64	F	1b	8,920	2.22/0.90	1 (7.7)	A
19	65	F	1b	89,500	1.56/2.88	2	A
20	59	F	1b	41,300	10.3/3.75	2	A

*Viral load in IU/mL.

†HCV kinetics pattern 24 hours after graft reperfusion. 1, increase in viral load; 2, unchanging viral load; 3, decrease in viral load. HCV doubling time is shown in parentheses in patients with pattern 1.

‡A, cyclosporine A or tacrolimus associated with corticosteroids; B, tacrolimus, mofetil mycophenolate, and a MAb anti-IL2 receptor.

longed anhepatic phase of 20 hours, a sharp decrease in viral load (1.99 $\log_{10}$ IU/mL) occurred after reperfusion of the second graft (viral load decay slope −0.18), with a deduced HCV elimination half-life (3.75 hours) significantly shorter than that of the anhepatic phase (Fig. 2, Table 2). The transfusion requirements after reperfusion (2 U red blood cells and 0.5 L plasma) were similar to those during the prolonged anhepatic phase.

The HCV elimination half-life after graft reperfusion was unusually long in 3 patients (patients 9, 10, and 12). We did not find any remarkable differences between these 3 patients and the remaining individuals regarding pretransplantation viral load and transfusion requirements. In 2 of them, however, there was significant ischemia-reperfusion injury of the graft that caused a remarkable elevation of aspartate transaminase and alanine transaminase values (>1,000 IU/mL) within the first 24 to 48 hours. In the only patient in whom viral load did not decrease immediately after graft reperfusion (patient 17), ischemia-reperfusion injury was also remarkable.

After this phase of viral load decline, we observed 3 different HCV kinetic patterns during the first week after LT (Table 2). The first one (pattern 1) was characterized by a rapid increase in HCV viral load (~2 $\log_{10}$). The second pattern (pattern 2) was identified by unchanging viral load (increase in viral load <1 $\log_{10}$). The third pattern (pattern 3) was characterized by a progressive decrease in HCV RNA. HCV-RNA concentrations increased rapidly to pretransplantation levels in 10 patients (Fig. 1A) with a viral load increase slope of 0.07 (95% CI 0.04-0.09) and a mean HCV doubling time of 13.8 hours (95% CI 6.6-21 hours). HCV RNA remained at similar levels in 4 patients (Figs. 1B and 2) and followed a second-phase decline in 6 patients (Fig. 1C). Interestingly, 5 of the 6 patients with a second-phase decline in HCV viral load were part of a group of 7 patients whose immunosuppressive regimen did not include corticosteroids (regimen B). In contrast, only 1 of the 13 patients who received corticosteroids as part of their immunosuppressive regimen showed a second-phase decline in HCV viral load ($P < .01$) (Table 2). Viral load kinetic patterns were not determined by pretransplantation HCV-RNA concentrations.

After the first week of transplantation, viral kinetics followed a similar pattern in most patients. We observed a progressive increase in viral load during weeks 2, 3, and 4 after LT, even in patients with viral kinetic patterns 2 and 3. In general, this increase in viral load coincided with higher and more stable levels of cyclosporine or tacrolimus. However, this was not the case in all individuals, such as in patients 12 and 19 (data not shown).

We did not find any significant differences in the early HCV kinetics among patients undergoing living-related (patients 4, 6, 9, and 13) or cadaveric liver transplantation (the remaining patients), or among patients infected with different HCV genotypes.

Viral Kinetics Based on Samples Taken From the Hepatic Veins. Previously published data suggested that HCV RNA remained undetectable in most patients up to 48 to 72 hours after liver transplantation.[12] To determine if HCV RNA could be detected earlier in blood drained directly from the liver, we catheterized the hepatic veins of 10 patients shortly after graft reperfusion. We did not find, however, significant differences between HCV-RNA concentrations in the peripheral circulation and the hepatic veins and viral kinetics followed a similar pattern in both sites (Fig. 1).

Discussion

We studied viral kinetics in 20 patients undergoing LT for HCV-related disease. Our results show that removal of the infected liver causes a significant decrease in HCV-RNA concentration, which can be explained in part by the lack of virion production.[12] The decline in viral load during the anhepatic phase varied significantly from patient to patient, most likely because of the large number of variables that may influence viral load during surgery. Among these variables, we found a clear correlation between the decrease in viral load and the amount of blood loss and transfusion requirements during surgery, suggesting that the viral load decay values observed during this phase are maximum estimates. One of the limitations of the analysis is that only 1 sample at the beginning and at the end of a short anhepatic phase does not allow detection of the presence of 2 or more phases in the viral kinetics.

After the implantation of the new graft, HCV viral load continued to decrease exponentially in all but 1 patient. Although we did not find a relationship between the viral load decay and the transfusion requirements, we cannot exclude some contribution of this variable to the decrease in HCV-RNA concentrations. We hypothesize that after graft reperfusion, massive entrance of HCV into the hepatocytes or HCV uptake by the liver reticuloendothelial system is the cause, at least in part, of HCV clearance. Hepatic clearance of hepatitis C virions after graft reperfusion is strongly supported by the data obtained in 1 patient with a prolonged anhepatic phase of 20 hours. In this patient the elimination half-life of HCV was significantly longer during the anhepatic phase than after graft reperfusion. This indicates that viral clearance occurs relatively slowly in the absence of liver, whereas it increases significantly after the implantation of a graft. The lower clearance rates after graft reperfusion observed in a significant number of patients might be explained, in part, by active virus production after implantation of the new graft. Regarding the lack of significant differences between the viral load in the systemic circulation and the hepatic veins, several reasons may explain this finding. First, our study was not aimed to measure HCV hepatic clearance and we did not determine viral load in the portal vein. Second, hepatic vein catheterization immediately after transplantation was difficult and blood samples were not available for most patients during the viral load decay slope that followed graft reperfusion. Finally, it is possible that the massive hepatic blood flow minimizes slight differences in viral load. This is supported by the lack of significant differences in HCV-RNA concentrations between the hepatic veins and the systemic circulation during the sharp increase in HCV viral load that occurred the first days after LT.

The elimination half-life of hepatitis C virions during the anhepatic phase is somewhat shorter in our study than in the study published by Fukumoto et al.[12] However, HCV half-life in the latter study was based on the decrease in viral load during both the anhepatic and after reperfusion phases. In fact, the elimination half-life of HCV after graft reperfusion in our study is similar to that reported by Fukumoto et al.[12] and other studies.[17,20] In 3 patients, the HCV elimination half-life was unusually long (over 6

hours), and in 2 of them ischemia-reperfusion injury of the graft was remarkable. It is possible that ischemia-reperfusion damage of hepatocytes impairs HCV entrance and prolongs the elimination half-life of circulating virions. This might also explain the lack of viral load decay immediately after graft reperfusion in 1 patient.

Differently from previous reports,[12] we found that HCV RNA remained detectable in almost all patients after LT. These differences are most likely explained by the use of a more sensitive test to detect HCV RNA. Our data indicate that virus particles are constantly present in the blood stream during the anhepatic phase and cause infection of the new graft.

HCV viral load increased as soon as 12 hours after graft reperfusion, reaching pretransplantation levels by day 4 after transplantation in a significant proportion of patients. The rapid increase in HCV viral load indicates that viral replication is highly efficient after LT and proves the high capacity of HCV to adapt to a completely new environment. Not in all patients, however, did HCV kinetics follow the same pattern. In 6 patients, HCV-RNA concentrations continued to decline during the first days after LT. Differences in the immunosuppressive regimen appeared to influence HCV kinetics immediately after LT. In fact, HCV-RNA concentrations increased rapidly in patients receiving corticosteroids as part of the immunosuppressive therapy,[21-23] whereas it continued to decrease in most patients in whom corticosteroids were not part of the immunosuppression therapy. Although this observation needs to be confirmed in other studies, it is possible that some immunosuppressive regimens might be more appropriate if early antiviral therapy to eradicate HCV is considered.

In general, we found that HCV-RNA concentrations increased progressively after the first week of transplantation and reached a plateau by the first month. Apart from the possible effect of the immunosuppression on HCV-RNA concentrations, other variables might influence this increase in viral load. It is possible that the HCV quasispecies populations require some time to reach an equilibrium in a context of a preestablished cellular and humoral immune response.[24]

Based on our data on HCV kinetics, it seems clear that any immunoprophylaxis attempt should start during the anhepatic phase, before circulating hepatitis C virions can infect the hepatocytes of the new graft. Regarding antiviral therapy, it might be more efficient if initiated during or immediately after LT, when viral load reaches its lowest level. It would be relevant to analyze if this therapeutic strategy is more efficient than the current antiviral preemptive regimens beginning a few weeks after LT.[9-11]

In summary, in patients undergoing LT, HCV viral load decreases during the anhepatic phase and after graft reperfusion because of lack of virus production, blood loss, and hepatic viral clearance. Despite the viral load decay, hepatitis C virions continue to circulate and infect the new graft rapidly. HCV replication in the liver graft begins as soon as a few hours after LT in most patients. These results might be helpful to design more efficient therapeutic strategies to eradicate HCV early after LT.

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Viral Kinetics of Hepatitis C: New Insights and Remaining Limitations

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Evaluation of a patient with a chronic viral illness includes the determination of serum viral load. This value provides no information regarding production rate of the virus, its half-life, or the turnover rate of viral infected cells. Such data require perturbation of the steady state and detailed observation of viral levels over time. Adequate interpretation of such results requires consultation with colleagues in theoretical mathematics. This is the wise direction that Dr. David Ho et al. embarked upon in the mid 1990s.[1] Such collaboration dramatically changed our understanding of the life cycle of human immunodeficiency virus (HIV) infection and improved therapeutic strategies. The input of mathematicians such as Perelson et al.,[1,2] Nowak et al.,[3] and Wei et al.[4] allowed seminal observations of the production rate of HIV, its serum clearance, and the elimination rate of infected CD4 cells. Using mathematical models, further predictions were made regarding the mechanism of action of antiretroviral therapy, the evolution of resistant viral strains, and the length of time necessary to eradicate all infected sites with perfect therapy. This interactive collaboration between clinician scientists, theoretical mathematicians, and pharmaceutical scientists markedly improved therapy resulting in an increased life expectancy and improved quality of life. Limitations of this approach should be noted; interpretation of data still requires verification from work performed by cell biologists, virologists, and immunologists.

Have similar collaborations in the field of hepatitis C virus (HCV) research led to significant findings? Lam, in conjunction with Neumann and Perelson et al.,[5] showed in 1997 that interferon alfa 2b (IFN-α2b) caused a dose-dependent 0.5 to 2.0 log decline in viral RNA levels within 24 hours of the first dose of IFN. Who would have thought that IFN, initially proposed to act in hepatitis B virus (HBV) infection by enhancing the immune response to infected liver cells,[6] would cause such a rapid lowering of HCV-RNA serum levels? The rapid, dose-dependent exponential decline in viral levels, which has been noted with all IFN products,[7,8] has now also been observed with pegylated interferon (PEG-IFN).[9] Neumann et al. proposed that such a rapid decline in viral levels over 24 hours could only be explained if IFN inhibited viral production,[8] termed IFN effectiveness, and if circulating virions were rapidly cleared (serum half-life). Indeed, the serum half-life of HCV has been calculated to be 2 to 3 hours, which is significantly shorter than either HIV or HBV. The mathematical prediction that IFN acts by inhibiting HCV production in a dose-dependent manner has now been substantiated in HCV replication models using different doses of IFN.[10]

Can differences in the degree of IFN effectiveness in blocking viral production account for differences in treatment response? Indeed, IFN effectiveness in blocking viral production is significantly greater in genotype 2– and 3–infected subjects compared with genotype 1–infected patients,[11] which may well explain in part the improved therapeutic response seen in genotype 2– and 3–infected patients. In fact, the drop within the first 24 hours of treatment is 1 log greater in genotype 2–infected patients compared with genotype 1–infected patients. In the latter patients, the extent of IFN effectiveness varies widely with some patients having less than a 0.5 log decline over 24 hours despite doses of IFN equal to or greater than 10 MU[11] while other patients have greater than a 2.0 log decline within 24 hours. Interestingly, in African American patients infected with genotype 1 virus, the extent of IFN effectiveness in blocking viral production is 50% less than in white patients,[12] which may explain in part the lower rate of sustained viral response (SVR) seen in African Americans.[13]

The mechanism(s) whereby IFN inhibits HCV viral production is not entirely known. IFN is known to activate double-stranded RNA-activated protein kinase (PKR), 2′,5′-oligoadenylate synthetase, and other path-

Abbreviations: HIV, human immunodeficiency virus; HCV, hepatitis C virus; IFN, interferon; HBV, hepatitis B virus; PEG-IFN, pegylated interferon; SVR, sustained viral response; ALT, alanine transaminase.

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ways that are known to inhibit viral production.[14,15] Interestingly, certain HCV proteins have been shown *in vitro* to interact with PKR and impair its activation by IFN or with the Jak-Stat pathway upstream of PKR activation, which would in theory impact IFN effectiveness.[16] Thus, variations in IFN sensitivity may relate to differences in IFN pharmacokinetics, cell receptor activation, intracellular signaling, and viral interactions with critical antiviral signaling processes.

Recently, it has been suggested by two separate groups that a low IFN effectiveness (<90% effectiveness; <1 log drop) after the first drug dose may predict non-SVR with over a year of IFN therapy. This observation was made for patients treated with IFN monotherapy,[17] combination therapy,[18] and pegylated IFN therapy.[19] In these studies, lack of a 1.0 log within 24 hours or a less than 30% decrease in viral load was associated with failure to clear virus early in therapy as well as non-SVR. While this was not examined by Buti et al.,[9] it can be noted in Fig. 4 of their article that patients d and e and in Fig. 6 that patients c, d, and e had little or no IFN effectiveness and subsequently had no further decline in viral levels with over a year of therapy. These composite results require additional confirmation, particularly for patients infected with genotype 1 virus and in populations of patients who are notoriously IFN resistant, such as African Americans. If true, a year-long treatment with significant side effects and cost could be avoided.

After the initial rapid lowering of viral RNA levels, viral decline slows and becomes quite variable between patients. Some patients exhibit a rapid second phase viral decline and clear virus within 1 month, whereas other patients have no further decline in viral levels (flat response) and never clear virus. Some show an initial decline but with time viral levels rebound. Preliminary data suggest that others have a slower decline and eventually clear virus.[20] These patients appear to be at greater risk for relapse. Furthermore, it has been suggested that the rate of the decline in viral levels in the second phase over the first month of treatment is the best predictor of SVR.[24] What determines this variation in viral decline? From a mathematical point of view the second phase of viral decline may be determined by death of infected cells (δ) by natural killer or cytotoxic T cells[8] or elimination of virus from infected cells by antiviral cytokines without cell turnover. Neumann et al. predicted from mathematics that the rate of decline in the second phase was dependent on the death rate of infected liver cells.[8] Indeed, in patients with a faster second slope decline, the calculated delta was higher. In their initial observation, the calculated death rate of infected liver cells was directly correlated with the baseline alanine transaminase (ALT) level, which was also noted by Zeuzem et al. in studies using PEG-IFN.[21] This suggests that patients with preexisting immunologic recognition of infected liver cells would have a faster lowering of viral levels in the second phase of viral clearance due to immunologic destruction of infected liver cells. If this theory is correct, one might predict an elevation in ALT values early in therapy for patients with a fast second phase viral decline. However, this has not been observed, and in fact the contrary generally holds true: as viral levels fall, so do ALT values. An alternate theory is that antiviral cytokines released from T cells can clear the cells of virus in a noncytopathic manner. This mechanism has been proposed by Guidotti and Chisari[22] in a series of studies examining how HBV is cleared from infected hepatocytes. This approach would have the homeostatic advantage to the host of a slower loss of liver cells. While mathematics has led us towards truth in this issue, truth will only be determined by careful immunologic and cell culture studies. Possibly, it is a little bit of both.

Although the biphasic model of HCV decline following the initiation of treatment has held true regardless of the type of IFN product, we[8] and others[23] have observed that after the rapid initial drop in HCV-RNA levels, there is a leveling off or slight increase in viral levels despite daily doses of IFN. Buti et al.[9] note a similar increase in HCV-RNA levels after 72 hours with PEG-IFN. Interestingly, Layden et al. showed that the rate of viral decline in the second phase is dependent on the serum viral levels at the end of the first phase; *i.e.,* the lower the viral level at the end of 24 hours, the faster the second phase viral decline.[17] In recent preliminary studies, Bergmann et al. has shown that a triphasic decline in HCV-RNA levels occurs in 25% to 30% of patients treated with IFN with or without ribavirin.[24] In this model, the first phase is followed by a second plateau phase that is variable between patients. The length of this plateau phase is dictated by the viral level at the end of the first phase; *i.e.,* those with a lower level will have a shorter plateau phase. The theoretical background for this triphasic model has not been presented.

The principles of viral kinetics have now been applied in great detail to study the recurrence of HCV after liver transplantation by Forns et al.[25] in a recent issue of HEPATOLOGY. While others have published reports on the kinetics of HCV after transplantation,[26] this is clearly the most comprehensive study. In the anhepatic phase, the mean elimination half-life of virus was 2.2 hours and the mean log drop was 0.5 log. Interestingly, with reperfusion, there was a first order kinetic log decline of 1.5 over 8 to 24 hours. In 1 patient with a prolonged anhepatic phase the elimination half-life of virus was significantly

longer in the anhepatic phase compared with the reperfusion phase suggesting that the liver is the major organ that clears virus. In 3 patients there was no significant decline in viral levels in the reperfusion phase, and of note, in 2 of these patients significant reperfusion injury had occurred. The lack of decline in these 2 patients probably reflects impaired uptake by the injured liver. After this rapid decline, viral levels in the first week either increased in a rapid exponential manner with a mean doubling time of 14 hours, remained relatively the same, or decreased. In half of the patients a new steady state was reached within 1 week of transplantation. The rapid increase in viral levels was seen more frequently in patients on corticosteroids reflecting a permissive effect of these drugs on viral production. In the patients who had stable or declining RNA levels in the first week after transplantation, viral levels soon increased reaching a steady state within a month of transplantation.

What does this study teach us about new infection after transplantation that may be useful in prophylactic studies? First, it is very clear that the liver is a very efficient organ responsible for viral clearance. Second, viral production is restored rapidly, and corticosteroids are a source that fuels the fire. Thus, it would be ideal to have an agent that impairs hepatic uptake immediately after the anhepatic stage. Unfortunately, neither IFN nor ribavirin appear, from a kinetic point of view, to act in this manner. There is some suggestion from kinetic studies that amantadine may impair HCV uptake, although its efficiency is quite low.[27] As IFN acts to impair HCV production, its administration within 24 to 48 hours of transplantation may blunt the rapid restoration of viral production. However, in this debilitated patient population, IFN can present problems. It is very clear that within a week to a month following transplantation, viral infection has reached a steady state with production equaling serum clearance. Although it is clear in standard acute infection that IFN can lead to cure in nearly 100% of patients,[28] in acute infection following transplantation this response has not been seen.[29,30] Possibly, these differences in treatment response are because of the size of the HCV inoculum, the speed at which viral steady state is reached, or the effect of immunosuppressants on viral replication coupled with the state of the immune system. Nevertheless, these kinetic results suggest that earlier therapy after transplantation increases the likelihood of a treatment response.

Mathematical modeling has dramatically altered our understanding of the life cycles of 3 very prevalent chronic viral infections. It has also provided us with information on how antiviral agents act to reduce viral levels and clear virus, and it has provided predictive information on whether a patient is likely to clear virus. Mathematics leads us to formulate tenable questions and hypotheses whose proof or rebuttal challenges the clinician and basic scientist.

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Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation☆

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See Editorial, pages 435–436

***Background/Aims*: After liver transplantation (LT) infection of the graft with the hepatitis C virus (HCV) is almost universal and chronic hepatitis and cirrhosis develop in a significant proportion of patients. One of the possible strategies to prevent HCV infection recurrence is to eradicate HCV before LT.**

***Methods*: We evaluated the efficacy and safety of antiviral therapy to prevent HCV recurrence in 30 HCV-cirrhotic patients awaiting LT. At the time of inclusion 15 patients were Child–Pugh A and 15 Child–Pugh B/C. The infecting genotype was 1b in 25 patients. Treatment with interferon α-2b 3 MU/day and ribavirin 800 mg/day was initiated when the expected time for LT was less than 4 months and continued until LT. The median duration of treatment was 12 weeks.**

***Results*: Nine patients (30%) achieved a virological response and 21 did not respond to therapy. In nine (43%) of the 21 non-responders viral load decreased ≥ 2 $\log_{10}$ during treatment. A viral load decrease ≥ 2 $\log_{10}$ at week 4 of treatment was the strongest predictor of virological response. All nine virological responders have already undergone LT; six patients remain free of infection after a median follow-up of 46 weeks and HCV infection recurred in three patients after LT. In one of these patients HCV-RNA was still detectable in the explanted liver. Side effects were frequent and dose reduction was necessary in 19 (63%) of the 30 patients; no patient died while on therapy.**

***Conclusions*: Our data support the utilization of antiviral therapy in HCV-infected patients awaiting LT as one of the strategies to prevent hepatitis C recurrence after transplantation.**

***Keywords*: Interferon; Ribavirin; Liver graft; Virological response; Viral load**

1. Introduction

Hepatitis C virus (HCV) infection is the leading cause of cirrhosis and hepatocellular carcinoma in the Western world and Japan and HCV-related liver disease accounts for more than half of the indications of liver transplantation in most transplant programs [1]. Regretfully, infection of the liver graft with HCV occurs almost universally after transplantation and chronic hepatitis and cirrhosis develop in a significant proportion of patients a few years after transplantation [2–4]. Recent studies have shown that graft and patient survival are significantly lower in patients undergoing transplantation for HCV-related cirrhosis compared to patients undergoing LT for other causes [5,6]. Therefore, prevention of hepatitis C recurrence in the liver graft has become one of the major goals of most transplant programs.

Treatment of HCV infection is usually initiated after liver transplantation, but the optimal timing for initiation of

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antiviral therapy is unknown. Early treatment initiated a few weeks after LT does not appear to be the best approach given the rapid increase in viral load that follows graft reperfusion [7–9]. Antiviral therapy is commonly initiated a few months (or years) after LT, when follow-up liver biopsies demonstrate disease progression. Most studies, however, indicate that the efficacy of antiviral therapy in liver transplant recipients is low; sustained virological response occurs in less than 20% of treated patients and adverse effects force treatment interruption in a significant proportion of cases [10–12].

A different strategy to prevent HCV disease recurrence in the liver graft is eradication of HCV before LT. Most clinical trials using combination therapy have shown that the response rates in patients with significant fibrosis is quite high, exceeding 40% when pegilated interferon and ribavirin are used [13,14]. Advanced cirrhosis, however, is considered a contraindication for antiviral therapy, due to the numerous and potentially severe side effects caused by interferon and ribavirin administration. For these reasons very few studies have analyzed the efficacy of antiviral therapy in HCV-infected patients awaiting liver transplantation [15,16].

We have analyzed the safety and efficacy of interferon and ribavirin therapy in a cohort of 30 patients with HCV-related cirrhosis on the waiting list for liver transplantation. Our results show that virological response occurs in one third of these patients and, although adverse effects are very common, a close clinical follow-up might prevent life-threatening complications.

2. Patients and methods

2.1. Patients

Thirty patients with HCV-related cirrhosis awaiting liver transplantation were included in the study. The study was approved by the Ethical Committee of our Institution and by the Spanish Health Ministry. All patients gave their written informed consent before entering the study. The inclusion criteria were: a positive anti-HCV test, positive HCV-RNA in serum, platelet count $> 50 \times 10^9$/l (or 75×10^9/l if prothrombin activity was $< 40\%$), neutrophil count $> 1.2 \times 10^9$/l, hemoglobin > 9 g/dl, and expected time on the waiting list shorter than 4 months. Exclusion criteria were: participation in other clinical trials, recurrent hepatic encephalopathy, renal failure (creatinine > 1.5 mg/dl or blood urea nitrogen > 30 mg/dl), infection with the hepatitis B virus or the human immunodeficiency virus, previous organ transplantation, and all other common causes that contra-indicate interferon and ribavirin treatment.

2.2. Treatment regimen and dose modification

Treatment was initiated when the expected time for liver transplantation was less than 4 months and continued until the day of LT. Patients were treated with interferon α2b 3 MU/day (Intron A; Schering-Plough, Inc., Kenilworth, NJ) and ribavirin 800 mg/day (Rebetol; Schering-Plough, Inc., Kenilworth, NJ). Interferon dose was reduced to 1.5 MU/day if the neutrophil count decreased below 0.75×10^9/l or the platelet count below 40×10^9/l. If neutrophil or platelet counts did not increase above 0.75×10^9/l or 40×10^9/l after 1 week, respectively, interferon was further reduced to 1.5 MU/48 h. Treatment was interrupted with platelet counts below 25×10^9/l and was reinitiated at the lowest dose (1.5 MU/48 h) once platelet counts were above 40×10^9/l. If neutrophil counts descended below 0.5×10^9/l filgrastim (Neupogen 30, Amgen SA, Barcelona) was initiated at a dose of 300 μg/48 h s.c. until counts reached 1.2×10^9/l and maintained at 300 μg weekly if counts descended below 0.75×10^9/l. Ribavirin dose was reduced to 600 mg/day when the hemoglobin level decreased below 8 g/dl and to 400 mg/day if hemoglobin levels did not increase after 2 weeks. In the latter case or if anemia was symptomatic, erythropoietin (epoetinum α, Epopen, Esteve SA, Barcelona) was initiated at, 2000 IU/48 h s.c.; this dose was maintained until hemoglobin reached 10 g/dl. Ribavirin administration was interrupted if despite epoetinum α therapy transfusion was required to maintain hemoglobin levels above 8 g/dl. Ribavirin was also discontinued in patients with upper gastrointestinal hemorrhage until they resumed oral ingestion. Neither interferon nor ribavirin doses were modified if ascites or mild hepatic encephalopathy occurred during therapy. In case of spontaneous bacterial peritonitis or other infections, therapy was not suspended unless accompanied by renal failure (serum creatinine above 1.5 mg/dl), severe hepatic encephalopathy, ileus, sepsis or neutropenia.

2.3. Follow-up

A complete clinical history, physical examination and biochemical and hematological work-up was performed every week. All patients could contact a staff member of our unit at any time during the treatment period.

2.4. HCV-RNA and genotype determination

HCV-RNA was quantified before initiating treatment and thereafter every 2 weeks until transplantation (one determination just before LT). After liver transplantation, viral load was determined every 2 weeks during the first 2 months and monthly thereafter. HCV viral load in serum was determined by a quantitative assay (Cobas Amplicor HCV Monitor 2.0; Roche Diagnostics, Branchburg, NJ, USA) and by a qualitative assay (Amplicor HCV 2.0; Roche Diagnostics) when HCV-RNA was below the detection limit of the quantitative test. HCV genotype was determined by restriction fragment length polymorphism after amplification of the 5 non-coding region of the HCV genome, as previously described [17].

In a few patients, HCV-RNA was determined in the explanted liver. After hepatectomy, tissue samples were immediately frozen in liquid nitrogen and kept at -80 °C before analysis. Liver RNA was extracted from 50–100 μg of tissue with the guanidium isothiocyanate procedure (Trizol, Gibco BRL Life Technologies, Barcelona, Spain) and the polymerase chain reaction was performed using primers of the 5 non-coding region, as previously described [7]. The sensitivity of the assay ranged between 100 and 1000 IU/ml. One positive control (liver tissue from a patient with detectable HCV-RNA in serum) and four negative controls (liver tissue from an anti-HCV negative alcoholic patient undergoing LT) were included in the procedure. The quality of the assay was controlled by simultaneous extraction of glucose 3-phospho-dehydrogenase mRNA.

2.5. Response to therapy

Virological response was defined as negativization of HCV-RNA confirmed by a sensitive qualitative HCV-RNA test, that persisted until liver transplantation. The remaining situations were considered non-response.

2.6. Statistical analysis

All quantitative variables are expressed as median (range). For categorical variables, differences between groups were calculated by the Fisher's exact test; for quantitative variables differences between groups were analyzed using a non-parametric test (Mann–Whitney).

3. Results

3.1. Patients' baseline characteristics

From June 2001 to September 2002, 50 patients who

were not participating in other ongoing clinical trials were evaluated for this study. Nineteen patients (38%) were excluded due to laboratory abnormalities (thrombocytopenia and/or neutropenia in 17 and renal failure in two) and one (2%) refused to participate. Therefore, 30 patients comprised the final study cohort. Eligibility to participate in this study for patients included in other clinical trials was similar (50%). The demographic, clinical, hematological and virological baseline characteristics of our patient cohort are shown in Table 1.

3.2. Response to treatment

The median duration of antiviral therapy was 12 weeks (ranging from 2 to 33). Some patients were treated for less than 4 weeks due to donor-recipient size incompatibility or temporal exclusion from the waiting list of preceding recipients. Of the 30 treated patients nine (30%) achieved a virological response, and 21 (70%) did not respond to therapy (Fig. 1). Among the 21 non-responders, nine (43%) patients achieved a decrease in viral load of ≥ 2 $\log_{10}$ during therapy, but without persistent HCV-RNA negativization.

Treatment with interferon and ribavirin did not appear to modify liver function tests. Bilirubin levels and prothrombin index were similar before and after therapy (2.1 vs. 1.9 mg/dl and 69 vs. 72%, respectively). There was a decrease in albumin levels during treatment, but it did only reach statistical significance in non-responders (34 vs. 31 g/l, $P = 0.001$).

Table 1
Baseline characteristics of 30 HCV-infected patients awaiting liver transplantation

Sex (M/F)	25/5
Age (years)	57 (36–66)
Indication of liver transplantation	
End-stage liver disease	13 (43%)
Hepatocellular carcinoma	17 (57%)
Child–Pugh score	
A	15 (50%)
B	13 (43%)
C	2 (7%)
Serum ALT (UI/l)	104 (29–207)
Bilirubin (mg/dl)	2.1 (0.7–4.6)
Albumin (g/l)	34 (17–48)
Prothrombin index (%)	72 (49–98)
Hematological parameters	
Hemoglobin (g/d)	13.5 (10–16.6)
WBC (10^9/l)	4.9 (3–7.9)
Platelets (10^9/l)	73 (50–203)
Genotype	
1a	3 (10%)
1b	22 (73.3%)
2	1 (3.3%)
3	4 (13.3%)
Viral load (IU/ml)	5.88×10^5 (10^2–3.3×10^7)
$< 800\,000$	22 (73%)
$> 800\,000$	8 (27%)
Previous interferon therapy	6 (20%)

3.3. Variables related to virological response

Virological responders were younger, had lower ALT values before initiating therapy and were more frequently infected by non-1 genotypes than non-responders; none of these differences, however, achieved statistical significance (Table 2). Pre-treatment viral load was significantly lower in responders (median 2.88×10^5 IU/ml) than in non-responders (median 6.4×10^5 IU/ml) ($P = 0.01$). Interestingly, a viral load decrease of ≥ 2 $\log_{10}$ at week 4 occurred in 9/9 (100%) responders but only in two (12%) of the 16 non-responders in whom a serum sample was available at week 4 ($P < 0.001$) (Table 2 and Fig. 2). The positive and negative predictive values of an early decrease in viral load were 82 and 100%, respectively. Treatment duration was not different between virological responders and non-responders; full-dose therapy was longer in responders (8 weeks) than in non-responders (4 weeks) but the difference did not reach statistical significance.

Among non-responders, we identified nine patients (numbers 10–18) in whom viral load decreased ≥ 2 $\log_{10}$ during treatment. Interestingly, in most of these patients viral load remained at very low levels (between 10^3 and 10^4 IU/ml) despite antiviral therapy (Fig. 1). In four patients (numbers 10, 13, 14 and 17) treatment was maintained at full-dose, whereas in five interferon was reduced to 1.5 MU/day (number 18) or to 1.5 MU/48 h (numbers 11, 12, 15 and 16). In the latter two patients treatment was interrupted.

3.4. Follow-up after liver transplantation

All but four patients have already undergone liver transplantation. Three patients died shortly after LT; the causes of death were primary non-function of the graft (patient 13), early recurrence of HCC (patient 8) and massive hemorrhage (patient 23). The latter patient had a history of previous abdominal surgery; platelet counts at time of LT were not significantly lower than pretreatment counts (48×10^9/l vs. 53×10^9/l, respectively). Two additional patients died 2 and 10 months after transplantation due to sepsis.

As expected, in all patients with detectable HCV-RNA before transplantation HCV infection recurred immediately after the procedure. As previously described [7], viral load increased rapidly above pre-LT levels following transplantation (Fig. 1C). Regarding the nine patients who achieved a virological response, all of them have already undergone LT. HCV infection recurred in three patients at weeks 2, 4 and 5 after transplantation (numbers 7, 8 and 9, respectively) (Fig. 1B). These three patients were infected with genotype 1b. The remaining six patients are HCV-RNA negative after a median follow-up of 46 weeks (range

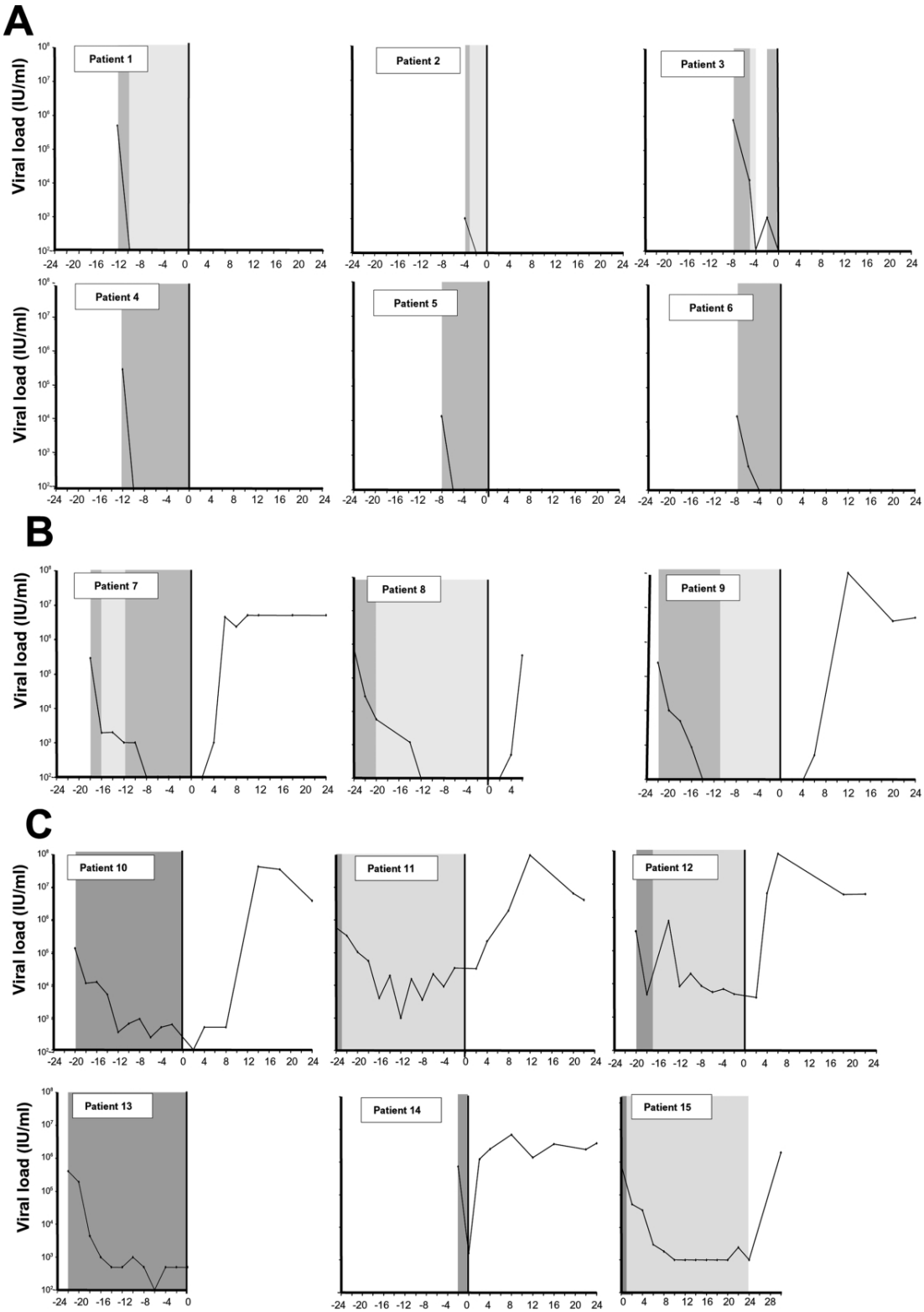

Fig. 1. Evolution of HCV-RNA concentrations in 30 HCV-infected patients on waiting list for liver transplantation treated with interferon and ribavirin. Viral load is expressed in IU/ml and depicted in the y axis in a logarithmic scale. A viral load value of 10^2 IU/ml is equivalent to a negative HCV-RNA test by a sensitive qualitative assay. Time is represented in the *x* axis, in weeks. Liver transplantation is represented as a vertical line (time 0). Weeks before transplantation are shown as negative values, except in patients who have not undergone liver transplantation. Dark shaded

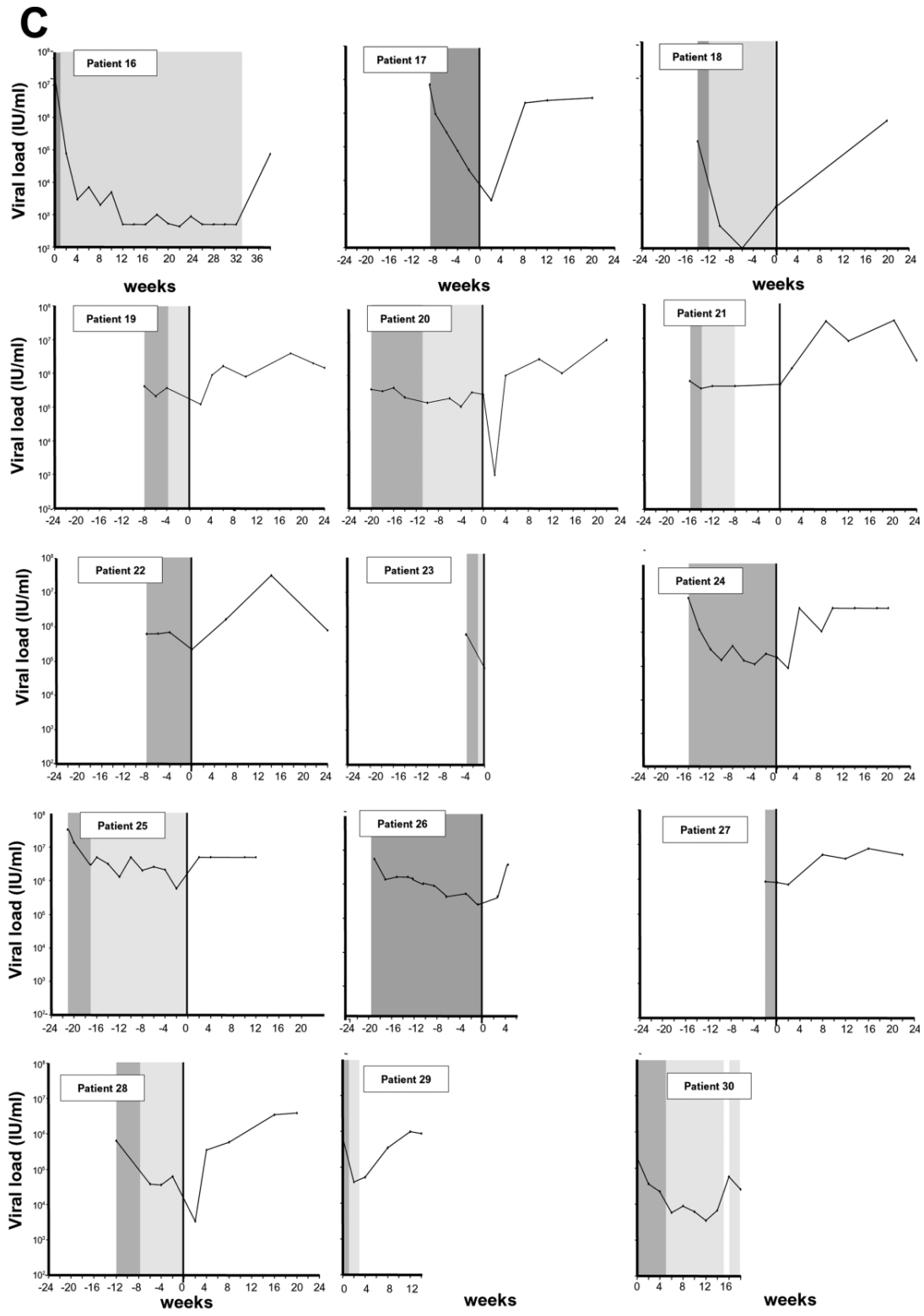

Fig. 1. (continued) areas indicate treatment with full interferon and ribavirin dosages; light shaded areas indicate treatment at reduced doses. Non-shaded areas before transplantation indicate treatment interruption. (A) Patients who achieved virological response (HCV-RNA negativization) during therapy that persisted after liver transplantation. (B) Patients with virological response during therapy and HCV infection recurrence after transplantation. (C) Patients who did not achieve virological response during therapy.

Table 2
Clinical, biochemical and virological differences between patients according to the virological response to therapy

	Response ($n = 9$)	Non-response ($n = 21$)	P value
Sex (M/F)	7/2	18/3	0.6
Age	55 (36–62)	58 (44–66)	0.1
Indication of LT (CH/HCC)	4/5	9/12	1
Child–Pugh score (A/BC)	4/5	11/10	1
ALT	83 (29–167)	105 (31–207)	0.1
Genotype (1/2–3)	6/3	19/2	0.1
Viral load pre-LT	2.88×10^5 ($10^{2-}7.9 \times 10^5$)	6.4×10^5 (1.28×10^5–3.3×10^7)	**0.01**
>2 log10 decrease VL at week 4 of therapy[a] (Y/N)	9/0	2/14	**0.00**
Weeks of treatment	12 (4–27)	15 (2–33)	1
Weeks of full-dose treatment	8 (1–12)	4 (1–21)	0.2
Previous interferon therapy (Y/N)	1/8	5/16	0.6

[a] Available in 25 patients.

24–80). Therefore, six (20%) of the 30 patients achieved a virological response that persisted after liver transplantation.

Interferon dose was reduced in the three relapsers and in three of the six sustained responders; ribavirin dose was reduced in one patient of each group. Immunosuppression regimen was similar in all treated patients (cyclosporine or tacrolimus plus corticosteroids); none of the three relapsers had a rejection episode and therefore did not require stronger immunosuppression.

Liver HCV-RNA was determined in the explanted liver of seven patients: five virological responders (patients 1, 2, 3, 7, and 9) and two non-responders with a decrease in viral load ≥ 2 $\log_{10}$ during therapy (patients 13 and 14). HCV-RNA was detectable in the explanted liver of the two non-responders and in one virological responder (patient 7) in whom HCV infection recurred 2 weeks after LT. In the remaining patients HCV-RNA was undetectable in the explanted liver in three independent experiments using different liver fragments.

3.5. Safety of combination antiviral therapy

Clinical adverse events were common during therapy (Table 3). Two patients developed sepsis; in one the cause was a pneumonia with respiratory failure caused by *Streptococcus pyogenes* and in the second a catheter-related thrombophlebitis caused by *Staphylococcus aureus*. The baseline Child–Pugh score of these two patients was 7. In both cases neutrophil counts were above 1.2×10^9/l at the time of hospital admission and infection resolved after adequate antibiotic treatment. During therapy four patients (three Child–Pugh B and one Child–Pugh C) presented de novo hepatic decompensation: hepatic encephalopathy in two cases, ascites in one case, and variceal bleeding associated with hepatic encephalopathy and ascites in one case.

Hematological adverse effects were very common among our patients. Neutrophil count decreased below 1.2×10^9/l in 18 patients and platelet counts decreased below 50×10^9/l in 15. Interferon dose reduction was necessary in 18 (60%) of 30 patients. Anemia occurred in eight patients and ribavirin dose reduction was necessary in seven (23%) of 30 patients. Eleven patients required filgrastim due to neutropenia and eight erythropoietin due to anemia. Filgrastim was administered for a median time of 5 weeks (range 1–19) and was efficient in increasing the neutrophil counts in all patients. Erythropoietin was administered for a median time of 10 weeks (range 2–20) and was efficient in increasing the hemoglobin values in all patients. We did not observe adverse side effects that could be attributed to erythropoietin administration. Acute rejection occurred in only five (19%) of the 27 patients with a follow-up longer than 1 month after transplantation.

Treatment was discontinued in six patients (numbers 3, 15, 16, 21, 29 and 30); in four therapy was interrupted definitively (numbers 15, 16, 21 and 29). The causes of treatment discontinuation were thrombocytopenia (4 patients) and sepsis (two patients). Child–Pugh score at initiation of therapy did not influence treatment dose reduction or discontinuation.

Importantly, no patient died while on therapy and the deaths that occurred after transplantation were not related to the treatment.

4. Discussion

The increasing incidence and severity of HCV recurrence after LT has prompted our and other groups to analyze all possible strategies to prevent HCV infection of the graft. One of these strategies is to initiate antiviral therapy before liver transplantation. In our study, interferon was administered daily to avoid the peaks and valleys of serum interferon concentrations that occur with standard therapy regimen and therefore to improve its pharmacokinetics [18,19]. Although pegilated interferon would have been a good alternative, we were afraid of a longer duration of

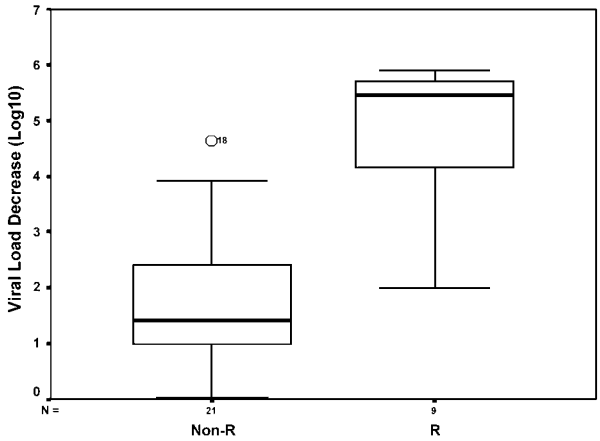

Fig. 2. Decrease in viral load during treatment in responders and non-responders to antiviral therapy. Viral load is expressed in IU/ml and depicted in the *y* axis in a logarithmic scale. Viral load decrease in each group is represented as box plot (the top and the bottom of the boxes being the 25th and 75th percentiles and the line through the middle of the box the median). Median viral load decrease was 5.45 $\log_{10}$ IU/ml in responders versus 1.41 $\log_{10}$ IU/ml in non-responders ($P < 0.001$).

hematological side effects in patients waiting for a non-scheduled major surgery procedure.

The results of our study support the utilization of antiviral therapy in patients awaiting LT. One third of the patients achieved a virological response while on therapy and 20% of the patients remained free of infection after LT. These results are similar to those recently reported by Crippin et al. and Everson et al. [15,16]. In the latter study [16], 102 HCV-cirrhotic patients were treated with interferon and ribavirin for one year with a low accelerating dose regimen. Half of these patients were Child A and half Child B or C, resembling the distribution of our study cohort. The end-of-treatment virological response in this study was 40% and the sustained virological response 20%. However, in genotype 1 infected patients sustained virological response was only 11%. Thirty-two patients have already undergone liver transplantation; in none of the ten sustained responders who underwent transplantation HCV infection recurred.

Table 3
Clinical and hematological adverse events in HCV-infected patients awaiting LT while on therapy

Clinical adverse events	
Hepatic encephalopathy	3 (10%)
Ascites	2 (7%)
Variceal hemorrhage	1 (3%)
Fever	5 (17%)
Asthenia	5 (17%)
Infection[a]	4 (13%)
Rash	2 (7%)
Diarrhea	2 (7%)
Other[b]	2 (7%)
Hematological adverse events	
Neutropenia	18 (60%)
Thrombocytopenia	15 (50%)
Anemia	8 (27%)

[a] Two patients developed sepsis.
[b] One patient presented ALT elevation (>5 times above the upper normal limit) and one patient presented gingival hemorrhage.

The results of our study indicate that antiviral efficacy is high in individuals with favorable virological variables, particularly those with a low HCV viral load. There was a clear trend towards a more favorable response in patients infected with non-1 genotypes, but the differences were not statistically significant due to the sample size. Importantly, an early viral load accurately predicted the type of virological response. This is specially relevant to interrupt treatment in patients with low response probability, in whom side effects are extremely frequent and sometimes severe.

One of the main differences between our study and the data reported by Everson et al. [16] is the duration of antiviral treatment. Our rationale to initiate treatment when the expected time for transplantation was less than 4 months was: (a) we assumed that most virological responders would achieve HCV-RNA negativization by week 12, and (b) to avoid a long treatment course in patients prone to develop severe side effects. Despite our results, it is still unclear which treatment schedule is more convenient for patients awaiting LT. A short antiviral therapy course appears a good strategy when the date of transplantation is known (living donor LT) or when the waiting time can be predicted.

Recurrence of HCV infection after LT in three of the nine virological responders was disappointing. Liver HCV-RNA was detected in the explanted liver of one of these patients, whereas it was undetectable in the liver of another. It is not surprising that even in patients with very low viremia before transplantation, circulating virions can initiate infection of the graft [7]. However, in patients with undetectable HCV-RNA in serum and in the explanted liver recurrence is more difficult to explain. One possibility is that our detection methods are not sensitive enough to detect very low levels of HCV-RNA. Another possibility is the persistence of virions in a second compartment, such as peripheral blood mononuclear cells or the bone marrow. There are data supporting HCV replication in a second compartment during the anhepatic phase of LT and during the first hours following graft reperfusion [20].

Based on our results, we can assume that > 50% of HCV-infected patients in the waiting list are adequate candidates for antiviral therapy. The clear association between HCV infection and decreased patient and graft survival after liver transplantation [5,6] strongly suggests that the implementation of antiviral therapy in these patients might be cost-effective, even with a sustained virological response rate of around 20%. We do not know the impact that a significant reduction in viral load during therapy would have on graft survival, as a low viral load before transplantation has been associated with a reduced severity of post-transplantation liver disease [21].

One of our main concerns was the incidence and severity of side effects, which are important limitations of this

therapeutical strategy [15]. Although the incidence of side effects in our study was similar to that reported by others, frequent clinical and laboratory evaluation proved useful for early detection and treatment of complications. The high proportion of Child A patients might have influenced our results towards a better tolerance of antiviral therapy. In addition, careful adjustment of drug dosage and utilization of G-CSF or erythropoietin might be helpful to decrease the incidence of hematological adverse effects. Bacterial infection is probably the most threatening complication of antiviral therapy in cirrhotic patients with decreased neutrophil counts. Although infection occurred in four of our patients, neutrophil counts were not below 1.2×10^9/l in any of these patients when the infection was diagnosed. As ours was not a controlled study, it is impossible to know if the incidence of infections would have been lower in non-treated patients. Importantly, no patient died while on therapy and deaths after LT were not related to antiviral treatment.

In summary, we believe that antiviral therapy in patients awaiting liver transplantation should be considered as one of the possible strategies to prevent HCV disease recurrence after LT. Although the incidence of adverse events is high, a very close clinical follow-up has proven helpful to rapidly detect and treat life-threatening complications.

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Editorial

Treatment of patients with decompensated post-hepatitis C cirrhosis before liver transplantation: strategy to prevent hepatitis C virus (HCV) recurrence?

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See Article, pages 389–396

After liver transplantation (LT), hepatitis C virus (HCV) recurrence is almost universal particularly if HCV RNA is detectable at the time of transplant and can lead in a great number of patients to recurrent cirrhosis and graft failure [1,2]. This recurrence is often rapid [1]. Several studies have shown that the treatment by combination therapy using interferon alfa and ribavirin is possible after liver transplantation but the virological response rate is low and the treatment is usually associated with major side effects, requiring dose reduction or stopping treatment [3].

Another strategy is the eradication of HCV RNA before LT in order to prevent HCV recurrence after LT and reduction in the level of HCV RNA to reduce the severity of post-transplantation liver disease. Forns et al. [4] evaluated the efficacy and safety of antiviral therapy in 30 patients with post-hepatitis C cirrhosis awaiting liver transplantation. Only patients having an expected time on the waiting list shorter than four months were included. Patients with hepatic encephalopathy, renal failure or co-infection by hepatitis B virus or human immunodeficiency virus were excluded. Patients were treated with interferon alfa 2b 3 MUI/day and ribavirin 800 mg/day. The reduction dose was realized according to the laboratory recommendations.

Fifty patients were screened during a 15 month period, but 19 (38%) were excluded due to contra-indication or refusal. The median duration of treatment was 12 weeks (2–33). Virological response was observed in nine patients (30%). Variables associated with a good response to treatment were age, ALT level, genotype non 1 and low viral load. A decrease of viral load ≥ 2 log had a positive predictive value of 100% at week 4. After liver transplantation, among the nine patients with virological response, HCV infection recurred in only three patients at week 2, 4, 5, respectively after liver transplantation. All these patients were infected with genotype 1b. Six patients became HCV RNA negative after a mean follow-up of 46 weeks (24–80). Indeed, 4/5 patients also tested in the liver were HCV RNA negative. Side effects were frequent. Two patients developed sepsis; in both cases, neutrophil counts were above 1.2×10^9/l at the time of hospital admission. Interferon dose reduction was necessary in 60% of cases and ribavirin dose reduction in 24% of cases. Eleven patients required filgrastim due to neutropenia and eight erythropoïetin due to anemia. No patients died during therapy.

Assessment of interferon in patients with decompensated chronic hepatitis C was until now based on limited small case series. A gradually increasing dose regimen of combination therapy with interferon and ribavirin has been used in patients with both compensated and decompensated cirrhosis due to hepatitis C by Everson et al. [5]. Patients were started on low dose of interferon (1.5 MUI, tiw) and ribavirin (600 mg/day) with slowly increasing dose of both drugs every 2 weeks as tolerated. Preliminary results of treating 91 patients, the majority infected with genotype 1, were recently reported. On-treatment virological responses occurred in 38% and a sustained virological response in 22% of patients. Sustained responses were more common in patients treated for more than 6 months. Eight patients who were treated and were HCV RNA negative at the time of transplantation remained virus free post-transplantation. On the other hand, recurrent and persistent HCV infection of the allograft was observed in all patients with detectable HCV RNA at the time of transplantation. No significant change was observed regarding the hepatic synthetic function and/or Child Pugh score. Indeed, 27 of non-responders were reported to develop adverse events. Less favorable

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outcome has been reported by Crippin et al. [6] in a collaborative study of five US liver transplant centers. Patients were treated with a common protocol using low dose of interferon with or without low dose of ribavirin. Only half the patients screened for the study were enrolled, many being excluded because of severe cytopenias. All patients had advanced liver disease with a mean Child-Pugh score of 12, as well as elevated serum bilirubin, prolonged prothrombin time and moderated impaired renal function. On treatment, 33% of patients became HCV RNA negative. Two patients underwent liver transplantation and both developed recurrent infection. Adverse events were common and sometimes severe, including profound thrombocytopenia, marked neutropenia, new-onset hepatic encephalopathy and life-threatening infections witch ultimately led to the early termination of the study. Of course, because both studies did not included an untreated control group for comparison, it is unclear whether interferon and ribavirin combination therapy per se precipitated these life-threatening infections or whether they merrily represented complications of end stage liver disease. All together these three studies suggest that antiviral therapy with post-hepatitis C cirrhosis awaiting liver transplantation is possible and can prevent HCV disease recurrence in several patients especially in patients with favorable predictive factors of response. However, recurrence of HCV infection after LT is possible even if HCV RNA is negative in the serum or the liver at the time of transplantation. Two explanations can be proposed to explain this discrepancy: first the method of detection of HCV RNA was not sensitive enough; in this case it would be interesting to compare this result with a more sensitive method of detection such as real-time PCR. The second explanation could be the persistence of the virus in a second compartment such as peripheral blood mononuclear cells; to confirm this hypothesis, it is necessary to study quasispecies distribution in each compartment. The best results observed by Everson et al. [5] and Forns et al. [4] suggest that the treatment is better tolerated in patients with Child A and B than in patients with Child C and leads to less severe complications such as neutropenia and thrombocytopenia. All these studies clearly show also that it is necessary in some cases to use growth factors including GM-CSF and erythropoïetin to boost peripheral blood cell counts in patients with severe neutropenia and erythropenia to prevent profound cytopenias and infections. From these studies, it seems very difficult to define the best regimen. In Forns et al. study [4], authors used daily dose of recombinant interferon. By contrast, Everson et al. [5] as well as Crippin et al. [6] used low doses of interferon three times a week. There are no data on the safety and/or efficacy of Peginterferon with or without ribavirin in patients with decompensated post hepatitis C cirrhosis. Indeed, the combination of Peinterferon plus ribavirin was only tested in patients with severe fibrosis (F3 and F4) and was well tolerated. [7] However, because peginterferon regimens are associated with higher rate of neutropenia and thrombocytopenia, treatment is likely to be associated with even greater infection complications than regimens using standard infection interferon and slower recovery from these complications when the interferon is stopped. However, it will be very interesting in the future to compare these different regimens. Indeed the best duration of treatment remains to be defined. The rational for Forns et al. [4] to treat for a short time was that most virological responders had a viral load decrease of ≥ 2 log 10 at week 4 and were HCV RNA negative by week 12. However these results are very surprising, especially in patients with genotype 1b and were not found by others and we do not know which treatment schedule is more convenient. In conclusion, in patients with decompensated HCV cirrhosis, antiviral therapy as suggest by Wright et al. [8] in the last American consensus conference, should be considered experimental and not be administered outside of prospective trials. If the results of these prospective trials are confirmed, this strategy could be then used in patients with post-hepatitis C cirrhosis without severe hepatocellular insufficiency awaiting LT.

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Hepatitis C Recurrence Is More Severe After Living Donor Compared to Cadaveric Liver Transplantation

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Preliminary reports suggested that hepatitis C virus (HCV) infection has a more aggressive course following living donor liver transplantation (LDLT) compared to cadaveric liver transplantation (CLT). The aim of this prospective study was to establish if HCV disease recurrence differs between LDLT and CLT. A cohort of 116 consecutive HCV-infected patients undergoing 117 LTs in a single center from March 2000 to August 2003 were followed-up, including systematic liver biopsies. Severe recurrence (SR) was defined as biopsy-proven cirrhosis and/or the occurrence of clinical decompensation. After a median follow-up of 22 months (2.6–44 months), 26 (22%) patients developed SR (decompensation in 12), involving 17 (18%) of 95 patients undergoing CLT and 9 (41%) of 22 undergoing LDLT. The 2-year probability of presenting SR was significantly higher in LDLT compared to CLT (45% vs. 22%, $P = .019$). By univariate analysis LDLT ($P = .019$) and an ALT higher than 80 IU/L 3 months after LT ($P = .022$) were predictors of SR. In 93 patients from whom a liver biopsy was available 3 months after LT, a lobular necroinflammatory score >1 ($P < .01$), LDLT ($P < .01$), and biliary complications ($P = .046$) were associated with SR. However, the only variables independently associated with SR were LDLT (odds ratio [OR], = 2.8; 95% CI,1.19-6.6; $P = .024$) and a lobular necroinflammatory score >1 (OR, 3.1; 95% CI, 1.2-8; $P = .013$). In conclusion, HCV recurrence is more severe in LDLT compared to CLT. Although our results were based on a single-center experience, they should be considered in the decision-making process of transplant programs, since severe HCV recurrence may ultimately compromise graft and patient survival. (HEPATOLOGY 2004; 40:699–707.)

Hepatitis C virus (HCV)–related cirrhosis is the leading indication for liver transplantation (LT) in the United States and Europe.[1,2] More than half of the patients on the waiting list are infected with HCV. Regretfully, HCV recurrence is universal after LT[3] and leads to chronic hepatitis and liver cirrhosis in a significant proportion of patients.[4,5] Although initial reports failed to demonstrate an impact of HCV infection on survival, Forman et al. have recently shown that graft and patient survival after LT were significantly lower in HCV-infected patients compared to noninfected individuals.[6]

Several variables have been associated with a more severe HCV disease recurrence after LT, such as a high pretransplantation viral load, old donor age, the presence of significant graft steatosis, and the administration of steroid boluses.[4,7–9] It is important to state, however, that most studies are retrospective and that there is a lack of homogeneity in the definition of severe HCV recurrence after LT.

With the limited pool of cadaveric donors, living donor liver transplantation (LDLT) has become the most feasible alternative to cadaveric liver transplantation (CLT) for patients with end-stage liver disease or hepatocellular carcinoma (HCC). Nowadays, more than 3,000 LDLTs have been performed worldwide using the right hepatic lobe. Despite this high number of procedures, the enthusiasm for LDLT is tempered by the need for a highly

Abbreviations: HCV, hepatitis C virus; LT, liver transplantation; LDLT, living donor liver transplantation; CLT, cadaveric liver transplantation ; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; HBV, hepatitis B virus; SR, severe recurrence.

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skilled group of senior liver surgeons, the elevated surgical-related morbidity, and the rare but potential donor mortality.[10–12] In addition, the applicability of LDLT is low, and only one fourth or less of the potential recipients undergo the procedure.[13,14]

The scientific community assumed that outcomes after LDLT and CLT were comparable, and this assumption provided the rationale to propose LDLT in patients awaiting CLT. Consequently, cost-effectiveness analyses were run with those assumptions.[15–17] Although there are studies reporting similar outcomes for both groups,[18–20] others suggest that HCV disease recurrence has an earlier and more severe course in LDLT compared to CLT.[21,22] We started a LDLT program in March 2000 in patients on the waiting list for CLT. This program was expanded to patients with HCC exceeding the conventional criteria in 2001.[23] The present prospective study was aimed at assessing if the outcome of HCV infection differed between CLT and LDLT. For this purpose, a cohort of 116 consecutive HCV-infected patients undergoing LT in a single institution between March 2000 and August 2003 were followed, including the performance of systematic liver biopsies.

Patients and Methods

Patients

HCV-infected patients who underwent LT for end-stage cirrhosis or HCC between March 2000 and August 2003 were included in the study. Exclusion criteria were (1) double kidney and liver transplantation, (2) coinfection with the human immunodeficiency virus (HIV) or hepatitis B virus (HBV), (3) recipients of a nonbeating-heart donor, (4) undetectable HCV-RNA before transplantation, and (5) survival shorter than 3 months following transplantation. Patients fulfilling these criteria entered the study, which was approved by the Investigation and Ethics Committee of the hospital.

Data from 10 LDLT recipients not infected with HCV who underwent transplantation during the same period of time were recorded following the study protocol described below, except for protocol liver biopsies.

Study Protocol

During hospital admission, patients were managed according to a previously published schedule.[3,24] In brief, induction immunosuppression was cyclosporine A or tacrolimus, and prednisone. Ten patients were treated with tacrolimus and anti–interleukin-2 receptor antibodies. Mycophenolate mofetil was given to patients who required cyclosporine or tacrolimus dose reduction or discontinuation. Immunosuppression therapy was recorded throughout the study. Acute rejection episodes were documented by liver histology[25,26] and treated with steroid boluses if moderate or severe. After discharge, patients were visited at the outpatient clinic, monthly for the first 3 months, with complete record of clinical and analytical variables (including viral load), and every 2 months thereafter. Patients underwent protocol liver biopsies 3 months after LT and yearly thereafter, as well as when clinically indicated. Liver biopsies were evaluated by a single pathologist (M.B.) with wide experience in the histopathology of LT. Necroinflammatory activity and fibrosis stage were assessed according to Scheuer's classification.[27]

Definition of Severe Recurrence

Severe HCV recurrence was defined as the presence of liver cirrhosis in a liver biopsy and/or the development of clinical decompensation secondary to liver disease with portal hypertension (ascites, variceal bleeding, hepatic encephalopathy).

Prognostic Factors of Severe HCV Recurrence

A total of 29 variables potentially associated with severe HCV disease recurrence were prospectively recorded. Pretransplantation variables included recipient age and gender; Child-Pugh and model for end-stage liver disease scores; presence of HCC; HCV genotype; and pretransplantation viral load. In case of antiviral treatment before LT, the duration and doses of interferon and ribavirin were recorded. Variables related to the donor included age, graft steatosis, and the type of donor (cadaveric or living). The presence of graft steatosis was evaluated in a postreperfusion liver biopsy and assessed by a single pathologist (M.B.). Graft steatosis was classified as absent, mild (<25% of hepatocytes), moderate (25%-50% of hepatocytes), and severe (>50% of hepatocytes).

Recorded peritransplant variables were cold ischemia time and transfusion requirements.

Post-transplantation variables included the doses and levels of immunosuppressive drugs; rejection episodes; administration of corticosteroid boluses; cytomegalovirus infection or disease, antiviral treatment after LT; post-transplantation viral load; alanine aminotransferase levels; and vascular and biliary complications. Biliary complications were defined as any leak or stenosis documented by transhepatic or endoscopic retrograde cholangiography, requiring either surgery or interventional radiology/endoscopy.

The graft weight/recipient body-weight ratio was analyzed in patients undergoing LDLT. In addition, we calculated the increase in graft volume using magnetic resonance imaging volumetry measured prior to transplantation and 1 month after the procedure.[28]

HCV-RNA Quantification and Genotyping

Blood samples were collected before transplantation and at weeks 1, 4,12, 24, and 48 following the procedure. HCV viral load was determined using a commercially available assay (Amplicor Monitor v2.0, Roche Diagnostics, Branchburg, NJ). For negative samples serum was retested using a more sensitive qualitative test (Amplicor HCV v2.0, Roche Diagnostics). HCV genotype was determined by restriction fragment length polymorphism (3).

Statistical Analysis

The primary end-point was severe HCV recurrence. Baseline characteristics of the patients are expressed as median (range). Differences between qualitative variables were assessed by the Chi-square or the Fisher exact test; differences between quantitative variables were analyzed by a nonparametric test (Mann-Whitney). Cumulative probability curves of severe HCV disease recurrence according to the Kaplan-Meier method were compared by the Cox-Mantel test. Stepwise forward Cox regression analysis of severe recurrence (SR) was used to evaluate baseline and postoperative variables found to be significant ($P < .05$) or near significant ($P < .1$) in the univariate analysis. The cutoff level chosen for quantitative variables was the median value, unless stated. Follow-up was maintained until death or retransplantation or was censored at the last visit before November 2003. The software used for statistical analysis was SPSS 10.0 (SPSS Inc., Chicago, IL).

Results

Baseline Data

A total of 283 liver transplantations were performed in our center from March 2000 until August 2003, 151 (53.4%) in 140 HCV-infected patients. Ten retransplantations performed within 3 months after implantation of the first graft were excluded from the analysis (Fig. 1). Twenty-four patients were excluded due to double liver-renal transplantation (3), HIV coinfection (1), nonbeating donor (2), anti–HCV positive but HCV-RNA negative (4), and survival less than 3 months after LT (cause of death not related to HCV recurrence) (14). Therefore, a total of 117 consecutive liver transplantations performed in 116 HCV-infected recipients were included in the study, 95 (81%) corresponding to CLT and 22 (19%) to LDLT.

Characteristics of the entire cohort and of patients undergoing CTL and LDLT are summarized in Table 1. Baseline features of CLT and LDLT recipients were similar, except for some variables inherently linked to LDLT, such as donor age and graft steatosis (Table 1). Regarding

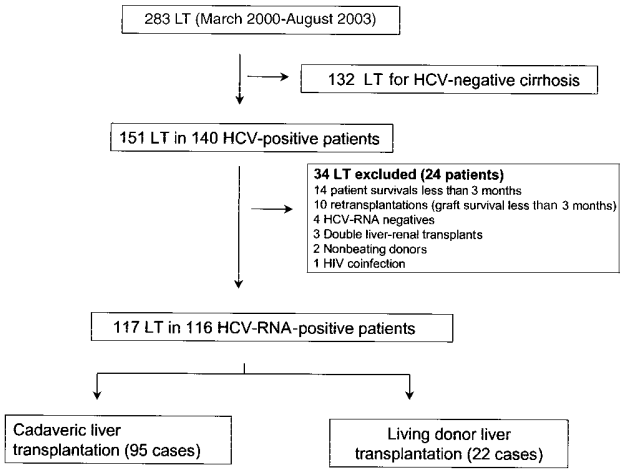

Fig. 1. Study flowchart. LT, liver transplantation; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

posttransplantation variables, biliary complications were significantly more frequent in LDLT than in CLT (73% vs. 22%, $P < .01$). Importantly, 15 (68%) of 22 LDLT recipients underwent double or multiple biliary anastomoses. Patients undergoing LDLT received tacrolimus more frequently than recipients of a cadaveric graft (86% vs. 41%, $P < .01$). Blood levels of tacrolimus, however, were similar between both groups at week 1 (9.8 ng/mL vs. 8.1 ng/mL, $P = .1$), month 1 (13.9 ng/mL vs. 13.3 ng/mL, $P = .6$), month 3 (10.9 ng/mL vs. 8.3 ng/mL, $P = .2$), month 6 (8.9 ng/mL vs. 8.2 ng/mL, $P = .4$), and month 12 (8.6 ng/mL vs. 7.2 ng/mL, $P = .3$), respectively. Similarly, the length and cumulative doses of steroid therapy were comparable between both groups. Acute rejection was diagnosed (2 weeks after LT; range, 1-52) in 29 (30.9%) and 9 (42.9%) CLT and LDLT recipients, respectively. Only in 2 cases acute rejection occurred after the third month (both in CLT recipients). Follow-up after transplantation was identical in both groups.

Incidence of Severe HCV Recurrence

After a median follow-up of 22 months (2.6-44 months), 26 (22.2%) patients developed SR, involving 17 (18%) of 95 patients receiving a CLT and 9 (41%) of 22 undergoing LDLT. The cumulative probability of being free of SR was 71% at 2 years and 67% at 3 years (Fig. 2). Diagnosis of SR relied on histology in 14 patients and on clinical decompensation in 12 (ascites in 11 patients, and variceal bleeding in 1). At the time of decompensation, 10 of the 12 patients underwent a liver hemodynamic study; hepatic venous pressure gradient was >10 mm Hg in all cases.

Table 1. Baseline and Posttransplantation Characteristics of Patients Undergoing LT According to the Type of Transplantation

	All (n = 117)	CLT (n = 95)	LDLT (n = 22)	*P* Value
Baseline variables				
Recipient age (years)*	59 (24–68)	59 (38–66)	59 (24–68)	.76
Gender (male)	71 (61%)	58 (61.1%)	13 (59.1%)	1
Donor age* (years)	42 (13–86)	47 (13–86)	31 (19–58)	<.01
Graft steatosis				
No	75 (64.1%)	58 (61.1%)	17 (77.3%)	.02†
<25%	25 (21.4%)	20 (21.1%)	5 (22.7%)	
25%-50%	12 (10.3%)	12 (12.6%)	0	
>50%	5 (4.3%)	5 (5.3%)	0	
Indication for LT (HCC)	58 (49.6%)	45 (47.4%)	13‡ (59.1%)	.35
Child-Pugh (A)	56 (47.9%)	47 (49.5%)	9 (40.9%)	.48
MELD*	11 (2–28)	11 (2–28)	11 (5–24)	1
Pre-LT viral load* (log_{10} IU/mL)	5.4 (1.70–6.6)	5.4 (1.70–6.6)	5.7 (3.2–6.5)	.43
Genotype 1	106 (91%)	86 (90.5%)	20 (90.9%)	1
Antiviral therapy before LT	17 (14.5%)	12 (12.6%)	5 (22.7%)	.31
Post-LT variables				
CyA	59 (50.4%)	56 (58.9%)	3 (13.6%)	<.01
MMF	41 (35%)	33 (34.7%)	8 (36.4%)	1
Prednisone	107 (91.5%)	85 (89.5%)	22 (100%)	.2
Prednisone length* (months)§	9 (0–29)	9 (0–29)	9 (3–23)	.3
Acute rejection	38 (33%)	29 (30.9%)	9 (42.9%)	.31
Corticosteroid boluses	23 (19.6%)	16 (17%)	7 (31.8%)	.09
Antiviral treatment after LT	33 (28.2%)	26 (27.4%)	7 (31.8%)	.79
Biliary complications	37 (31.6%)	21 (22.1%)	16 (72.7%)¶	<.01
Follow-up after LT (months)*	22 (2.6–44)	22 (2.6–42.5)	21.9 (3.6–44)	.94

Abbreviations: MELD, model for end-stage liver disease; CyA, cyclosporine A; MMF, mycophenolate mofetil.
*Quantitative variables expressed as median (range).
†Difference between mild (<25%) vs. moderate or severe (>25%) steatosis.
‡In 6 cases, the indication was using expanded criteria.
§Median doses of prednisone (mg) at 1, 3, 6, 9, and 12 months were 20, 15, 10, 5, and 0, respectively, in both CLT and LDLT recipients.
¶Three patients had minor leakages that solved after single endoscopic papillotomy (2) or percutaneous drainage (1).

Predictors of Severe HCV Recurrence

Entire Cohort (N = 117). *Univariate analysis.* We analyzed the prognostic value of pretransplantation and posttransplantation variables on the development of SR. LDLT was the only baseline variable predictive of SR by univariate analysis ($P = .019$), whereas there was a nonsignificant trend in patients with pretransplantation viral load above 5.43 log_{10} IU/mL ($P = .08$) (Table 2). Donor age, graft steatosis, recipient age, pretransplantation Child-Pugh and model for end-state liver disease scores, HCV genotype, and the administration of antiviral therapy before transplantation did not show any value as predictors of severe HCV recurrence (Table 2). Regarding posttransplantation variables, an alanine aminotransferase value 2 times the upper limit of normal (80 IU/L) 3 months following LT was predictive of SR ($P = .022$), whereas biliary complications ($P = .09$) and infection with cytomegalovirus ($P = .072$) showed a nonsignificant trend. None of the variables related to the type and intensity of immunosuppression influenced the severity of HCV disease recurrence (Table 2), even when the analysis was restricted to the LDLT group (data not shown). Patients who developed severe HCV recurrence received antiviral treatment more frequently than patients who did not (61.5% vs. 18.7%, $P < .01$). There was no rejection

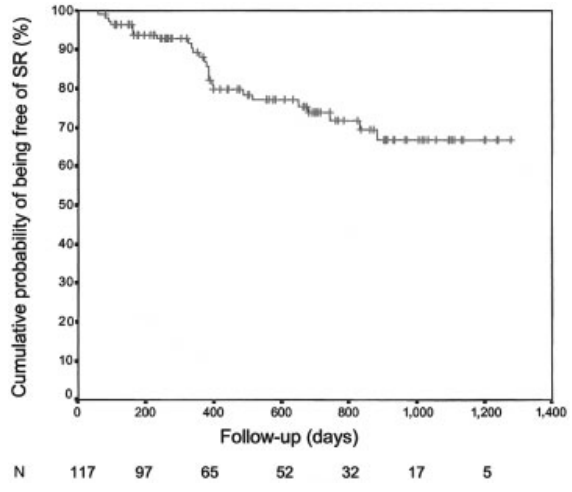

Fig. 2. Cumulative probability of being free of severe recurrence after LT in the entire cohort (N = 117). SR, severe recurrence. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 2. Prognostic Factors Associated With the Development of SR Following Liver Transplantation. Univariate Analysis

	Non–severe HCV Recurrence (n = 91)	Severe HCV Recurrence (n = 26)	*P* Value (log rank)
Baseline variables			
Recipient age >60 years	37 (40.4%)	15 (57.7%)	.18
Graft steatosis (moderate/severe)	12 (13%)	5 (19%)	.52
Type of donor (LDLT)	13 (14%)	9 (35%)	.019
Donor age >45 years)	42 (46.2%)	11 (42.3%)	.82
Indication for LT (HCC)	45 (49.5%)	13 (50%)	.99
Child-Turcotte-Pugh (A)	46 (50.5%)	10 (38.5%)	.27
MELD score >10	53 (58%)	16 (61%)	.82
Pre-LT viral load (>5.43 $\log_{10}$ IU/mL)	40 (45.5%)	16 (66.7%)	.082
Genotype 1	81 (89%)	25 (96%)	.451
Antiviral therapy before LT	14 (15.4%)	3 (11.5%)	.76
Post-LT variables			
ALT >80 IU/L (3rd month)	38 (42%)	19 (77%)	.023
Lobular necrosis*	28 (41%)	16 (73%)	.006
CyA (vs. tacrolimus)†	47 (52%)	12 (46%)	.6
MMF	32 (35.2%)	9 (34.6%)	1
Prednisone	84 (92.3%)	23 (88.5%)	.7
Prednisone length (months)‡	9 (0–22)	9.5 (0–29)	.71
Acute rejection	30 (34%)	8 (31%)	.719
Corticosteroids boluses	18 (19.7%)	5 (19%)	.82
CMV infection	6 (7%)	4 (15%)	.072
Biliary complications§	26 (28.6%)	11 (42.3%)	.090
Antiviral treatment after LT¶	17 (18.7%)	16 (61.5%)‡	<.01
Follow-up after LT (months)	21	23	.64

Abbreviations: MELD, model for end-stage liver disease; ALT, alanine aminotransferase; CyA, cyclosporine A; MMF, mycophenolate mofetil; CMV, cytomegalovirus.

*Necroinflammatory index >1 in a liver biopsy performed 3 months after LT. Available in 93 patients.

†Blood levels of tacrolimus and cyclosporine were comparable between both groups at week 1, and month 1, 3, 6, and 12 after LT.

‡Median doses of prednisone (mg) at month 1, 3, 6, 9, and 12 were 20, 15, 10, 5, and 0 in patients without SR and 20, 10, 10, 5, and 2.5 in patients with SR (nonsignificant at all points).

§In patients undergoing cadaveric liver transplantation, SR occurred in 4 (19%) of 21 with biliary complications and in 13 (18%) of 74 without biliary complications (log rank = 0.5).

¶Seven patients received antiviral treatment before and 9 after the diagnosis of SR.

episode related to antiviral therapy. Follow-up after LT was comparable between patients with and without SR (Table 2).

Multivariate analysis. The type of transplantation was the only independent predictor of SR (odds ratio, 2.5; 95% CI, 1.13-5.68; $P = .025$) (Table 3). Multivariate analysis including the variable biliary complications provided identical results. Therefore, the 2-year probability of presenting SR was significantly higher in LDLT compared to CLT (45% vs. 22%, $P = .019$) (Fig. 3).

Table 3. Prognostic Factors Associated With the Development of SR Following LT in the Entire Cohort (n = 117) and in Patients Who Underwent Liver Biopsy 3 Months After LT (n = 93). Cox Regression Analysis

Entire Cohort (n = 117)	Odds Ratio	95% CI	*P* Value
Type of transplantation (LDLT vs. CLT)	2.5	1.13–5.68	.025
ALT >80 IU/mL			.084
Liver Biopsy Available 3 Months After LT (n = 93)	**Odds Ratio**	**95% CI**	***P* Value**
Type of transplantation (LDLT vs. CLT)	2.82	1.19–6.66	.024
Lobular necroinflammation >1	3.1	1.2–8	.013
Biliary complications			.21

Abbreviation: ALT, alanine aminotransferase.

Patients With Liver Biopsy Available 3 Months After LT (n = 93). Ninety-three patients underwent a protocol liver biopsy 3 months after transplantation. In the remaining 24 patients it was not performed due to a nonprotocol biopsy obtained between the 1st and 2nd month after LT for clinical indication (13 cases), biliary complications (8 cases), and patient denial (3 cases). In this subgroup of 93 patients, the univariate analysis disclosed that LDLT ($P = .004$), biliary complications ($P = .046$), and a lobular necroinflammatory score >1 in the third-month liver biopsy ($P = .006$) were predictors of SR (Fig. 3). By Cox regression analysis, only the type of transplantation and the presence of necroinflammatory changes showed independent predictive value for SR (Table 3).

We performed a similar analysis using liver histology (F3-F4) as an end-point. Thirty patients (25.6%) developed stage 3 or stage 4 fibrosis during follow-up: 20 (21%) of 95 patients receiving a CLT and 10 (45.4%) of 22 undergoing LDLT ($P = .02$). The results of the uni-

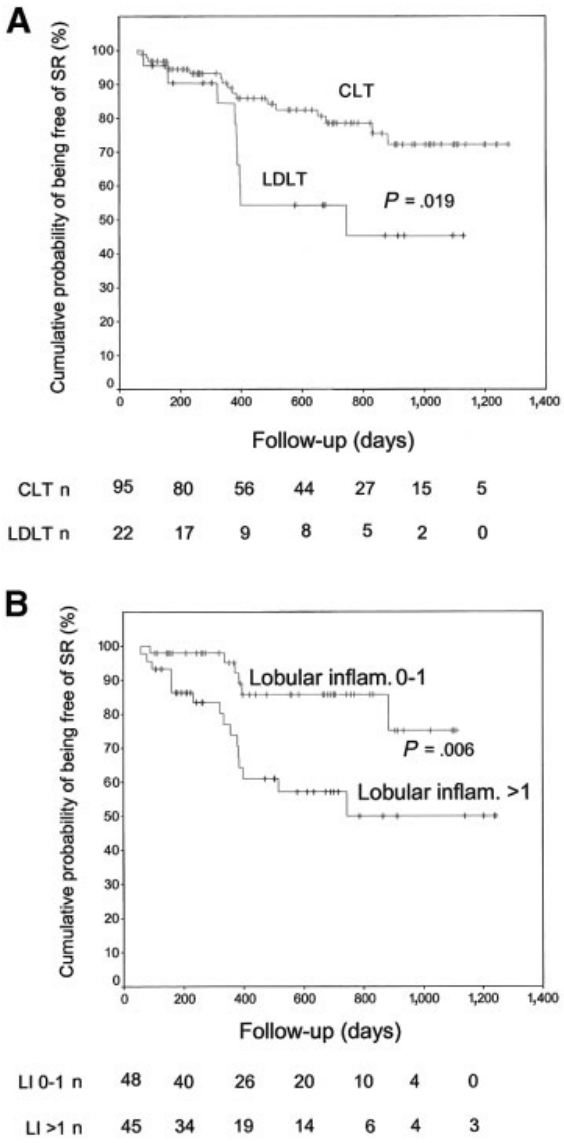

Fig. 3. Cumulative probability of being free of severe recurrence after LT by (A) the type of transplantation (cadaveric vs. living donor) and by (B) the degree of lobular inflammation 3 months after transplantation (0–1 vs. >1). SR, severe recurrence; CLT, cadaveric liver transplantation; LDLT, liver donor liver transplantation; LI, lobular inflammation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

variate and multivariate analysis were identical to those presented above, and LDLT and a lobular necroinflammatory score >1 in the third-month liver biopsy were independently related to the development of F3-F4.

Analysis of HCV Disease Recurrence in CLT vs. LDLT

Alanine aminotransferase at 1 and 3 months after transplantation was significantly higher in LDLT compared to CLT recipients (113 IU/L vs. 50 IU/L, $P < .01$, and 121 IU/L vs. 65 IU/L, $P = .016$, respectively). Similarly, GGT values were significantly higher in LDLT than in CLT recipients both at 1 and 3 months after transplantation (420 IU/L vs. 128 IU/L, $P < .01$, and 647 IU/L vs. 119 IU/L, $P < .01$, respectively). Severe acute cholestatic hepatitis (lobular necroinflammatory score >2, and cholestasis) was confirmed by liver biopsy in 9 of 93 patients; its incidence was significantly higher in LDLT (6 of 17, or 35%) compared to CLT (3 of 76, or 4%) ($P < .01$). Regarding viral kinetics, HCV viral load at weeks 1, 4, 12, and 24 after transplantation was higher in LDLT compared to CLT recipients, though the differences did not reach statistical significance.

Patients who underwent LDLT received the right lobe of a living donor in all cases. The median weight of the right lobe was 759 g (range, 550-1045), and the right lobe/recipient weight ratio was 1.07 (range, 0.76-1.66). The median increase in liver volume during the first month following transplantation (measured by magnetic resonance imaging volumetry) was 67% (range, 4.5%-161%). SR occurred more frequently in patients with an increase in liver volume above the median value (6 of 11) compared to patients with an increase in liver volume below the median value (3 of 11) ($P = .1$).

Follow-up of Anti–HCV Negative LDLT Recipients

We analyzed the outcome of 10 HCV-RNA negative patients who underwent LDLT during the same period of time. Indication for LT was alcoholic cirrhosis (5), HBV-related cirrhosis (2), alfa-1-antitrypsin deficiency (1), Caroli's disease (1), and cryptogenic cirrhosis (1). Except for follow-up liver biopsies, anti–HCV negative patients were followed with the same protocol as HCV-infected patients. Baseline and posttransplantation characteristics of LDLT recipients with and without HCV infection were similar, except for the presence of HCC (only in 2 of 10 anti–HCV negative patients). Importantly, the incidence of biliary complications was identical (72% in HCV infected patients, 70% in noninfected patients). Median follow-up was 14 months (3.5-31 months). Liver fibrosis was absent in 1 or more follow-up liver biopsies available in 3 of the 10 anti–HCV negative patients (all 3 with biliary complications). Moreover, none of 10 individuals presented with clinical decompensation or ultrasonographic evidence of ascites during follow-up.

Discussion

The outcome of HCV disease recurrence after LDLT is still controversial.[18,19,21,22,29] Our data, though limited to a single center, show that LDLT is a strong and independent predictor of severe HCV disease recurrence following transplantation. Accord-

ingly, the 2-year probability of presenting SR was significantly higher in LDLT compared to CLT (45% vs. 22%). This study has some relevant differences from previous reports. First, we designed a prospective study specifically aimed at assessing whether HCV disease recurrence was different between both types of transplantation. Although the numbers of patients (116) and events (26) are small, the strength of the differences after a median follow-up of 22 months prevents us to expand the series with additional cases. More importantly, severe recurrence was defined by the presence of cirrhosis in a follow-up liver biopsy or by the occurrence of clinical decompensation. This definition allows an unbiased classification of patients and represents a relevant event in the natural history of HCV disease recurrence.[30] Finally, patients were recruited in a single center, and the same standard of care was established. Additionally, all relevant variables that might influence HCV disease recurrence were included in the analysis, and, except for factors inherently related to living donation (and the type of calcineurin inhibitor), patients receiving the graft of a living donor or a cadaveric donor were comparable.

The mechanisms that might explain the more aggressive course of HCV recurrence after LDLT are unknown. Theoretically, there are variables specifically linked to LDLT that might prevent from severe HCV disease recurrence, such as the young donor age, the lack of significant steatosis of the graft, and the short ischemia time during surgery.[8,31] On the contrary, other variables might affect negatively HCV disease recurrence, such as an increased HLA donor-recipient matching, the type of immunosuppression, a high incidence of biliary complications following transplantation, and liver regeneration.[21,32,33]

Our initial hypothesis was that either biliary complications or liver regeneration (or both) would accelerate liver fibrosis in patients undergoing LDLT. Biliary complications are frequent in the latter group, and it is well known that persistent cholestasis induces fibrogenesis. Despite the lack of homogeneity in the definition of biliary complications among reported series, the incidence of biliary leaks or stenosis in our cohort was very high. Three reasons might explain this high incidence: first, the prospective nature of data collection (including even minor leakages); second, the high frequency of double and multiple biliary anastomoses in our LDLT series[34]; and third, the learning curve.[35] Despite this center-specific issue, neither the multivariate analysis nor the follow-up of a small cohort of anti–HCV negative LDLT recipients support an independent predictive value of biliary complications on the severity of HCV disease recurrence. However, a synergistic effect of persistent cholestasis on HCV-infected grafts cannot be excluded.

Biochemical markers of hepatitis increased earlier and reached significantly higher levels in patients undergoing LDLT compared to CLT, strongly suggesting that hepatitis C recurrence is a distinct process in those patients. In fact, the occurrence of cholestatic hepatitis was significantly more frequent in LDLT compared to CLT. One of the key differences between LDLT and CLT is liver regeneration.[36] *In vitro*, HCV internal ribosome entry site activity and replication were found to be higher in actively dividing cells, and it is possible that viral translation may be enhanced by factors that stimulate the regeneration of hepatocytes.[37,38] Moreover, there are experimental data suggesting that liver regeneration induces LDL receptor expression,[39] which might facilitate HCV entrance into the hepatocytes.[40] The absence of significant differences in viral load between LDLT and CLT at the analyzed time points does not exclude increased viral production in regenerating cells, as viral load also depends on the number of cells producing viral particles and on viral clearance mechanisms.

Regretfully, this study presents several caveats. First, the limited number of patients who underwent LDLT did not allow a thorough analysis of the variables that predict SR among them. Our data suggest that patients who experienced a greater increase in liver volume during the first weeks following LDLT had a higher probability of developing SR afterward. Though the increase in liver size cannot be use as a direct marker of the degree of regeneration, this increase might support a negative influence of liver regeneration on HCV disease recurrence. These results should be further confirmed in extensive series. Second, the relatively short follow-up of this cohort did not allow a consistent analysis of graft and patient survival. However, it is well established that around 40% of patients with compensated HCV graft cirrhosis will develop clinical decompensation within the first year following the diagnosis. Once decompensation occurs, survival is lower than 50% at 1 year,[30] whereas retransplantation leads to disappointing results.[41] The expected decrease in graft or even patient survival derived from this scenario might make LDLT a non-cost-effective approach in HCV-infected patients. Possible alternatives would be to restrict LDLT to very long waiting times and/or high dropout rate settings,[17] limiting LDLT to non-HCV-infected patients (at least during the learning curve), or to treat HCV infection before LDLT.[42–44] Implementation of any of these strategies would require confirmation of our results in other prospective series.

In summary, our data indicate that LDLT is a strong predictor of severe HCV disease recurrence after trans-

plantation. Although the data need to be validated, the more aggressive course of HCV infection in LDLT compared to cadaveric transplantation should be considered in LDLT programs, since it may ultimately compromise graft and patient survival.

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Is Severe Recurrent Hepatitis C More Common After Adult Living Donor Liver Transplantation?

Mark W. Russo and Roshan Shrestha

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End-stage liver disease from chronic hepatitis C infection is the leading indication for adult to adult living donor liver transplantation (LDLT). The proportion of LDLTs performed for end-stage liver disease from chronic hepatitis C infection in this country has been relatively stable over the past 4 years, at about one third (Fig. 1), which is similar to the proportion of deceased donor liver transplants performed for end-stage liver disease from chronic hepatitis C. Potential benefits of LDLT include reducing waiting time mortality in an era of deceased donor organ shortage, curing patients with hepatocellular carcinoma, and rapid post-operative recovery. These benefits may be offset if outcomes are worse post-transplantation compared to deceased donor liver transplantation. Because end-stage liver disease from hepatitis C infection is the most common indication for LDLT, if recurrent disease is more common or more aggressive in LDLT recipients compared to deceased donor recipients then this would have a significant impact on how we approach our patients.

Studies have emerged comparing rates of recurrent hepatitis C in LDLT recipients and deceased donor recipients (Table 1).[1–11] But what is recurrent hepatitis C? There is no uniform or standardized definition of recurrent hepatitis C after liver transplantation. Investigators have used different outcomes to define recurrent hepatitis C in studies comparing outcomes after LDLT and deceased donor liver transplant. Outcomes used in studies have been based upon histological findings on liver biopsy consistent with recurrent hepatitis C, cholestatic hepatitis C on liver biopsy, cirrhosis from hepatitis C, and patient and graft survival. Comparing studies on recurrent hepatitis C after LDLT is difficult because there is no consensus on definition of recurrent hepatitis C and different outcomes have been used.

In this issue of HEPATOLOGY, Garcia-Retortillo et al. demonstrate that severe recurrence of hepatitis C is higher in LDLT recipients.[12] Severe recurrence was defined as the development of cirrhosis or clinically decompensated liver disease. The difference in the 2-year probability of developing severe recurrence is quite striking where 22% of deceased donor recipients and 45% of LDLT recipients developed severe recurrence, $P = .019$. The association between LDLT and severe recurrence remained significant after the authors adjusted for confounding variables known to be associated with recurrent hepatitis C.

A unique aspect of this study included using cirrhosis or decompensated liver disease as the outcomes and that protocol liver biopsies were performed. One problem with other studies that have used histological recurrence of hepatitis C as the outcome is that there is no gold standard or uniform pathologic definition for recurrent hepatitis C. Many of the pathological features of acute or chronic cellular rejection and recurrent hepatitis C overlap. Because there is no uniform definition for histological recurrence of hepatitis C on liver biopsy we believe using objective outcomes, such as cirrhosis, decompensated liver disease, or graft failure, would avoid the problems associated with using histological recurrence of hepatitis C as the outcome, until a standardized, validated definition of histological recurrence of hepatitis C is developed.

Other studies have reported higher rates of recurrent hepatitis C in LDLT recipients compared to deceased donor recipients, but they have several limitations (Table 1).[3,6] Most studies have not performed protocol liver biopsies, and biopsies were performed only when clinically indicated. A strength of the current study is that protocol liver biopsies were performed that would detect recurrent hepatitis C or advanced liver disease in patients with normal liver tests. Most of the other studies have been small, single-center studies that include their early experience with LDLT. Including the early experience with LDLT may bias the results against this group. An analysis of data from the United Network from Organ Sharing (UNOS) liver transplant database

Abbreviations: LDLT, living donor liver transplantation; UNOS, United Network from Organ Sharing.

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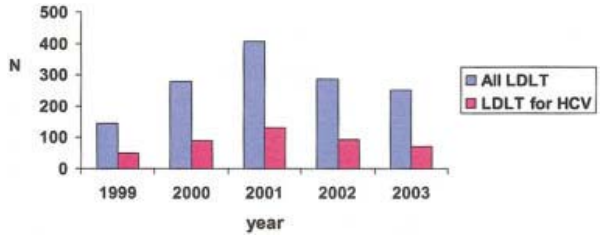

Fig. 1. Total number of adult living donor liver transplants and number for end stage liver disease from hepatitis C, by year. From http://www.optn.org/latestData/advancedData.asp; accessed June 8, 2004.
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comparing LDLT and deceased donor recipients with HCV demonstrated that graft survival after LDLT increased from 1999 to 2001 suggesting improvements in outcomes were seen with experience.[10] However, follow-up was relatively short and detailed data on important factors such as histology, viral characteristics, and immunosuppression are either not provided or they are missing in the UNOS and Scientific Registry of Transplant Recipients (SRTR) databases.

One question raised by the current study is why is the rate of cirrhosis relatively high. After a median follow-up of 22 months, the rate of cirrhosis or decompensated cirrhosis was 22% in the entire cohort and 18% in the deceased donor group. These rates are strikingly higher than rates reported in a recent U.S. study that performed protocol liver biopsies and compared histologic recurrence of hepatitis C after LDLT and deceased donor transplantation.[1] In the United States none of the patients in the LDLT group developed cirrhosis after a median of 3 years of follow-up. The differences between the studies do not appear to be due to differences in immunosuppression, genotype, or corticosteroid use. There is literature that suggests there may be geographical differences in the behavior of hepatitis C after liver transplantation. A study from Spain reported more rapid fibrosis rates during recent years in deceased donor liver transplant recipients with chronic hepatitis C.[13] The study included liver transplant recipients from Spain and the United States. The recipients from Spain had more rapid fibrosis progression compared to patients from the United States. Unlike the studies from Spain, studies from the United States, United Kingdom, and Italy have demonstrated that histological recurrence of hepatitis C or graft failure is not more common in recent years.[14,15]

Other factors need to be considered before higher recurrence rates of hepatitis C are attributed to LDLT. A greater proportion of recipients who developed severe recurrence were treated with antiviral therapy in the current study (61% vs. 19%, $P < .01$). The authors state that no rejection episode was related to antiviral therapy, but interferon may induce acute and chronic cellular rejection leading to graft loss, and the pathological findings may be difficult to distinguish from recurrent hepatitis C.[16,17]

There are a number of plausible biological mechanisms to suggest that LDLT recipients would have higher recurrence rates of hepatitis C compared to deceased donor recipients. Donor and recipient are likely to be related with LDLT and share HLA homology. Genetic matching may be associated with an increased risk of histologic recurrence of hepatitis C.[18] Translation of hepatitis C is mediated by the internal ribosomal entry site (IRES).[19]

Table 1. Characteristics of Studies Comparing LDLT and Deceased Donor Liver Transplantation (DD) in Patients With End-Stage Liver Disease From Chronic Hepatitis C Virus (HCV)

Study	N	Country	Protocol Biopsies	Findings
No differences between LDLT and DD				
Shiffman[1]	23	USA, single center	yes	No patients developed cirrhosis *on liver biopsy* @ 3 years
Russo[10]	279	USA, UNOS database	no	2-year graft survival 72% LDLT and 75% DD
Bozorgzadeh[11]	35	USA, single center	no	No difference in HCV recurrence on liver biopsy
Gordon[5] (abstract)	19	USA, single center	no	Survival 92% in both groups at 36 months
Pan[7] (abstract)	15	USA, single center	no	HCV recurrence *on liver biopsy* 47% LDLT vs. 65% DD
Fahmy[8] (abstract)	33	USA, single center	no	Graft loss from recurrent hepatitis C 3% LDLT vs. 7.7% DD
Vlierberghe[9] (abstract)	17	Belgium, Italy	no	HCV recurrence on *liver biopsy* 35% LDLT vs. 38% DD
Trotter[4] (abstract)	41	USA, 2 centers	no	No difference in rate of graft loss from HCV in LDLT and DD group
LDLT worse				
Garcia-Retortillo[12]	22	Spain	yes	Cirrhosis or decompensated liver disease in 45% LDLT vs. 22% DD at 2 years, $P = .019$
Gaglio[6]	23	USA, single center	no	HCV recurrence *on liver biopsy,* graft failure were similar in LDLT and DD group, cholestatic hepatitis higher in LDLT group, 17% vs. 0%, $P = .001$
Ghobrial[3] (abstract)	9	USA, single center	no	HCV recurrence on *liver biopsy* in 86% LDLT vs. 30% DD, $P = .004$

IRES activity is greatest in dividing cells, and rapidly proliferating hepatocytes after LDLT may promote HCV viral replication. It is unclear if or how immunosuppression affects recurrent hepatitis C, but immunosuppression may play a role. LDLT recipients metabolize tacrolimus differently compared to deceased donor recipients and have higher drug levels early after transplant.[20] Whether this impacts on recurrence of hepatitis C after LDLT is unknown.

Final conclusions about the association between LDLT and recurrent hepatitis C can not be made, because the two best designed studies have discrepant results. Studies from the United States reporting higher recurrence rates of hepatitis C in LDLT recipients have not performed protocol liver biopsies. Although the current study was well designed and performed protocol liver biopsies, the much higher rates of cirrhosis and decompensated liver disease over a relatively short time period suggest the results may not be generalizeable to other populations. The benefits of LDLT should not be overlooked and include reducing waiting time mortality and providing a cure for some patients with hepatocellular carcinoma who might otherwise develop incurable disease awaiting deceased donor liver transplantation.[21] These real benefits must be considered before making a premature decision about the risk of recurrent hepatitis C with LDLT.

The next step should be to develop a standardized, validated definition of histological recurrent hepatitis C on liver biopsy. We should strive not only to look for histological recurrence of hepatitis C, but to also use objective outcomes, such as cirrhosis and graft survival. Data on factors associated with recurrent hepatitis C, including transplant center, should be collected and adjusted for. The multicenter adult to adult living donor liver transplantation cohort study (A2ALL) sponsored by the NIH, ASTS, and HRSA may be well positioned to answer some of the questions about recurrent hepatitis C rates after LDLT and deceased donor liver transplantation.

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7. DISCUSIÓN GENERAL

La recurrencia de la infección crónica del VHC se ha convertido en uno de los principales problemas para todos los programas de trasplante hepático del mundo. Dicha recurrencia se produce de forma universal y precoz y conduce al desarrollo de una hepatitis crónica avanzada o a la cirrosis en una proporción significativa de los receptores a los pocos años del trasplante. La inmunosupresión provoca una progresión de la hepatopatía relacionada con el VHC mucho más rápida que en los sujetos inmunocompetentes. En los últimos años se está detectando un aumento en la velocidad de progresión de este proceso posiblemente en relación al uso de potentes agentes inmunosupresores y al uso de órganos procedentes de donantes cada vez más añosos [91]. Todo ello hace prever que en los próximos años se produzca una pérdida importante de injertos y de pacientes en relación a la recidiva grave del VHC. Los resultados del retrasplante por esta indicación son poco satisfactorios y la escasez relativa de órganos ha avivado el debate ético sobre hasta qué punto deben ser empleados órganos para estos pacientes cuando la mortalidad en la lista de espera alcanza un 15% en pacientes que esperan un primer hígado. En contra de una visión restrictiva de la indicación de trasplante para los pacientes infectados por el VHC, se deben investigan cuáles son los factores que condicionan la recurrencia grave y cuáles podrían ser las estrategias profilácticas o terapéuticas más efectivas. En los últimos años numerosas investigaciones se han dirigido al estudio de este tema.

En los trabajos que conforman esta tesis se ha abordado la recurrencia de la infección por el VHC de forma secuencial: primero, el estudio de la

recurrencia en la fase inmediata tras el trasplante; segundo, la puesta en marcha, en base a los resultados del primer estudio, de un abordaje terapéutico consistente en erradicar el VHC antes del trasplante y análisis de la eficacia del mismo; por último, la identificación de pacientes con mayor riesgo de presentar una recurrencia grave. Delimitando la población de riesgo las medidas profilácticas o terapéuticas se podrían dirigir de forma más eficiente, evitando costosos tratamientos o exploraciones invasivas en el resto.

7.1.La cinética de la recurrencia del VHC tras el trasplante hepático.

En el primer estudio de la tesis se analiza la cinética de la recurrencia del VHC en la fase precoz post-trasplante. Se incluyeron 20 pacientes infectados por el VHC que se sometieron a trasplante hepático de forma consecutiva en nuestro centro. Se extrajeron muestras de suero a de forma seriada, inmediatamente antes del trasplante, durante la fase anhepática y durante la fase de reperfusión. Además, se recogieron muestras de suero durante los primeros días post-trasplante (primeros 5 días post-trasplante). En todas las muestras se procedió a la determinación de la carga viral del VHC. En los pacientes que habían permanecido hemodinámicamente estables durante el trasplante, se colocó un catéter en las venas suprahepáticas a las 24 horas del trasplante lo que permitió tomar muestras de sangre procedente de las venas suprahepáticas a los mismos tiempos que se obtenían de la circulación general, durante los primeros días post-trasplante. Las venas suprahepáticas son los vasos de drenaje directo del hígado. El objetivo de la colocación del catéter en estos vasos era investigar si existían diferencias en cuanto a la carga viral comparada con las determinaciones en sangre periférica. Si tras el trasplante hepático el injerto es la fuente principal de producción de viriones, la

carga viral detectada en las muestras de sangre procedente de las venas suprahepáticas durante las primeras horas post-TH debería ser superior a la observada en sangre de la circulación periférica.

Los datos obtenidos demostraron que durante la fase anhepática, la carga viral del VHC disminuye debido, por un lado, a la falta de producción de viriones (al estar ausente la principal fuente productora que es el hígado) y por otro lado, a la pérdida sanguínea producida durante la técnica quirúrgica. La caída de la carga viral continúa tras la reperfusión del nuevo injerto. Además de la ausencia de producción viral durante esta fase, es probable que el aclaramiento viral que se produce como consecuencia de la entrada masiva de viriones en el nuevo órgano, contribuya a este descenso de la viremia. Esta última hipótesis se ve reforzada por los resultados obtenidos en el caso de una paciente que excepcionalmente tuvo que permanecer en fase anhepática durante 20 horas. Durante este periodo, la carga viral disminuyó de forma paulatina pero no a la velocidad que cabría esperar si asumimos que la vida media de eliminación de las partículas virales es de tan sólo unas horas (2,2 h) y de que el hígado es la única fuente de producción viral. En cambio se observó una brusca disminución de la viremia tras la reperfusión del segundo injerto. Por otro lado, el hecho de que durante la fase anhepática no disminuyera la carga viral a la velocidad esperada teniendo en cuenta la vida media de los viriones podría traducir la presencia de replicación viral extrahepática. Existen datos que sugieren la presencia de replicación viral en un segundo compartimento durante la fase peri y post-trasplante [92] . La existencia de estos reservorios podría contribuir o explicar la recurrencia del VHC que se produce tras el trasplante en pacientes sin viremia detectable en el momento inmediatamente anterior al trasplante (ver segundo trabajo de la

tesis). A diferencia de otros estudios, en nuestro trabajo se ha demostrado la presencia de ARN circulante durante todo el proceso del post-trasplante inmediato. Los viriones circulantes en suero serían los responsables de la infección del nuevo órgano. Aunque en algunos pacientes el ARN fue indetectable tras la reperfusión, ello fue excepcional y el límite de detección de las técnicas empleadas podría explicar este hecho. En efecto, en la mayoría de los pacientes en los que las determinaciones del ARN del VHC cuantitativas fueron negativas, se pudo detectar ARN viral al repetir la determinación con técnicas más sensibles.

Tras la fase de reperfusión y la caída brusca de la carga viral, la replicación en el injerto aparece de forma rápida, tan sólo 12-24 horas tras la reperfusión y se traduce en un aumento rápido de la viremia. Dicho aumento provoca que tan sólo 4 ó 5 días tras el trasplante la carga viral del VHC alcance o supere los niveles pre-trasplante en una proporción significativa de los casos. Sin embargo, no todos los pacientes presentaron un mismo patrón de evolución de la carga viral. En realidad, el estudio ha permitido diferenciar tres patrones de cinética viral. En el primer patrón, se produce un incremento rápido de la viremia que alcanza el máximo hacia el día 4 ó 5 del post-trasplante, llegando a niveles superiores a los de la fase pre-trasplante. Este fue el patrón que siguieron la mayoría de los pacientes y no se detectaron diferencias entre receptores de donante vivo o cadavérico. En los pacientes que siguieron el patrón 2, no existió modificación alguna de la viremia durante los primeros días post-trasplante y por último, los pacientes que presentaron el patrón 3 experimentaron una progresiva disminución de la carga viral durante los primeros días post-trasplante. Aunque no está claro a qué pueden deberse dichas diferencias, la inmunosupresión inicial podría jugar un papel en esta

fase temprana modulando los niveles de replicación viral. En efecto, aquellos pacientes que siguieron un patrón 3, con una segunda fase de caída de la carga viral, fueron pacientes que mayoritariamente recibieron inmunosupresión libre de corticoesteroides (5 de los 6 pacientes con patrón 3). A pesar de las diferencias observadas en cuanto a la cinética viral durante la primera semana post-trasplante, a partir de este momento, se asistió en todos los casos a un progresivo aumento de la carga viral hasta alcanzar una fase de meseta hacia el primer mes post-trasplante (incluso en aquellos pacientes que habían presentado un patrón 2 ó 3).Así pues, deben existir otras variables implicadas en la cinética viral post-trasplante. Es posible que las diferencias observadas en la fase precoz post-trasplante traduzcan, por ejemplo, el tiempo que algunas cuasiespecies seleccionadas tras el trasplante necesitarían para adaptarse a un nuevo entorno [93]. La lesión producida por la isquemia-reperfusión del injerto también podría desempeñar un papel en el aclaramiento del VHC en la fase inmediata post-trasplante. En tres de los pacientes en los que no se observó un descenso significativo de la carga viral tras la reperfusión, se produjo una lesión de isquemia-reperfusión confirmada por biopsia y que se acompañó de elevación de las transaminasas por encima de 1000 U/L. La disfunción de las células del sistema retículoendotelial que aparece en el contexto de la isquemia-reperfusion podría actuar impidiendo la captación de viriones de la circulación sistémica y prolongando la vida media de eliminación de los mismos.

No se observaron diferencias en la carga viral detectada en sangre periférica comparada con la procedente de las venas suprahepáticas a lo largo de todo el periodo de estudio. Tal vez el enorme flujo sanguíneo a través de las venas suprahepáticas hayan enmascarado cualquier diferencia al ejercer un

efecto dilutorio. También cabe la posibilidad de que al colocar el catéter a las 24 horas tras el trasplante no se hayan detectado diferencias que pudieran aparecer en la fase inmediatamente posterior al trasplante. Sin embargo, los resultados obtenidos demuestran que el hígado es el lugar principal de producción viral.

Los datos de cinética viral post-trasplante inmediato son de gran relevancia a la hora de diseñar estrategias terapéuticas o profilácticas que eviten la infección del injerto por el VHC tras el trasplante. Dado que la replicación viral se inicia tan sólo a las 12 horas tras la reperfusión del injerto, las medidas profilácticas deberían iniciarse en la fase anhepática o inmediatamente tras la reperfusión, cuando aún no se ha producido la infección masiva de los hepatocitos y cuando la carga viral alcanza niveles mínimos. Por otro lado, debería investigarse de qué manera los diferentes regímenes de inmunosupresión son capaces de alterar la cinética viral inmeditamente tras el trasplante. Esta información sería de gran importancia para el diseño de pautas inmunosupresoras más apropiadas para pacientes trasplantados infectados por el VHC.

7.2. Eficacia y tolerancia del tratamiento con interferón más ribavirina en pacientes cirróticos-VHC en lista de espera para trasplante hepático.

El tratamiento antiviral en pacientes cirróticos descompensados se considera una contraindicación debido al riesgo de aparición de efectos adversos y a su menor eficacia en cirróticos compensados respecto a pacientes con hepatitis crónica. Por este motivo, prácticamente no existen estudios que hayan evaluado la seguridad y la eficacia del tratamiento antiviral en pacientes en lista de espera para trasplante hepático [94,95].

La mejor estrategia para prevenir la infección del injerto tras el trasplante hepático sería erradicar el virus antes de que tome contacto con el nuevo órgano, es decir, en la fase pre-trasplante. Por ello, se diseñó un estudio multicéntrico en el cual se incluyeron de forma consecutiva todos los pacientes en lista de espera para trasplante hepático que estuvieran infectados por el VHC. Un total de 30 pacientes formaron parte del estudio. La indicación de trasplante hepático fue la presencia de un hepatocarcinoma en el 50% de los casos. Tal vez por ello, el 50% de los pacientes del estudio presentaba una función hepática relativamente conservada (Child A). El momento en el cual se iniciaba el tratamiento era aquel en el que se preveía que el tiempo restante en lista de espera sería de unos 4 meses. A partir del inicio, se mantenía hasta el mismo día del trasplante. Se administró interferón alfa-2b y ribavirina a dosis de 3 MUI/día sc y 800 mg/día vo, respectivamente. El motivo por el cual se empleó interferón estándar (y no la forma pegilada) fue para favorecer un mejor control de los efectos adversos; al tener el primero una vida media mucho más corta, la dosis se podía modificar más rápidamente que la del interferón pegilado. Además, la pauta diaria de administración evitaba los picos y valles de niveles de interferón estándar comparado con la pauta de tres dosis a la semana. Este hecho es importante si se tiene en cuenta que se trataba de pacientes en los que era fundamental que llegaran al momento del trasplante con ARN del VHC indetectable. Los pacientes realizaban semanalmente un seguimiento clínico y analítico en Consultas Externas de forma que cualquier problema pudiera detectarse a tiempo. Con ello se pretendía minimizar la aparición y mejorar el control de los efectos secundarios, sobretodo de aquellos considerados potencialmente graves.

La duración media del tratamiento fue de 12 semanas. El tratamiento consiguió la negativización del ARN-VHC en nueve pacientes (30%) los cuales se sometieron a trasplante hepático sin que se detectara viremia en el suero extraído pre-TH. Los pacientes respondedores recibieron tratamiento a dosis plenas durante más tiempo que los pacientes no respondedores si bien esta diferencia no fue estadísticamente significativa. Los pacientes respondedores presentaron una carga viral basal significativamente menor que los no respondedores. Los genotipos no-1 se presentaron con mayor frecuencia entre los pacientes respondedores. Sin embargo esta diferencia no alcanzó significación estadística probablemente debido al tamaño de la muestra.

El mejor índice pronóstico de respuesta virológica fue el descenso de la carga viral medido en la cuarta semana tras el inicio del tratamiento. En efecto, todos los pacientes (100%) respondedores presentaron un descenso precoz de la carga viral de más de dos logaritmos frente a sólo 2/16 (12%) pacientes no respondedores de los que dispusimos suero en la semana cuatro de tratamiento. Seis de los 9 pacientes cuya viremia fue indetectable en el momento del trasplante, consiguieron erradicar la infección viral como lo demuestra la no detección de ARN-VHC tras el trasplante y después de un seguimiento medio de 46 semanas. Así pues, en un 20% se evitó la recurrencia de la infección por el VHC tras el trasplante.

La eficacia del tratamiento alcanzada en nuestro estudio es similar a la descrita en los dos únicos estudios publicados al respecto [94,95]. La diferencia fundamental de nuestro estudio frente a los anteriormente citados es la menor duración del tratamiento antiviral en nuestros pacientes. Acordamos iniciar el tratamiento cuando el tiempo previsto en lista de espera fuera de cuatro meses porque: 1) se intentaba evitar tratamientos largos en enfermos más proclives al

desarrollo de efectos adversos graves , 2) porque asumimos que gran parte de los pacientes que respondieran serían ARN-VHC negativos tras 12 semanas de tratamiento y 3) no era necesario esperar alcanzar una RVS ya que la fuente principal de viriones (el hígado) iba a ser eliminada con el trasplante. Hasta la fecha, la duración y el régimen de tratamiento antiviral que debería utilizarse en estos pacientes sigue sin estar bien definido.

Uno de los hallazgos más importantes y sorprendentes del estudio fue la recurrencia de la infección en 3 de los 9 pacientes que habían negativizado el ARN-VHC antes del trasplante. Existen varias hipótesis para explicar este hecho. Por un lado, es posible que en alguno de estos pacientes la carga viral estuviera por debajo de los límites de sensibilidad de la técnica empleada. En dos de los pacientes con ARN-VHC negativo en suero en el momento del trasplante y recurrencia posterior dispusimos de tejido hepático del explante y se pudo determinar la presencia de ARN-VHC en el mismo. En uno de los pacientes se detectó ARN-VHC en tejido hepático, sin embargo, la determinación fue negativa en el otro paciente. En este último caso, la infección del injerto hepático podría explicarse por la producción de partículas virales en un segundo compartimento donde persistiera el VHC, como las células mononucleares de sangre periférica o de médula ósea. El VHC, acantonado en este segundo compartimento, podría ser el responsable de la infección del injerto al ver favorecida su replicación por la inmunosupresión post-trasplante.

Los efectos adversos atribuídos al tratamiento fueron muy frecuentes tal y como se había descrito en otros estudios [94,95]. Dos de nuestros pacientes desarrollaron complicaciones infecciosas graves en forma de sepsis que requirieron hospitalización para tratamiento antibiótico endovenoso. Ambas se resolvieron de forma satisfactoria. Hay que destacar que, en el momento del

diagnóstico de la infección, el recuento de neutrófilos se encontraba por encima de $1200x10^9$/L y ambos pacientes presentaban una función hepatocelular aceptable (Child B de 7 puntos). Cuatro de los pacientes que iniciaron tratamiento presentaron descompensaciones *de novo* de su hepatopatía (en un caso, ascitis, dos casos de encefalopatía y un episodio de hemorragia por varices esofágicas). Dada la ausencia de grupo control en el estudio, desconocemos si la incidencia de estas complicaciones es superior a lo esperable en pacientes con función hepatocelular similar.

Los efectos secundarios a nivel hematológico también fueron frecuentes pero la rápida modificación de dosis de los fármacos antivirales junto a la administración de G-CSF(factores estimulantes de las colonias granulocíticas) y eritropoyetina recombinante consiguieron un adecuado cumplimiento del tratamiento en una proporción muy elevada de los pacientes. A pesar de la alta frecuencia de efectos secundarios, no se produjeron muertes durante el periodo de tratamiento. Es muy probable que el estrecho seguimiento clínico/analítico llevado a cabo en Consultas Externas sea fundamental para la detección precoz de los efectos adversos y su rápido tratamiento.

En resumen, este estudio demuestra que el tratamiento antiviral en cirróticos en lista de espera para trasplante hepático infectados por el VHC es eficaz para la prevención de la recurrencia de la infección en un 20% de los casos tratados. Nuestros datos sugieren que el tratamiento sería especialmente efectivo en el subgrupo de pacientes con carga viral baja (independientemente del genotipo) y en aquellos infectados por genotipos virales no-1. La disminución rápida de la carga viral durante el tratamiento constituye el mejor índice pronóstico de respuesta virológica y facilita la interrupción del

tratamiento en aquellos pacientes con baja probabilidad de eliminar el virus con lo que se evitarían efectos adversos potencialmente graves.

La aplicabilidad del tratamiento antiviral en nuestra serie fue superior al 50%. Las principales contraindicaciones para el tratamiento fueron la leucopenia, la trombocitopenia y la insuficiencia renal. Sin embargo y, vistos los resultados, es posible que el tratamiento pueda extenderse a otros pacientes que fueron excluídos por utilizar criterios demasiado estrictos al ser éste un estudio piloto. A pesar de que sólo se obtuvo una respuesta virológica sostenida en el 20% de los pacientes, la asociación entre la infección del VHC y la menor supervivencia tanto del injerto como del paciente sugieren que el tratamiento antiviral en pacientes en lista de espera de trasplante hepático puede ser una estrategia coste-efectiva.

7.3.La gravedad de la recurrencia de la infección por el VHC: factores pronósticos. Donante vivo/donante cadavérico.

El progresivo incremento del tiempo en lista de espera para trasplante hepático que se ha producido en los últimos años ha provocado la búsqueda de alternativas para aumentar el número de órganos aptos para el trasplante. Esta necesidad ha promovido la creación de programas de trasplante hepático de donante vivo, los cuales han ido surgiendo a lo largo de la última década. Los avances en las técnicas quirúrgicas han permitido que sea ésta una modalidad aceptada como alternativa válida al trasplante de hígado procedente de donante cadavérico. El debate ético que envuelve la donación a partir de donantes vivos gira entorno al riesgo-beneficio del procedimiento. En efecto, se debe tener en cuenta tanto el pronóstico del receptor como la morbi-mortalidad en el donante a la hora de aceptar la indicación de trasplante con donante vivo.

La mayoría de las series cifran la morbilidad en el donante en un 10-25%, incluyendo las complicaciones leves . También se han producido algunos fallecimientos de donantes como consecuencia del procedimiento. Esta mortalidad, a pesar de ser baja (entre un 0,4 y un 1%), es cualitativamente relevante si se tiene en cuenta que son sujetos sanos que se someten a un procedimiento destinado a tratar pacientes con una hepatopatía terminal. Los resultados iniciales de los grupos de trasplante de donante vivo demuestran que se trata de una técnica segura con una supervivencia a corto plazo, tanto del injerto como del paciente, similares a la obtenida en el trasplante a partir de órganos cadavéricos.

En un primer momento se asumió que la recurrencia de la infección por el VHC seguiría el mismo curso en los receptores de donante vivo que en los receptores de donante cadavérico. Sin embargo, algunos estudios retrospectivos que incluyeron un número reducido de pacientes, alertaron de una mayor agresividad de la recurrencia del VHC entre los receptores de donante vivo. Otros estudios, en cambio, no han logrado establecer diferencias entre ambas modalidades de trasplante hepático en cuanto a la gravedad de la recurrencia del VHC. La controversia viene favorecida por el hecho de que la mayoría de los estudios se han llevado a cabo sin una definición homogénea de recurrencia grave y sin biopsias realizadas por protocolo. Todo ello hace que sea difícil extraer conclusiones sólidas al respecto.

El objetivo de nuestro tercer trabajo fue analizar de forma prospectiva si existen factores predictivos de recurrencia grave del VHC post-trasplante hepático y, en especial, si existen diferencias en cuanto a dicha gravedad entre los receptores de donante vivo versus los receptores de donante cadavérico. Para ello se procedió a la inclusión en el estudio de todos aquellos pacientes

trasplantados con infección crónica por el VHC, desde marzo de 2000 hasta agosto de 2003. Se excluyeron los receptores de doble trasplante (hepático y renal), los coinfectados con el virus de la inmunodeficiencia humana (VIH) o con el virus de la hepatitis B (VHB) y los receptores de órganos procedentes de donantes a corazón parado. Para excluir aquellas complicaciones sin relación a la recidiva C, también se excluyeron del análisis los receptores de trasplante hepático con supervivencias inferiores a tres meses.

Se recogieron numerosas variables relacionadas con el huésped (edad, sexo, función hepatocelular antes del trasplante, presencia de hepatocarcinoma), con el donante (edad, esteatosis, donante vivo/cadavérico), con el procedimiento quirúrgico (tiempo de isquemia, requerimientos transfusionales), con el VHC (carga viral basal, genotipo, tratamiento viral antes del trasplante, tratamiento viral post-trasplante) y con la evolución del injerto (tipo de inmunosupresión, evolución del perfil hepático, infecciones, complicaciones biliares o vasculares, etc).

En nuestro centro existe un protocolo asistencial de seguimiento para los trasplantados infectados por el VHC que incluye la realización de biopsias hepáticas a los 3, 12 meses y anualmente tras el trasplante, independientemente del estado clínico y analítico del paciente. Asimismo, se realiza un estudio hemodinámico al mes 3 y 12 después del trasplante. Estas exploraciones, permiten disponer de numerosos datos referentes al curso clínico, histológico, bioquímico y hemodinámico de los pacientes que han ayudado a una mejor definición de la recurrencia grave de la infección por el VHC post-TH. En concreto, en nuestro estudio se definió la recurrencia grave como la presencia de cirrosis en cualquiera de las biopsias llevadas a cabo

(por protocolo o por criterios clínicos) y/o el desarrollo de descompensación clínica de la hepatopatía secundaria a la hipertensión portal (ascitis, hemorragia por varices esofágicas, encefalopatía hepática), siempre que estos hallazgos no se pudieran justificar por otras causas diferentes a la infección por el VHC.

Un total de 116 pacientes, con 117 injertos, se incluyeron en el estudio y se siguieron durante un tiempo medio de 22 meses. A lo largo del periodo de seguimiento, 26 pacientes desarrollaron una recurrencia grave por el VHC según la definición anteriormente descrita. La probabilidad acumulada de desarrollar recurrencia grave a los dos y tres años del trasplante fue de un 29% y un 33%, respectivamente. Estos porcentajes son significativamente más altos que los descritos en la mayoría de series americanas y bastante parecidos a los ya documentados en nuestra área geográfica [96]. Se ha especulado que la mayor gravedad de la recurrencia de la infección podría ser debida a la mayor virulencia de las cepas del VHC en nuestra área geográfica. De los pacientes que desarrollaron recurrencia grave, 17 (18%) pertenecían al grupo de 95 receptores de donante cadavérico y 9 (41%) formaban parte del grupo de 22 receptores de donante vivo. No encontramos diferencias significativas entre los dos grupos de receptores de trasplante en cuanto a edad, sexo, función hepática antes del trasplante, indicación de trasplante, carga viral basal o genotipo infectante. Como era de prever, sí fueron diferentes en cuanto a variables inherentes al mismo proceso de la donación a partir de vivo. Por ello, en el grupo de receptores de donante vivo, la edad media de los donante fue menor, los injertos presentaron menor grado de esteatosis y el tiempo de isquemia fue también significativamente más corto que en los receptores de donante cadáver. Las complicaciones biliares (fugas biliares y estenosis de la

vía biliar) aparecieron con mayor frecuencia entre los receptores de donante vivo (72,7% versus 22,1%). Otra diferencia entre ambos grupos de trasplantados fue el hecho de que los receptores de donante vivo recibieron tacrolimus como inmunosupresor de base en la mayoría de los casos. Cuando comparamos los pacientes que desarrollaron recurrencia grave (independientemente del tipo de donante) frente a los que no, el análisis univariante demostró que el tipo de donante (peor el vivo frente al cadavérico) y la elevación de las transaminasas a los tres meses del trasplante fueron las únicas variables que correlacionaron con la recurrencia grave por el VHC. Además, los pacientes que desarrollaron recurrencia grave mostraron cargas virales basales (pre-TH) más elevadas, sin embargo, esta diferencia no alcanzó significación estadística.

En 93 pacientes de los 117 que formaron parte del estudio, se dispuso de una biopsia hepática a los tres meses del trasplante. En el análisis univariante, un índice necroinflamatorio por encima de 1 en dicha biopsia también se relacionó con una mayor gravedad de la recurrencia del VHC. Así, es de suponer que la elevación de las transaminasas al tercer mes post-TH no sería más que la traducción de un mayor grado de inflamación en la biopsia y constituiría un factor de mal pronóstico en cuanto a la recurrencia del VHC, tal y como ya había sido descrito en otros estudios [97,98].

Sorprendentemente no encontramos diferencias en cuanto a la edad del donante, el grado de esteatosis, la incidencia del rechazo, el tratamiento con bolus de corticoides, el tipo de inmunosupresión (tacrolimus versus ciclosporina) o la duración en meses del tratamiento con corticoides. En trabajos publicados recientemente, la esteatosis del injerto y la edad del donante aparecían como factores predictivos de recurrencia grave. La

naturaleza retrospectiva de dichos estudios puede, en parte, justificar el hecho de que no hayamos observado la misma relación en cuanto a estos factores. En efecto, a pesar de que los receptores de donante vivo recibieron un injerto procedente de donantes más jóvenes, con menos esteatosis y con menor tiempo de isquemia, la recurrencia de la infección por el VHC fue más grave. Una posible explicación sería que la potencia del factor " tipo de donante" podría haber enmascarado el efecto deletéreo de la edad del donante y de la esteatosis sobre la recidiva C. Sin embargo, cuando se analizó sólo el grupo de receptores de donante cadavérico, tampoco se encontraron diferencias significativas en cuanto a estas variables.

Las complicaciones biliares aparecieron con mayor frecuencia entre aquellos pacientes que desarrollaron posteriormente una recurrencia grave. Sin embargo las complicaciones biliares no aparecieron como factor predictivo independiente de recurrencia grave en el análisis multivariante. El impacto de dichas complicaciones biliares sobre la recurrencia de la hepatitis C post-TH es un tema controvertido. Se sabe que la colestasis crónica actúa estimulando la inflamación y la fibrogénesis hepática. La incidencia de las complicaciones biliares en nuestra serie fue significativamente mayor que en la de otros programas de trasplante. Existen varios factores que pueden haber contribuído a aumentar el número de complicaciones biliares en nuestro estudio. Por un lado, al ser un trabajo prospectivo se han registrado todas las complicaciones biliares, fueran tempranas o tardías, leves o que requirieran de técnicas invasivas para su resolución. En la mayoría de las series publicadas, las complicaciones leves de la vía biliar pueden haber sido obviadas, especialmente en los trabajos retrospectivos. Un hecho que apoya esta posibilidad es el progresivo aumento de la frecuencia de complicaciones

biliares descritas desde que hace unos años se procedió a la estandarización del método de recogida de estos datos. Con todo, la mayor incidencia de complicaciones biliares publicada por otros programas de trasplante es de hasta un 40%, lejos del 72% de nuestra serie. En segundo lugar, muchos de los receptores de donante vivo de nuestra serie presentaban variantes anatómicas de la vía biliar (del donante y del receptor) lo que supuso la realización de anastomosis biliares múltiples en una proporción significativa de los casos. Las anastomosis múltiples de la vía biliar se han relacionado con una mayor probabilidad de desarrollo de complicaciones biliares [99,100]. Por último, este trabajo incluye los primeros casos del programa de trasplante hepático de donante vivo realizados en nuestro centro. Este hecho sugiere que el elevado número de complicaciones biliares registrado pueda ser la traducción de la curva de aprendizaje. A pesar de todo lo expuesto y como se ha mencionado anteriormente, las complicaciones biliares no aparecieron como factor predictivo independiente de recurrencia grave. Lógicamente, no podemos excluir un posible efecto sinérgico de la infección por el VHC y la colestasis provocada por estas complicaciones.

Uno de los hechos diferenciales claves del trasplante hepático de donante vivo es la regeneración hepática que aparece inmediatamente después del implante del lóbulo hepático derecho del donante en el receptor. La regeneración hepática podría favorecer una mayor replicación viral, precisamente en la fase precoz post-TH cuando los niveles de inmunosupresión son mayores. Ello favorecería el desarrollo de una hepatitis más agresiva sobre el injerto, como sugiere el hecho de que en estos pacientes se produzca un pico de transaminasas más precoz y acusado que en los receptores de donante cadavérico (Fig 6).

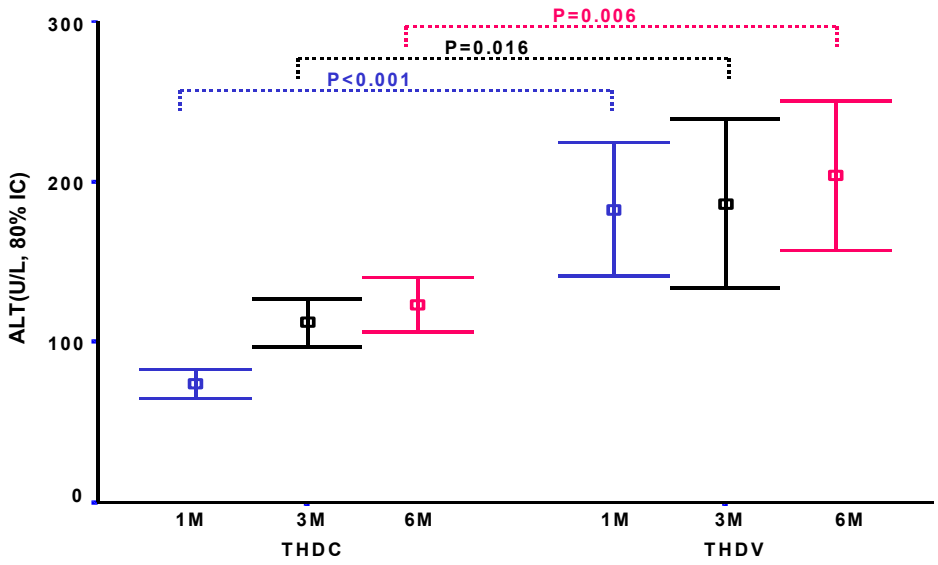

Fig 6. Evolución de los niveles de ALT post-TH en receptores de donante cadavérico (THDC) y receptores de donante vivo (THDV).

Existen datos obtenidos a través de experimentos *in vitro* que sugieren que el VHC aprovecharía los mecanismos del ciclo celular para activar su propia replicación [101,102]. Además, durante la regeneración hepática se estimula la expresión del receptor celular de las lipoproteínas de baja densidad (LDL), y ello podría facilitar la entrada del VHC en la célula. Cuando se compararon los niveles de carga viral en diferentes puntos post-TH en ambos grupos de pacientes, éstos fueron superiores en los receptores de donante vivo en todos los puntos analizados aunque las diferencias no alcanzaron significación estadística. Sin embargo, esta ausencia de diferencias en cuanto a la carga viral, no excluye la posibilidad de que en estos pacientes la replicación viral se produzca de forma más activa. En efecto, la carga viral depende de la

producción y del aclaramiento viral y éstos, a su vez, del número de células infectadas y de la capacidad del sistema retículoendotelial para eliminar partículas virales.

A pesar de que el número de pacientes incluídos es limitado, se trata del mayor trabajo prospectivo realizado sobre la recurrencia del VHC en el trasplante hepático de donante vivo. Refleja la experiencia de un solo centro , lo que implica la ventaja de un seguimiento y manejo de los pacientes más homogéneo. Sin embargo, estos resultados deberán ser validados por otros grupos de trasplante.

Así pues, la recurrencia de la infección por el VHC tras el trasplante hepático sigue un curso más agresivo en los receptores de donante vivo comparado con los receptores de donante cadavérico. Los resultados presentados tienen implicaciones clínicas relevantes. El descenso de la supervivencia tanto del injerto como del paciente que se podría derivar de este hecho cuestionaría si el trasplante de donante vivo es una opción coste-efectiva en sujetos infectados por el VHC. Algunas alternativas serían la de limitar esta modalidad de trasplante para programas con tiempos de espera muy prolongados y por tanto con elevada mortalidad en lista , para pacientes no infectados por el VHC (por lo menos mientras durase el efecto de la curva de aprendizaje) o realizar tratamiento antiviral antes del trasplante. Por tanto, los resultados de este estudio deberían tenerse en cuenta a la hora de establecer las indicaciones de trasplante hepático de donante vivo ya que la recurrencia de la infección por el VHC tras el trasplante podría comprometer la supervivencia del injerto y del paciente.

8. CONCLUSIONES.

1. La recurrencia de la infección por el VHC se produce de forma universal tras el trasplante hepático. Las partículas virales se detectan en sangre a lo largo de todo el procedimiento y la infección del injerto se produciría a partir de viriones circulantes.

2. Durante las fases anhepática y de reperfusión del injerto se produce un descenso significativo de la carga viral, que alcanza níveles mínimos a las 8-24 horas del trasplante. Este descenso se debe a las pérdidas hemáticas que se producen durante la intervención, a la ausencia de producción de viriones y al aclaramiento hepático de los mismos por el injerto.

3. La replicación viral en el nuevo órgano se inicia a las pocas horas de la reperfusión y, en la mayoría de los pacientes, la viremia alcanza niveles similares a la fase pre-trasplante a los pocos días. Ello demuestra la enorme capacidad del VHC para adaptarse a un nuevo entorno.

4. La cinética de la infección del injerto por el VHC debe tenerse en cuenta cuando se diseñen estrategias profilácticas (inmunoprofilaxis) y/o terapéuticas. Desde un punto de vista virológico, cualquier estrategia terapéutica debería tener en cuenta que la carga viral alcanza niveles mínimos durante las primeras horas del post-trasplante.

5. El tratamiento de pacientes cirróticos en lista de espera infectados por el VHC con interferón y ribavirina consigue una respuesta virológica en un 30% de casos, respuesta que se mantiene tras el trasplante en la mayoría de pacientes. A pesar de que la aplicabilidad del tratamiento antiviral es baja, éste evita la recurrencia de la infección por el VHC en una proporción significativa de pacientes.

6. El tratamiento antiviral en pacientes en lista de espera infectados por el VHC es especialmente efectivo en aquellos individuos con un perfil virológico favorable (baja carga viral y genotipo no-1).

7. Una vez iniciado el tratamiento, el mejor factor predictivo de respuesta virológica es la disminución de la carga viral (superior a 2 log) en la semana 4 de tratamiento. Este hecho permitiría suspender el tratamiento de forma precoz en aquellos pacientes con baja probabilidad de respuesta, con lo que se evitarían efectos adversos innecesarios.

8. El tratamiento antiviral en pacientes en lista de espera infectados por el VHC se acompaña de numerosos efectos adversos, algunos potencialmente graves. Sin embargo, un seguimiento clínico y analítico adecuado puede contribuir a minimizar el efecto de los mismos y el número de abandonos del tratamiento.

9. La recurrencia de la infección por el VHC tras el trasplante hepático sigue un curso agresivo que condiciona el desarrollo de cirrosis hepática en un tercio de los pacientes a los tres años del TH.

10. En nuestro programa de trasplante hepático, la recurrencia de la hepatitis C siguió un curso más agresivo entre los receptores de donante vivo comparado con los receptores de donante cadavérico.

11. La hepatitis aguda post-TH que se produce como consecuencia de la recurrencia de la infección por el VHC aparece de forma más precoz y grave en los receptores de donante vivo. A nivel histológico, ésta se caracteriza por un mayor índice necroinflamatorio lobulillar, lo que se traduce en niveles de transaminasas más elevados.

12. Aunque nuestro estudio no permitió el análisis de las causas de esta mayor agresividad, la regeneración hepática que se produce en receptores de donante vivo podría favorecer la replicación viral y una hepatitis más grave. La aparición de complicaciones biliares no fue una variable con valor predictivo independiente en la gravedad de la recurrencia de la hepatitis C. Sin embargo, no podemos descartar un efecto sinérgico entre la colestasis secundaria a dichas complicaciones y la hepatitis C.

13. Los programas de trasplante hepático deberían tener en cuenta el mayor riesgo de recurrencia grave de la hepatitis C en los receptores de donante vivo a la hora de seleccionar a los candidatos.

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