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ENFERMEDAD INJERTO CONTRA HUÉSPED CRÓNICA: FACTORES PRONÓSTICOS Y NUEVAS OPCIONES TERAPÉUTICAS

FERNANDO DA MOTA VEIGA MENDES DA SILVA

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Dña. Maria Dolores Caballero Barrigón

Profesora asociada del departamento de Medicina de la Universidad de Salamanca

Jefe de Sección de la Unidad Clínica del Servicio de Hematología del Hospital Clínico Universitario de Salamanca,

CERTIFICA:

Que el trabajo realizado bajo nuestra dirección por D Fernando da Mota Veiga Mendes da Silva titulado "ENFERMEDAD INJERTO CONTRA HUESPÉD CRÓNICA: FACTORES PRONÓSTICOS Y NUEVAS OPCIONES TERAPÉUTICAS", reúne, a nuestro juicio, las condiciones de originalidad requeridas para optar al grado de Doctor por la Universidad de Salamanca.

Y para que así conste, firmo la siguiente certificación en Salamanca, a 25 de Septiembre de 2010.

Firmado: Drª. Maria Dolores Caballero Barrigón

D. José Antonio Pérez-Simón
Jefe de Servicio
Servicio de Hematologia del Hospital Universitario Virgen del Rocio /
Instituto de Biomedicina de Sevilla (IBIS),

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Firmado: Dr. José Antonio Pérez-Simón

“When there is a will there is a way….”

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ABREVIATURAS

Alo-TPH Trasplante alogenico de progenitores hematopoyéticos
AIR Acondicinamiento de intensidad reducida
AMA Acondicionamiento mieloablativo
ANM Acondicionamiento no mieloablativo
ATG Globulina antitimocítica
BDP Dipropionato de beclometasona
BO Bronquiolitis obliterante
ClCr Clearence de creatinina
Células NK Células natural killer
CMV Citomegalovirus
CPA Células presentadoras de antígenos
CPH Células progenitoras hematopoyéticas
CPHSP Células progenitoras hematopoyéticas de sangre periférico
CTLs Linfocitos T citotóxicos
CU Cordón umbilical
CsA Ciclosporina A
DE Donante emparentado
DnE Donante no emparentado
DLT Depleción de linfocitos T
EBMT European Group for Blood and Marrow Transplantation
EH Enfermedad de Hodgkin
EICHa Enfermedad injerto contra huésped aguda
EICHc Enfermedad injerto contra huésped crónica
EICL/T Efecto injerto contra leucemia/tumor
EMR Enfermedad mínima residual
EBV Virus Epstein Barr
FA Fase acelerada
FC Fase crónica
FCH Factor de crecimiento hematopoyético
G-CSF Factor estimulante de colonias granulocíticas
GM-CSF Factor estimulante de colonias granulocito-macrofágicas
HLA Antígenos leucocitarios humanos
ICT Irradiación corporal total
IL Interleucina
ILD Infusión de linfocitos del donante
IU Unidades Internacionales
LLC Leucemia linfática crónica
LLA Leucemia linfoblástica aguda
LNH Linfoma no Hodgkin
LMA Leucemia mieloblástica aguda
LMC Leucemia mieloide crónica
MHC Complejo de histocompatibilidad mayor
MM Mieloma múltiplo
MO Médula ósea
MRT Mortalidad relacionada con el trasplante
MSC Células mesenquimales

MTX Metotrexate
NIH National Institute of Healths
PHSP Progenitores hematopoyéticos de sangre periférica
RC Respuesta completa
RC1 Primera respuesta completa
RC2 Segunda respuesta completa
REDMO Registro de Donantes de Médula Ósea
RP Respuesta parcial
SC Superficie corporal
SCU Sangre de cordón umbilical
SG Supervivencia global
SLE Supervivencia libre de enfermedad
SLP Síndrome linfoproliferativo
SMD Síndrome mielodisplásico
SNC Sistema nervioso central
SP Sangre periférica
TC Tomografia axial computarizada
TCR Receptor de células T
TPH Trasplante de progenitores hematopoyéticos
TPHSP Trasplante de progenitores hematopoyéticos de sangre periférica
TPHMO Trasplante de progenitores hematopoyéticos de médula ósea
VHA Virus de la hepatitis A
VHB Virus de la hepatitis B
VHC Virus de la hepatitis C
VHS Virus herpes simplex
VIH Virus de la inmunodeficiencia humana
VVZ Virus varicela zoster

ÍNDICE

INTRODUCCIÓN

1. INTRODUCCIÓN

El trasplante de progenitores hematopoyéticos (alo-TPH) puede ser el tratamiento de elección de muchos pacientes con enfermedades malignas y no malignas -congénitas y adquiridas- del sistema hematopoyético. Durante las últimas décadas el TPH ha evolucionado desde un procedimiento experimental hasta convertirse en el tratamiento estándar de algunas enfermedades como leucemias agudas, síndromes mielodisplásicos, linfomas, mieloma múltiple, talasemias, anemia aplásica, etc[1].

De acuerdo con los datos del European Group for Blood & Marrow Transplantation (EBMT), en 2008 se realizaron 26.766 trasplantes (60% autólogos y 40% alogénicos) en los diversos centros europeos. De los 16.028 trasplantes autólogos, solo 1,3% se realizaron a partir de médula ósea (MO) y el resto a partir de células progenitoras hematopoyéticas de sangre periférica (CPHSP). De los 10.738 trasplantes alogénicos en 23% la fuente fue MO, el 71% la sangre periférica (SP) y en el 6% el cordón umbilical (CU). En cuanto al donante, de los 10.738 trasplantes alogénicos, el donante fue familiar HLA idéntico en el 45% de los casos, familiar no idéntico en el 5% de los casos y donante no emparentado en el 50%. De acuerdo con los datos del EBMT los principales cambios en la epidemiología del trasplante en la última década es una mayor realización de trasplantes alogénicos en leucemias agudas, un aumento de trasplantes a partir de cordón umbilical y un mayor número de trasplantes con acondicionamiento de intensidad reducida (AIR), representando el 34% de los trasplantes alogénicos en 2006[2].

La enfermedad injerto contra huésped crónica (EICHc) representa la complicación tardía más importante de los pacientes sometidos a trasplante alogénico de progenitores hematopoyéticos y se asocia a una no desdeñable mortalidad, segundas neoplasias y una deficiente calidad de vida. En este sentido, la clasificación del National Institute of Health (NIH) de acuerdo al número y grado de severidad de los órganos afectados, permite individualizar la estrategia del tratamiento inmunosupresor de acuerdo al riesgo de los pacientes[3].

El uso de progenitores hematopoyéticos de sangre periférica ha aumentado la incidencia y modificado las características de la EICHc incrementando el número de pacientes que requieren varias líneas de tratamiento y el tiempo de duración del tratamiento inmunosupresor[4]. Si bien tras alo-TPH de médula ósea se han publicado estudios que identifican diversos factores pronósticos en pacientes con EICHc[5,6], tras trasplante de progenitores hematopoyéticos de sangre periférica (TPHSP) se requieren estudios que permitan identificar factores de riesgo.

El tratamiento estándar de la EICHc sigue basandose en el uso de inhibidores de calcineurina y corticosteroides. Sin embargo, como queda mencionado este tratamiento es insuficiente para un considerable número de pacientes, especialmente los que reciben progenitores hematopoyéticos de sangre periférico, por lo que la necesidad de nuevas opciones terapéuticas es evidente.

Con todos estos antecedentes, en este trabajo de tesis doctoral nos proponemos evaluar el valor pronóstico de la clasificación propuesta por el NIH, identificar nuevos factores pronósticos y predictivos para el desarrollo de EICHc severa o extensa y valorar nuevas opciones terapéuticas.

1.1 Generalidades sobre el trasplante de progenitores hematopoyéticos

El trasplante de progenitores de hematopoyéticos (TPH) se basa en la infusión de precursores hematopoyéticos obtenidos de la médula ósea, sangre periférico o cordón umbilical a un receptor que ha sido previamente acondicionado con quimio o radioterapia.

Desde la realización de los primeros trasplantes de progenitores hematopoyéticos de médula ósea en humanos (TPHMO) en 1957[7] la supervivencia de los enfermos ha mejorado ostensiblemente. En 1965 se documentó por primera vez la obtención de un injerto estable en un paciente con leucemia linfoblástica aguda que había recibido irradiación y quimioterapia seguida de infusión intravenosa de médula de seis donantes emparentados

distintos[8]. A mediados de la década de 1970 se publicaron por primera vez datos de supervivencia a largo plazo en un número significativo de pacientes con leucemias agudas sometidos a trasplante alogenico de progenitores hematopoyéticos de médula ósea (alo-TPHMO) en los que la terapia convencional había fracasado[9].

Desde entonces, se han producido pocos avances en relación a los régimenes de acondicionamiento pretrasplante, manteniéndose esquemas ya propuestos por Donald Thomas (irradiación corporal total (ICT) + altas dosis de ciclofosfamida o busulfan más ciclofosfamida)[1,10-12] como los más habitualmente empleados. Intentos posteriores de disminuir las recaídas mediante el aumento de la intensidad de los regímenes existentes o añadiendo nuevos fármacos se siguieron de un incremento de la mortalidad relacionada con el trasplante (MRT)[13-17], por lo que los esquemas clásicos son todavía los más utilizados en el trasplante convencional.

A pesar de los avances en el tratamiento de soporte y de la utilización de la sangre periférica como fuente de progenitores, que condiciona un injerto más rápido de neutrófilos y plaquetas, con menos infecciones y menos estancia hospitalaria[18] todavía la MRT no es desdeñable (7-11% en el día +100 y entre 16 y 27% al año, considerándose la edad límite los 50-55 años). Dado que la edad media de los pacientes con enfermedades potencialmente curables con trasplante alogénico es superior a los 60 años, esta estrategia no esta accesible a la mayoría de los enfermos con hemopatias malignas. Esto, unido a la constatación de que la eficacia del trasplante no solo depende de la quimioterapia de acondicionamiento sino del efecto antitumoral de las células del donante o efecto injerto contra leucemia (EICL), permitió el desarrollo de AIR o NMA, en un intento de ampliar este tratamiento a pacientes de edad superior a 50-55 años o con coomorbilidades que contraindicaban el trasplante con AMA debido a un riesgo inaceptable de MRT[19,20]. Así a mediados de los 90 los AIR fueron ampliamente introducidos en Europa y EEUU y desde entonces se han descrito numerosos estudios multicéntricos utilizando fármacos con capacidad inmunosupresora asociados a mieloablativos y/o ICT a dosis no

mieloablativas sobretodo en pacientes mayores[21-24]. Se han descrito hasta 39 regímenes de AIR de acuerdo con una revisión de Barret y Savani[25].

Recientemente Bacigalupo y col.[26] han propuesto una definición de los regimenes de acondicionamiento de acuerdo con su intensidad mieloablativa y/o inmunosupresora en 3 categorías basándose en la duración de las citopenias y el requerimiento de soporte con células progenitoras hematopoiéticas (CPH): 1- acondicionamiento mieloablativo (AMA): producen citopenias irreversibles y el soporte con CPH es mandatario; 2- acondicionamiento no mieloablativo (ANM): causan citopenias leves o moderadas y se pueden administrar sin necesidad de soporte con CPH; 3- acondicionamiento de intensidad reducida (AIR): aquellos regímenes que no presentan criterios de AMA o ANM y que producen citopenias de duración variable aunque no sean irreversibles y que requieren rescate con CPH.

Respecto a los resultados de este tipo de trasplante es evidente que han abierto un campo a pacientes mayores (de hasta 70 años) que pueden alcanzar supervivencias prolongadas con este procedimiento aunque la MRT, la recaída y la EICH, al igual que tras el trasplante mieloablativo siguen siendo las limitaciones fundamentales de este procedimiento. No existen estudios randomizados entre alo-TPH convencional y de intensidad reducida que permitan definir las diferencias en términos de supervivencia global y libre de enfermedad entre ambos acondicionamientos aunque en algunos estudios retrospectivos se describen una mayor incidencia de recaídas y una menor mortalidad con AIR[15-17,27-32].

Actualmente este procedimiento se ha extendido a un amplio grupo de patologías hematológicas y no hematológicas[33]. Además, la morbilidad y mortalidad relacionada con el TPH ha mejorado en los últimos años debido a los avances en el conocimiento del sistema HLA, las mejorías en el tratamiento de soporte así como en el conocimiento de la fisiopatología y tratamiento de la EICH.

1.2 Enfermedad injerto contra huésped

1.2.1 Definición

La EICH constituye la complicación mas frecuente tras el trasplante alogénico y tiene un impacto significativo en la supervivencia y calidad de vida de estos pacientes[34]. En este sentido, > 50% de la mortalidad no relacionada con la recaída tras trasplante alogénico es producida por la EICH[35]. La EICH es una consecuencia de la interacción entre las células presentadoras de antígenos del receptor y las células T del donante lo que da lugar a una respuesta inmune responsable de las manifestaciones clínicas caracteristicas de la enfermedad. Clásicamente la EICH se ha dividido en aguda y crónica en función de que su aparición se produjera antes o después del día +100 postrasplante[36]. Sin embargo, de acuerdo con la clasificación propuesta por Filipovich y col[3], actualmente son las manifestaciones clínicas más que el momento de aparición las que permiten establecer el diagnóstico de EICH aguda ó crónica.

1.2.2 Fisiopatología

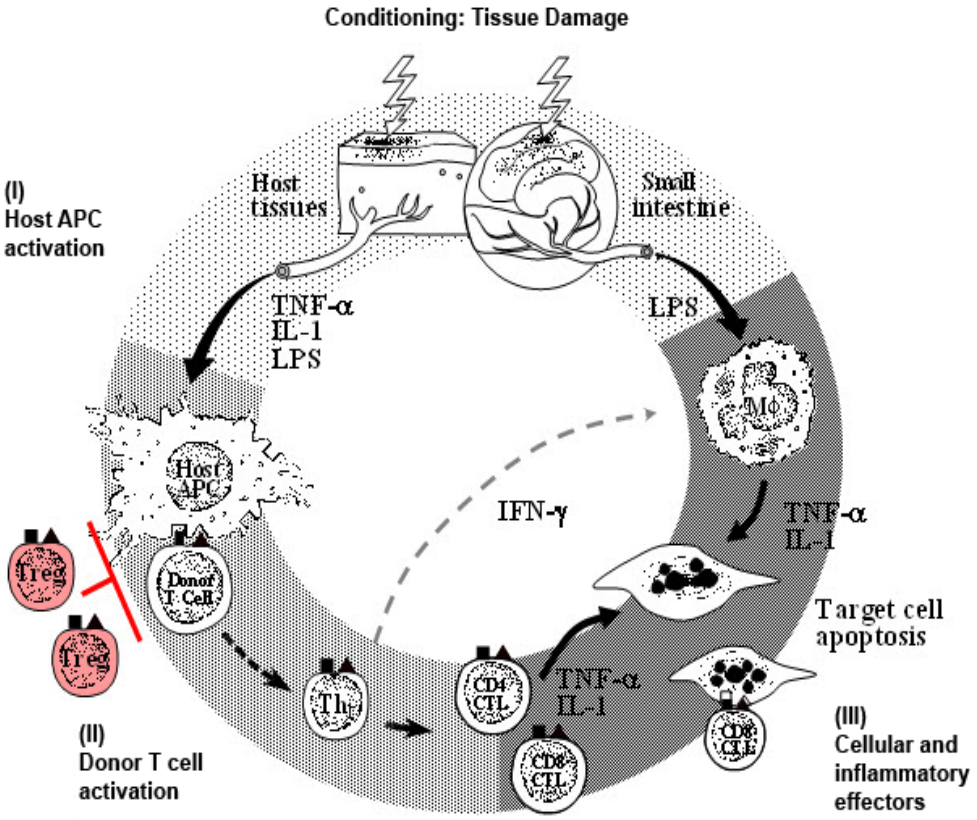
A diferencia de la EICH aguda (EICHa) para la cual existe un modelo fisiopatologíco ampliamente aceptado basado en 3 fases claramente definidas (daño tisular y liberación de citocinas, activación linfocitaria y fase celular efectora)[37], en la EICH crónica los mecanismos subyacentes no están claramente definidos debido tanto, a su complejidad como a no disponer de modelos experimentales adecuados. Dado que el primer factor para desarrollar una EICH crónica es el haber padecido una EICH aguda, a continuación se decribiran brevemente los mecanismos implicados en el desarrollo de la misma.

Para que se produzca la EICH se deben cumplir tres condiciones[38]:

1- La presencia de células inmunocompetentes en el implante
2- La presencia en el receptor de antígenos que difieran de los del donante
3- Incapacidad del receptor de producir una respuesta inmune contra el injerto

De acuerdo con el modelo propuesto por Ferrara[37], la fisiopatología de la EICHa se desarrolla en 3 fases consecutivas (figura 1):

Figura 1. GVHD pathophysiology. (JLM Ferrara-2009).



GVHD Pathophysiology. In Phase I, the recipient conditioning regimen damages host tissues and causes release of inflammatory cytokines such as TNFα, IL-1 and IL-6. Increased levels of these cytokines leads to activation of host antigen presenting cells (APCs). In Phase II, host APCs activate mature donor cells. The subsequent proliferation and differentiation of these activated T cells produces additional effectors that mediate the tissue damage, including Cytotoxic T Lymphocytes, Natural Killer (NK) cells, TNFα and IL-1. Lipopolysaccharide (LPS) that has leaked through the damaged intestinal mucosa triggers additional TNFα production. TNFα can damage tissue directly by inducing necrosis and apoptosis in the skin and GI tract through either TNF receptors or the Fas pathway. TNFα plays a direct role in intestinal GVHD damage which further amplifies damage in the skin, liver and lung in a "cytokine storm."

Los tratamientos previos, las infecciones y en particular el régimen de acondicionamiento producen un daño tisular, dando lugar a la liberación de grandes cantidades de citocinas proinflamatorias como el factor de necrosis tumoral alfa (TNF-α), interleucina-1 (IL-1) e IL-6 y factores de crecimiento como el GM-CSF) que aumentan la expresión de moléculas de adhesión, antígenos del sistema HLA y moléculas co-estimuladoras en las CPA del receptor facilitando el reconocimiento de antígenos menores de histocompatibilidad en los tejidos del huésped por parte de los linfocitos T del donante. Otro factor que favorece la activación de las CPA es la presencia en la circulación sistémica de

productos microbianos como lipopolisacaridos (LPS) producidos como consecuencia del daño en la mucosa intestinal.

2ª Fase- Activación de las células T: los linfocitos T (principalmente CD4+) del donante interactúan con las CPA del receptor: las CPA presentan el complejo MHC clase II-péptido al receptor de células T (TCR) del donante en presencia de señales co-estimuladoras que permitirán la activación de las células T, dando lugar a la proliferación, producción de citocinas como el IFN-γ, IL-2, TNF- α, etc, diferenciación y migración de los linfocitos T.

3ª Fase- Efectores celulares e inflamatorios: los efectores celulares de la EICHa son los linfocitos T citotóxicos (CTLs) y las células NK. Los mecanismos efectores de lisis celular empleados por estas células son el sistema Fas/FasL y el perforina/granzima, además de citocinas inflamatorias como el TNF- α e IL-1 que son producidas por los monocitos y macrófagos tras su estimulación. El TNF- α activa las células dendríticas y aumenta la presentación de aloantígenos, induce la producción de quemoquinas inflamatorias que a su vez reclutan linfocitos T, neutrófilos y monocitos a los órganos dañados y además, induce daño tisular directamente mediante la inducción de apoptosis y necrosis.

EICH crónica

Los mecanismos fisiopatológicos de la EICHc no están completamente establecidos. Mientras que la EICHa generalmente se presenta como un proceso inflamatorio que afecta fundamentalmente la piel, tracto gastrointestinal e hígado, las manifestaciones clínicas de la EICHc se asemejan a las observadas en las enfermedades autoinmunes como el lupus eritematoso sistémico, síndrome de Sjogren, esclerodermia o artritis reumatoide. Probablemente alteraciones en los mecanismos de regulación del sistema inmunológico favorecen la expansión de linfocitos T del donante en respuesta a alo o auto-antígenos que escapan a los mecanismos de inmunotolerancia a nivel tímico o en sangre periférica[39]. En estudios

experimentales y clínicos se han observado atrofia del timo, depleción de linfocitos y pérdida de la función del timo resultando en una timopoyesis aberrante con persistencia de clones autoreactivos[40,41]. Sin embargo, aún esta por clarificar el papel de la auto y/o alo-reactividad en el contexto de la EICHc. Tanto en modelos experimentales como en estudios clínicos se ha observado la formación de autoanticuerpos[42] de forma que se detectan anticuerpos antinucleares, antiDNA o antimúsculo liso en el 11 a 62% de los casos. Igualmente, se ha encontrado una relación entre la expresión del marcador de activación BAAF (B-cell activating factor)[43] que promueve la supervivencia y diferenciación de los linfocitos B o la generación de anticuerpos anti-H-Y[44](sobre todo en receptor varón y donante mujer) y el desarrollo de EICHc. Estos hallazgos junto a las semejanzas clínicas de la EICHc con las enfermedades autoinmunes pone de relieve el papel de la auto-reactividad así como de la respuesta inmune humoral en su aparición.

Por otro lado, la alo-reactividad frente a antígenos menores de histocompatibilidad (mHLA) explica la EICHc como una manifestación o fase tardía de EICHa[45] aunque el patrón de citocinas que intervienen en el desarrollo de la EICH aguda y crónica es diferente. Mientras que en la EICHa predominan las citocinas del tipo Th1 (TNF-α, IL-1, IL-6 y la IL-8) en la EICHc predominan las de tipo Th2 (IFNγ, IL-4, IL-10). Sin embargo, hay investigadores que no han encontrado diferencias tan significativas en el patrón de citocinas, por ejemplo se pudo demostrar en modelo murino que en las fases iniciales, tanto de EICH aguda como crónica, existía un aumento de citocinas tipo Th2 (IL-4 y IL-10)[46]. También se han descrito alteraciones a nivel periférico que contribuyen al desarrollo de la EICHc. En este sentido, tanto las células dendríticas del donante como las del receptor pueden contribuir al desarrollo de EICHc mediada por linfocitos CD4 en la piel o en el tubo digestivo, respectivamente. Finalmente diversos estudios describen la relación entre los linfocitos T reguladores (T_{reg}) y la EICH[47,48]. Las T_{reg} son células T ($CD4^+CD25^+FOXP3^+$) que se desarrollan en el timo y su función es suprimir la proliferación de los linfocitos autoreactivos y controlar la respuesta inmune normal. Estudios en modelos murinos han demostrado que la depleción de las

células T_{reg} produce una proliferación de linfocitos T y destrucción autoinmune de varios órganos y tejidos[49]. El alo-TPH afecta la función timica y la reconstitución de células T de manera que la pobre reconstitución de linfocitos T_{reg} puede dar lugar a la incapacidad de suprimir las células auto y aloreactivas contribuyendo a la aparición de la EICH. En un estudio reciente en pacientes sometidos a alo-TPH (de los cuales un grupo de pacientes tenia EICHc activa y otro sin EICHc) y donantes sanos se demostró que los linfocitos T_{reg} así como la expresión de FOXP3 se encuentran disminuidos en los pacientes con EICHc activa[50]. La causa de la disminución de esta población de linfocitos T en el contexto de la EICHc no está clara.

1.2.3 EICHc: Clasificación y manifestaciones clínicas

Clásicamente, la EICH se ha dividido en aguda o crónica en función de que su aparición se produjera antes o después del día + 100 postrasplante. Sin embargo, actualmente son las manifestaciones clínicas más que el tiempo de aparición las que permiten establecer el diagnóstico diferencial entre ambas, como se recoge en la tabla 1 propuesta por Filipovich y cols[3]. La mediana de aparición de la EICHc es de 201 días tras el trasplante de donante emparentado HLA idéntico, 159 días tras TPH de donante no emparentado y de 133 días tras trasplante de donante no emparentado con alguna disparidad HLA[51]. En la clasificación clásica de la EICHc descrita por Shulmann y col[36] hace tres décadas, los pacientes se categorizan de acuerdo al tiempo de inicio de los síntomas y/o signos (a partir del día + 100 posTPH) y de acuerdo con su extensión en limitada (estos pacientes no requieren tratamiento inmunosupresor sistémico) o extensa (estos pacientes sí requieren tratamiento sistémico (tabla 2). Aunque es fácilmente reproducible y ampliamente utilizada, esta clasificación tiene un escaso valor pronóstico y no permite distinguir pacientes con menor o mayor riesgo de fallecer por complicaciones secundarias a la EICHc. Además, la mayoría de los pacientes quedan finalmente incluidos dentro del grupo de EICHc extenso.

Diversos estudios han intentado identificar variables con valor pronóstico y se han descrito, entre estas variables, los cambios cutáneos liquenoides, la

Tabla 1: Clasificación de la EICH en aguda o crónica

Categoría	Momento de aparición	Síntomas característicos de EICHa	Síntomas característicos de EICHc
EICHa			
Clásica	< 100 días postrasplante	Sí	No
Persistente, recurrente o tardía	> 100 días postrasplante	Sí	No
EICHc			
Clásica	Sin límite temporal	No	Sí
Mixto ó compuesto	Sin límite temporal	Sí	Sí

afectación cutánea extensa, bilirrubina sérica elevada, EICHc progresiva, trombocitopenia, antecedentes de EICHa refractaria/dependiente de corticoides entre otras[5,6,52,53]. Akpek y col.[6] desarrollaron una clasificación con valor pronóstico basada en el análisis de 151 pacientes sometidos a TPH de médula ósea basada en 3 factores: afectación cutánea extensa (>50% de la superficie corporal), trombocitopenia (plaquetas <100 x 10^9/L) y EICHc progresiva. La probabilidad de supervivencia a los 3 años en los pacientes de bajo riesgo (presencia de 1 factor) fue del 92%, para los de riesgo intermedio (2 factores) del 71% y para los de alto riesgo (presencia de los 3 factores) 9%.

Tabla 2. Clasificación de la EICHc en limitada o extensa

EICHc limitada (1 y/o 2)	1. Afectación cutánea localizada (< 50 % de la superficie corporal) y/o 2. Afectación hepática limitada debido a EICHc (alteración de las pruebas de función hepática con bilirrubina total < 3 mg/dl)
EICHc extensa (1 ó 2 + a ó b ó c ó d ó e)	1. Afectación cutánea extensa (≥ 50 % de la superficie corporal), o 2. Afectación cutánea y/o hepática limitada a- Afectación ocular (test de Schirmer < 5 mm) b- Biopsia de mucosa oral o glándula salivar com histología diagnóstica de EICHc c- Biopsia hepática com histología compatible com EICHc (hepatitis crónica agresiva, puentes de necrosis, cirrosis hepática) con bilirrubina total ≥ 3 mg/dl d- Alteración pulmonar compatible con bronquiolitis obliterante sin evidencia de causa viral en el estudio histológico e- Afectación intestinal: malaabsorción y/o pérdida de peso > 15% debido a anorexia, sin causa evidente excepto la presencia de EICHc.

Tras tratamiento de primera línea, además de los factores anteriores, también el estado general del paciente (Karnofsky <50%) permitía distinguir a los pacientes con un peor pronostico.

Lee y col.[54] tras analizar más de 1500 pacientes del International Bone Marrow Transplant Registry (IBMTR) y el National Marrow Donor Program (NMDP) establecieron una clasificación pronóstica en función del estado general del paciente (Karnofsky < o ≥80), presencia de diarrea crónica, pérdida de peso, afectación cutánea y oral como se observa en la tabla 3.

Tabla 3. Modelo pronóstico de EICHc propuesto por Lee y col.

Indice Karnofsky*	**Factores pronósticos**	**Nº de factores presentes**	**Grupo de riesgo**	**SLE a 5 años**
≥80%	Diarrea	0	Bajo	72%
	Pérdida de peso	1	Intermedio	52%
		2	Alto	12%
<80%	Diarrea	0	Intermedio	52%
	Pérdida de peso	1 ó 2 + afección oral { Sí →	Intermedio	52%
	Afectación	{ No →	Alto	12%
	cutánea	3	Alto	12%

* Al diagnóstico de la EICHc. SLE- supervivencia libre de enfermedad.

Sin embargo, la mayoria de estos estudios se realizaron en pacientes sometidos a trasplante de progenitores hematopoyéticos de médula ósea.

El uso de progenitores hematopoyéticos de sangre periférica ha modificado tanto la incidencia como las características de la EICHc, de manera que los factores asociados a una mayor morbimortalidad también pueden ser diferentes en este grupo de pacientes. Pavletic y col.[55] en un estudio sobre pacientes que habían recibido TPHSP identificaron la cifra de plaquetas <100 x 10^9/L y el antecedente de EICH agudo hepático como factores pronósticos adversos.

Más recientemente, la clasificación establecida por el National Institute of Health (NIH) Consensus Development Project[3] define criterios mínimos para el diagnóstico de la EICHc y establece grupos pronósticos que permiten perfilar el tratamiento de acuerdo con el riesgo del paciente. Así, se definen los siguientes criterios:

- ***Diagnósticos***: aquellos signos o síntomas que permiten establecer el diagnóstico de EICHc sin más estudios adicionales.

- ***Distintivos o característicos***: las manifestaciones típicas de EICHc que normalmente no aparecen en el contexto de EICH aguda pero que no permiten establecer un diagnóstico definitivo sin pruebas adicionales.
- ***Otros criterios***: incluyen las manifestaciones raras, controvertidas o inespecíficas que no pueden utilizarse para establecer el diagnóstico de EICHc.
- ***Comunes***: las manifestaciones que aparecen indistintamente en la EICH aguda y crónica.

Aunque se recomienda tener una confirmación histológica para establecer el diagnóstico, esta no es obligatoria si el paciente presenta al menos un criterio "***diagnostico***" (tabla 4).

A cada órgano se le asigna una puntuación en una escala de 0 a 3 según el grado de severidad de la afectación por EICHc (tabla 5) y de este modo, la EICHc se divide en 3 categorías:

1- Leve: afectación de uno o dos órganos (excepto el pulmón) con una puntuación máxima de 1;

2- Moderada: 3 o más órganos afectados con una puntuación máxima de 1 o siempre que un órgano tenga una puntuación de 2 (salvo el pulmón en el que es suficiente una puntuación de 1);

3- Grave: cuando al menos un órgano alcanza una puntuación de 3 o bien el pulmón alcanza una puntuación de 2.

Las formas moderada y grave requieren tratamiento inmunosupressor sistémico, mientras la forma leve puede manejarse con tratamiento local/tópico. El tiempo medio dedicado a la exploración del paciente para realizar una evaluación completa sería de 36 minutos con 14 minutos adicionales dedicados a completar los cuestionarios de auto-valoración por el paciente. El NIH recomienda la utilización de formularios de evaluación que se encuentran disponibles en www.asbmt.org/GvHDforms (apéndices A a D). Para cada órgano se propone una medición objetiva que permita cuantificar el grado de respuesta.

Tabla 4: Criterios diagnósticos de EICHc

Criterios:	Diagnóstico	Característico	Otros	Común con EICHa
Piel	Poiquilodermia; liquen plano Cambios escleróticos Morfea, liquen escleroso	despigmentación	Alt sudación ictiosis Queratosis pilaris Hipo / hiperpigmentación	Eritema Rash maculopapular prurito
Uñas		Distrofia Estrías longitudinales Uñas quebradizas Onicolisis Pterigium ungueal pérdida ungueal		
Cuero cabelludo		Alopecia cicatricial Lesiones descamativas Lesiones papulo-escamosas	Fragilidad capilar, Encanecimiento prematuro	
Boca	Lesiones liquenoides Placas hiperqueratósicas (leucoplaquia) esclerosis	Xerostomía, mucocele, atrofia mucosa pseudomembranas, ulceras		Gingivitis Mucositis Eritema dolor
Ojos		Sequedad, dolor, conjuntivitis cicatricial, queratoconjuntivitis seca (requiere Schirmer)	Fotofobia, hiperpigmentación periorbital blefaritis	
Genitales	Liquen plano, cicatrices o estenosis vaginal	Erosión, fisuras, úlceras		
Digestivo	Membrana esofágica Estenosis hasta 1/3 medio (documentados por endoscopia o contraste)		Insuficiencia pancreática exocrina	Anorexia, nauseas, vómitos, diarrea, pérdida de peso, retraso crecimiento (niños)
Hígado				Bilirrubina total, fosfatasa > 2 veces el límite superior de la normalidad (N) AST ó ALT > 2 x N
Pulmonar	Bronquiolitis obliterante (BO) diagnosticada por biopsia	BO diagnosticada por espirometría y radiología		Bronquiolitis obliterante con neumonía organizada
Muscular, fascia	Fascitis, rigidez o contracturas articulares secundarias a esclerosis	Miositis ó polimiositis (requiere biopsia)	edema, calambres, artralgia, artritis	
Hematológico e inmunológico			Trombocitopenia, eosinofilia, linfopenia, hipo/hipergammaglobulinemia, autoanticuerpos	
Otros			Ascitis, derrame pleural o pericárdico, neuropatía, síndrome nefrótico, miastenia gravis, alt conducción a nivel cardíaco, miocardiopatía	

Tabla 5: Puntuación asignada a cada órgano para evaluar la severidad de la EICHc

	Puntuación: 1	Puntuación: 2	Puntuación: 3	Puntuación: 4
Estado general	Asintomático ECOG 0 Karnofsky 0	Sintomático; paciente ambulatorio, limitado unicamente con actividad intensa ECOG 1 Karnofsky 1	Sintomático, paciente ambulatorio, capaz de llevar a cabo medidas elementales de higiene personal ECOG 2 Karnofsky 2	Sintomático, capacidad limitada para llevar a cabo medidas de higiene personal ECOG 3 Karnofsky 3
Piel Rash maculopapuloso Similares a liquen plano, Ictiosis o lesiones papuloescamosas Hiperpigmentación Hipopigmentación Queratosis pilaris Eritema, Eritrodermia Poiquilodermia Cambios escleróticos Prurito Afectación capilar Afectación ungueal % SCA	Asintomático Sin hallazgos a la exploración	<18% SCA pero sin signos de esclerosis	19-50% SCA o lesiones superficiales escleróticas (la rigidez permite "pellizcar" la piel)	> 50% SCA o cambios escleróticos profundos (no se puede "pellizcar" la piel) o alteración de la movilidad ulceraciones o prurito severo
Boca	Asintomático	Sintomatología leve con signos de enfermedad en mucosa pero que no limita la ingesta	Sintomatología moderada con signos de enfermedad en mucosa y limitación parcial de la ingesta oral	Sintomatología severa con signos de enfermedad que limitan la ingesta
Ojos Schirmer > 10 mm 6-10 mm < 5 mm	Asintomático	Síntomas leves de ojo seco que no afectan la AC (requiere gotas oculares ≤ 3 / día) ó asintomático con signos de QS	Síntomas moderados que afectan parcialmente la AC (gotas > 3 / día) sin afectación de agudeza visual	Síntomas severos que afectan la AC ó incapacidad para trabajar debido a la sintomatología ocular ó pérdida de visión causada por QS
Tubo digestivo	Asintomático	Disfagia, anorexia, nauseas, vómitos, dolor abdomial o diarrea sin pérdida significativa de peso (< 5%)	Síntomas asociados a pérdida moderada de peso (5-15%)	Síntomas asociados a pérdida de peso > 15%, requiere aporte nutricional ó dilatación esofágica
Hígado	PFH normales	Alteración PFH < 2 x LSN	Bilirrubina > 3 mg/dL o enzimas hepáticos 2-5 x LSN	PFH > 5 x LSN
Pulmones FEV1 DLCO	Asintomático FEV1 > 80% ó PFP	Síntomas leves (disnea tras subir un piso de escaleras) FEV1 60-79% ó PFP 3-5	Síntomas moderados (disnea tras caminar en llano) FEV1 40-59% ó PFP 6-9	Síntomas graves (disnea de reposo) FEV1 < 39% ó PFP 10-12
Articulaciones y músculos	Asintomático	Tirantez en brazos o piernas, movilidad articular normal o levemente disminuida que no afectan la AC	Tirantez en brazos o piernas o contracturas articulares, eritema debido a fascitis, movilidad articular afectada moderadamente que limita la AC de manera leve o moderada	Contracturas con afectación severa de la movilidad articular que afecta severamente la AC (incapaz de atarse los zapatos, vestirse, etc)
Tracto genitourinario	Asintomático	Sintomático con signos de afectación leve que no afectan el coito; mínimas molestias a la exploración ginecológica	Sintomático con signos moderados de afectación a la exploración con dispareunia leve o molestias a la exploración	Sintomático con signos severos de afectación (estenosis, ulceración) con dispareunia severa o imposibilidad de introducir un espéculo ginecológico

Otros indicadores, manifestaciones clínicas o complicaciones relacionadas con EICHc (indicar puntuación 0 a 3 según que el grado de afectación sea leve, moderado o severo): Membrana, esofágica, Derrame pericárdico, Derrame pleural, Ascitis, Síndrome nefrótico, Neuropatía periférica, Miastenia gravis, Miocardiopatía, Eosinofilia > 0.5 x 10^9/L, Polimiositis, Alteraciones en la conducción, Afectación arteria coronaria, Plaquetas < 100x10^9 / L, Comienzo progresivo, Otros

SCA: superficie corporal afectada; AC: actividades cotidianas; PFH: pruebas de función hepática (bilirrubina, fosfatasa alcalina, transaminasas); QS: queratoconjuntivitis seca; LSN: límite superior de la normalidad; PFP: pruebas de función pulmonar (al FEV1 y DLCO se les asigna un valor de acuerdo al siguiente criterio: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6).

Para valorar la respuesta al tratamiento el *Response Criteria Working Group* propone los siguientes criterios:

- **Respuesta completa:** resolución de todas las manifestaciones reversibles de EICHc en todos los órganos afectos
- **Respuesta Parcial:** Mejoría de al menos el 50% en la escala usada para medir las manifestaciones clínicas de EICHc en un órgano afecto, sin empeoramiento de los demás
- **Progresión:** Empeoramiento de al menos un 25% en la escala usada para medir las manifestaciones en alguno de los órganos afectos sin mejoría en otros órganos.

Se consideran como cambios no reversibles, y por tanto no deben considerarse como ausencia de respuesta la persistencia de: ojos secos, bronquiolitis obliterante, lesiones escleróticas avanzadas y estenosis esofágica.

De acuerdo con el tipo de inicio de las manifestaciones clínicas la EICHc se clasifica en:

1. **Progresiva**: en el caso de pacientes que siguen recibiendo tratamiento con prednisona o ciclosporina A en dosis terapéutica debido a la EICH aguda y que evolucionan hacia EICHc sin resolución de los síntomas.
2. **Quiescente:** pacientes con antecedentes de EICHa con resolución de los síntomas o sin inmunosupresión en el momento del diagnostico de EICHc.

3. ***de* Novo:** aparición de EICHc en pacientes sin antecedentes de EICHa previo.

Varios estudios retrospectivos han intentado evaluar el valor pronóstico de la nueva clasificación propuesta por el NIH. En nuestra experiencia, basados en una serie de 171 pacientes sometidos a TPHSP de donante emparentado, la incidencia acumulada de EICHc leve, modera y severa es del 29%, 42% y 28%. Globalmente a los 5 años pos-trasplante el 68% de los pacientes no recibe tratamiento inmunosupresor y la ausencia de EICHa (HR = 2; p = 0,004) y el EICHc leve (HR = 4,2: p = 0,007) permiten identificar pacientes en los que, con mayor probabilidad, se podrá suspender el tratamiento inmunosupresor. Además, la presencia de EICHc severa tiene un efecto desfavorable en la supervivencia global (HR = 13,27; p = 0,001) mientras que, el tipo de comienzo *de novo* (HR = 0,094; p = 0,003) tiene un valor pronóstico favorable, de manera que la combinación de ambos factores (grado de severidad de la EICHc de acuerdo con la clasificación del NIH y el tipo de comienzo) permite identificar claramente 4 subgrupos de pacientes con supervivencia global del 82, 70, 50 y 25%, respectivamente[56].

1.2.4 Factores de riesgo

Se calcula que la incidencia global de la EICHc es de 22 a 80%, dependiendo su incidencia de la presencia o no de los factores de riesgo expuestos en la tabla 5. En este sentido, en los últimos años se constata un incremento en su incidencia debido al aumento del número de trasplantes de donantes no emparentados o con disparidades HLA entre donante y receptor, el aumento de TPH en pacientes de mayor edad, el aumento de los TPHSP y del empleo de ILD, así como el aumento de la supervivencia de los pacientes con complicaciones peri o postrasplante.

El factor de riesgo más importante para el desarrollo de EICHc es el antecedente de EICHa, de manera que el 25-35% de los pacientes sin EICHa previa desarrollan EICHc (*de novo*)[57] frente al 60% de incidencia entre los pacientes con EICHa grado I y el 80% en los pacientes con EICHa grados II-IV. Otro importante factor de riesgo para la aparición de la EICHc es la disparidad

Tabla 6. Factores de riesgo para el desarrollo de EICHc

EICH aguda
Disparidad HLA
Donante no emparentado
Edad avanzada
Sexo (donante mujer → receptor hombre)
Fuente de células progenitoras (CPSP>MO>SCU)
Depleción de células T (DLT →↓EICHa →↓EICHc)
Dosis de células $CD34^+$ infundidas (TPHSP)
Infusión de linfocitos del donante (ILD)

HLA entre donante y receptor, de manera que aproximadamente un 40% de los pacientes que reciben un trasplante de donante emparentado HLA idéntico, el 50% de los pacientes sometidos a trasplante de donante emparentado con disparidad HLA y hasta un 70% de los que reciben un trasplante de donante no emparentado desarrollaran esta complicación[58-59]. No obstante, a pesar de la clara relación causal entre disparidad HLA y EICH tanto aguda como crónica, no se conoce claramente qué disparidad específicamente incrementara el riesgo de aparición de una u otra.

El sexo y la edad también influyen en la incidencia de EICHc, de modo que en los pacientes varones con donante mujer el riesgo de EICHc es mayor e incluso se relaciona con un mayor riesgo de bronquiolitis obliterante (BO), lo que atribuye a la aparición de anticuerpos frente a antígenos menores codificados en el cromosoma Y[44]. Por otro lado, existe una relación directamente proporcional entre la edad e incidencia de EICHc, de modo que entre los 10 y 20 años su incidencia es de 13% mientras que en los mayores de 20 años su incidencia se incrementa hasta un 40% en pacientes sometidos a trasplante de donante emparentado HLA idéntico[58]; también la edad del donante influye en el riesgo de EICHc por lo que es un factor importante a tener en cuenta a la hora de seleccionar el mejor donante[60].

En relación a la fuente de progenitores hematopoyéticos, varios estudios han confirmado una mayor incidencia de EICHc extenso en los pacientes que reciben CPHSP[4]. Además, en estos pacientes la EICHc puede ser más resistente al tratamiento en comparación con los que reciben un trasplante de progenitores hematopoyéticos de médula ósea y por tanto, la duración del

tratamiento immunosupresor es mayor[61].

La depleción de linfocitos T (DLT) del inóculo disminuye de manera significativa la incidencia y severidad tanto de la EICH aguda como crónica. Los métodos de eliminación de linfocitos T del inóculo pueden ser: **1.** por procedimientos de manipulación *ex vivo* que reducen el contenido de linfocitos T del inóculo; **2**. DLT *in vivo* mediante el uso de globulina antitimocítica (ATG) ó alemtuzumab (CAMPATH-1H). En un estudio prospectivo, randomizado y multicéntrico en pacientes sometidos a TPH de DnE comparando la profilaxis estándar con ciclosporina A (CyA) y metotrexate (MTX) con o sin globulina antitimocitica se verificó en el grupo de pacientes que recibieron ATG una menor incidencia de EICHa grado II-IV (33% vs 51%) y de EICHc extensa (12% vs 42%) si bien no hubo diferencias significativas entre ambos grupos en terminos de mortalidad o supervivencia global[62]. El uso de alemtuzumab también ha demostrado su eficacia. En un estudio comparativo retrospectivo en pacientes que recibieron acondicionamiento de intensidad reducida, los pacientes que recibieron alemtuzumab presentaron una incidencia de EICHc extensa del 5% frente al 66% entre los pacientes que recibieron MTX ($p < 0,001$)[63].

También la cantidad de progenitores CD34+ es otra variable que influye en el riesgo de EICHc. En este sentido, la infusión de un número elevado de $CD34^+$ (generalmente > 8 x 10^6/kg) aumenta el riesgo de EICHc extensa en los pacientes que reciben sangre periférica como fuente de progenitores hematopoyéticos[64,65]; sin embargo, en los pacientes que reciben progenitores de médula ósea la dosis de $CD34^+$ no influye en el riesgo de desarrollar EICHc[66,67]. En un estudio multicentrico se analizaron prospectivamente 932 pacientes registrados en el NMDP y que habían sido sometidos a trasplante de CPHSP de donante no emparentado bajo acondicionamiento mieloablativo (n=611), intensidad reducida (n=160) y no mieloablativo (n=161) se verificó que dosis elevadas de $CD34^+$ infundidas (> 4,5 x 10^6/kg) no aumentaba el riesgo de desarrollar EICHa ni EICHc[68].

1.2.5 Tratamiento de la EICHc

Diversos estudios han evidenciado la relación existente entre la EICHc y el efecto injerto contra leucemia, de forma que el desarrollo de EICHc puede tener un efecto favorable en la supervivencia[31,69,70]. Por tanto, al definir la estrategia de tratamiento inmunosupresor es muy importante tener en cuenta el grado de severidad de la EICHc y el riesgo de recaída del paciente.

El tratamiento de primera línea para la EICHc consiste en la combinación de inhibidor de calcineurina (CyA o tacrolimus) y prednisona. En un estudio en pacientes de alto riesgo (aquellos con EICHc extenso y trombocitopenia) la supervivencia a los 3 años fue del 26% cuando se empleo prednisona como agente único[61]. Al añadir ciclosporina A a la prednisona en este subgrupo de pacientes la supervivencia aumenta al 52%[71], mientras que en los pacientes con riesgo estándar la adición de ciclosporina disminuye los efectos secundarios del esteroide pero no mejora la supervivencia de los pacientes[72]. Uno de los esquemas más utilizados es el descrito por el grupo de Seattle en el cual se emplea prednisona a 1 mg/kg/día y ciclosporina A a 10 mg/kg/día dividida en 2 dosis de acuerdo al peso ideal o actual del paciente. Tras dos semanas de tratamiento y una vez confirmada la ausencia de progresión de la EICHc, se inicia un descenso de la prednisona del 25% por semana hasta administrar 1 mg/kg a días alternos. La evaluación de la respuesta al tratamiento se realiza a las 8, 20 y 40 semanas. En caso de respuesta a la semana 20 se continúa el descenso de prednisona hasta alcanzar 0,5 mg/kg a días alternos seguido de un descenso de la dosis de ciclosporina. Sí a las 40 semanas alcanza remisión completa se realiza un descenso paulatino hasta suspender ambos fármacos. La adición de otras drogas o sustitución de estos fármacos dentro de la primera línea de tratamiento no ha ofrecido ninguna ventaja, hasta la actualidad. En un estudio reciente doble ciego, randomizado y multicéntrico con 230 pacientes, que analizaba la eficacia del mofetil micofenolato asociado al tratamiento de primera línea no hubo diferencias significativas entre los grupos de pacientes que recibían mofetil micofenolato y los que no, por lo que no recomiendan su asociación al tratamiento

inmunosupresor sistémico inicial[73].

Para los pacientes que no responden a la primera línea de tratamiento, no existe ninguna opción terapéutica que pueda considerarse estándar de manera que siempre que sea posible estos pacientes deben incluirse en ensayos clínicos controlados. Al no existir un orden preestablecido entre las distintas opciones terapéuticas y en ausencia de ensayos clínicos activos la estrategia terapéutica debe escogerse de manera individualizada en función de las características de cada paciente. Algunas de las opciones terapéuticas se indican a continuación:

a) Sirolimus: es un macrólido con propiedades antifungica, antitumoral e inmunosupresora que actúa uniéndose a la proteína de unión de FK, inhibiendo la ruta de activación linfocitaria de mTOR (mamalian target of rapamycin). Como tratamiento de rescate en una serie de 35 pacientes con EICHc resistente se obtuvo un 63% de respuestas globales incluyendo 6 remisiones completas y 16 respuestas parciales[74]. En otra serie de 47 pacientes, Jurado y col describen un 81% de respuestas, incluyendo 18 y 20 pacientes con respuestas completa y parcial respectivamente. Los efectos adversos son bastante comunes e incluyen alteración de la función renal, citopenias, síndrome hemolítico urémico, hipertrigliceridemia e hipercolesterolemia[75].

b) Micofenolato mofetil: es un antimetabolito que inhibe la inosina monofosfato deshidrogenasa interfiriendo en la síntesis de purinas y, en consecuencia, inhibe la proliferación linfocitaria. En una serie de 34 pacientes se constató un 35 y 44% de remisiones completas y parciales, respectivamente[76].

c) Rituximab: Cutler y cols.en una serie de 21 pacientes, describen un 70% de respuestas observadas principalmente a nivel cutáneo y músculo-esquelético, incluyendo 2 respuestas completas[77]. En otro estudio, con 38 pacientes con EICHc refractaria el Grupo Italiano de Trasplante reporta un 65% de respuestas globales (63% en piel, 48% en boca, 43% en ojos y 25% en hígado)[78]. Un reciente estudio prospectivo multicéntrico con 37 pacientes con EICHc refractaria se constató un 86% de respuestas principalmente a nivel cutáneo, oral y músculo-esquelético siendo 8 de ellas remisiones completas y

y 24 respuestas parciales; además en el 57% de los pacientes fue posible reducir o suspender los corticoides[79].

d) Fotoaféresis extracorpórea: aunque su mecanismo de acción no está completamente establecido, se sabe que induce apoptosis en las células presentadoras de antígenos y linfocitos T, tiene un efecto modulador sobre la producción de citocinas y favorece la expansión de células T reguladoras produciendo inmunotolerancia[80,81]. Se han descrito respuestas globales de 50 a 80% y una tasa de reducción o suspensión del tratamiento inmunosupresor en el 80% de los pacientes[82].

e) Pentostatina: es un análogo de nucleósidos con un potente efecto inhibitorio sobre la adenosina deaminasa, bloqueando el metabolismo de la 2`-deoxiadenosina. Aunque en el contexto de EICHc la experiencia es más limitada, en algunos estudios piloto se ha observado cierta eficacia en pacientes con EICHc refractaria a corticoesteroides y con un perfil de toxicidad aceptable. Jacobson y cols reportan en una serie de 58 pacientes con EICHc refractaria que recibieron pentotastina a dosis de 4 mg/m^2/día cada 2 semanas hasta completar ≥ 12 dosis un 55% de repuestas con una supervivencia del 70% a dos años[83].

f) Otras opciones terapéuticas

Imatinib

El Imatinib, un inhibidor de la tirosin kinase de 1ª generación, inhibe la señalización intracelular del TGFβ y PDGF, factores que están involucrados en los cambios fibróticos/escleróticos que tienen lugar en estos pacientes. Se han descrito hasta un 79% de respuestas (7 remisiones completas y 8 respuestas parciales) a los 6 meses tras inicio del imatinib a dosis de 100 mg/día en una serie de 19 pacientes con EICHc refractaria a por lo menos 2 líneas de tratamiento, incluyendo pacientes con afectación pulmonar severa[84].

Células mesenquimales

Estas células presentan un efecto inmunomodulador, inhibiendo la activación y proliferación de linfocitos T además de interferir en la diferenciación, maturación y función de las células dendríticas[85]. Por este motivo se ha utilizado en el tratamiento de la EICHa resistente a corticoides con

resultados favorables[86]. Sin embargo, la experiencia en EICHc aún es muy limitada.

Otros fármacos como la talidomida, hidroxicloroquina, daclizumab entre otros también se han utilizado con resultados variables.

Vitamina D

La vitamina D (vit D) es una prohormona liposoluble y sus principales formas son el ergocalciferol (vitamina D_2) y el colecalciferol (vitamina D_3). Las principales fuentes de vit D son el sol, los alimentos y suplementos. Su forma activa es el 1,25-hidroxicolecalciferol que se sintetiza en los riñones a partir de la forma circulante en la sangre 25-hidroxicolecalciferol que a su vez se forma en el hígado a partir de la vitamina D_2 o D_3. La vit D es importante para el desarrollo del esqueleto, masa ósea, función neuromuscular y en asociación con el calcio se utiliza para la prevención y tratamiento de la osteoporosis y osteopenia[87]. El receptor nuclear de la vit D (VDR) es expresado en varios tejidos y células del organismo incluyendo las células mononucleares periféricas (monocitos y macrofagos activados, células dendriticas, células NK, linfocitos T y B)[88]. Muchas de estas células tienen la capacidad de convertir la 25-hidroxicolecalciferol en 1,25 hidroxicolecalciferol. Estudios in vitro indican que la vitamina D inhibe la activación y proliferación de las células T dependientes de células dendríticas y disminuye la producción de citocinas producidas por las células Th1 como IL-2, IFNγ and TNFα lo que explica su efecto inmunomodulador[89-91].

Tratamiento tópico

El tratamiento tópico es una opción útil y siempre que sea posible e indicado se recomienda su uso. Entre los fármacos con acción tópica destacamos el dipropionato de beclometasona (BDP), un corticoesteroide con una débil afinidad de unión por receptores de glucocorticoides y que es metabolizado a nivel de la mucosa intestinal e hígado. Su metabolito activo, el 17-monopropionato de beclometasona (17-BMP), presenta una mayor afinidad a los receptores de glucocorticoides y es detectado en la circulación sistémica.

Debido a la absorción incompleta, a la hidrólisis intestinal por enzimas esterasas y a su rápido aclaramiento de la circulación reduce de forma considerable los efectos sistémicos. Se administra por vía oral y ejerce su efecto antiinflamatorio a nivel local sobre la mucosa intestinal. Se ha mostrado eficaz en el tratamiento de la EICH aguda gastrointestinal permitiendo evitar o reducir la exposición a los corticoides sistémicos[92-94]. La información disponible sobre el uso de la beclometasona en la EICHc gastrointestinal es escasa.

Medidas de soporte

También son importantes las medidas de soporte y las recomendaciones higiénico-dietéticas propuestas por el NIH Consensus Development Project[95]. Estas recomendaciones incluyen la educación del paciente, medidas preventivas, seguimiento adecuado de estos pacientes, prevención y tratamiento de las infecciones, principalmente frente a *Pneumocistis jiroveci* y microorganismos encapsulados mientras dure el tratamiento inmunosupresor, así como un adecuado manejo de las complicaciones derivadas del tratamiento para la EICHc y el manejo de los efectos neurocognitivos y psicosociales relacionados con la EICHc.

Es fundamental que un equipo multidisciplinar haga en manejo a largo plazo del paciente trasplantado, sobre todo cuando presenta EICH crónica.

HIPÓTESIS DE TRABAJO Y OBJETIVOS

2. HIPÓTESIS DE TRABAJO Y OBJETIVOS

2.1 Hipótesis de trabajo

La clasificación propuesta por el NIH junto con otros factores presentes en el momento del diagnostico de la EICH crónica permitirán establecer grupos de pacientes con diferente pronóstico. En este sentido, la reevaluación del día + 100 postrasplante podría ser de utilidad para identificar pacientes a riesgo de desarrollar formas severas de EICHc. Finalmente, en los pacientes con formas leves o moderadas de EICHc es posible controlar los síntomas de la enfermedad mediante el uso de fármacos de uso tópico u otros, sin efecto inmunosupresor sistémico.

2.2 Objetivos

1. Análisis de factores pronósticos en el contexto de la enfermedad injerto contra huésped crónica:
    a. Evaluación de forma retrospectiva de la clasificación NIH e identificación de otros factores pronósticos.
    b. Análisis retrospectiva del screening del día +100 como factor predictor de EICHc

2. Estudio prospectivo y retrospectivo de nuevas estrategias terapéuticas en la EICHc
    a. Tratamiento de la EICHc digestiva con beclometasona
    b. Papel de la vitamina D en el control de la EICHc

PACIENTES Y MÉTODOS

3. Pacientes y Métodos

3.1 Pacientes

Todos los sujetos incluídos en el presente trabajo de tesis son pacientes consecutivamente sometidos a trasplante alogénico de progenitores hematopoyéticos en la Unidad de Trasplante del Servicio de Hematología del Hospital Universitario de Salamanca entre el 01/01/1998 y el 31/12/2008. Durante este periodo de tiempo se llevaron a cabo 351 trasplantes alogénicos. A continuación se describen las caracteristicas de los pacientes incluídos en cada uno de los estudios llevados a cabo en el contexto del presente trabajo de tesis. Obviamente muchos de los pacientes han podido ser incluidos en más de uno de estos trabajos.

3.1.1 Pacientes en el estudio titulado "Prognostic factors of chronic graft-versus-host disease following allogeneic peripheral blood stem cell transplantation: the national institutes of health scale plus the type of onset can predict survival rates and the duration of immunosuppressive therapy"

Características de los pacientes al trasplante

	N=171
Edad: mediana (rango)	45 (14-6 años)
Diagnóstico	
LAM	44 (25,7%)
LAL	24
MM	21
LNH	20
SMD	19
LMC	14
LLC	10
EH	9
SMP/SMD	6
Aplasia Medular	2
Outros	2
Estado de la enfermedad al trasplante*	
Bajo riesgo	63 (37%)
Riesgo intermedio	75 (43%)
Alto riesgo	33 (20%)
Sexo**	
Masculino	100
Femenino	71
Acondicionamiento	
Mieloablativo	68 (39,7%)
Intensidad reducida	103
Profilaxis de EICH	
CsA + MTX	171
Fuente de progenitores hematopoyéticos	
SP	171
Índice de comorbilidad***	
Bajo	113 (66%)
Intermedio / bajo	37
Intermedio / alto	10
Alto	11
CD34 infundidas x 10^6 / kg	5.1 (1.9 – 13.2)

CsA: ciclosporina A; EH: enfermedad de Hodgkin; EICH: enfermedad injerto contra huésped; LAM: leucemia aguda mieloblástica; LAL: leucemia aguda linfoblástica; MM: mieloma múltiplo; LNH: linfoma no Hodgkin; SMD: síndrome mielodisplásico; LMC: leucemia mieloide crónica; LLC: leucemia linfoide crónica; MTX: metotrexate; SMD/SMP: síndrome mielodisplásico/síndrome mieloproliferativo; SP: sangre periférica; Otros: 1- sindrome hipereosinofílico, 2- leucemia aguda bifenotípica.
(*) Bajo riesgo: 1ª respuesta completa o fase crónica; Alto riesgo: recaída, progresión, crisis blástica; Riesgo Intermedio: las restantes.
(**) 39 varones recibierom CPSP de donantes mujeres
(***) Índice de comorbilidad de Charlson

3.1.2 Pacientes en el estudio titulado "Liver function tests and absolute lymphocyte count at day +100 are predictive factors for extensive and severe chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplant"

Características de los pacientes al trasplante e incidencia de EICHc

	n=165
Edad: mediana (rango)	49 (14-69 años)
Diagnostico	
LAM	41 (24,8%)
LAL	19
MM	25
LNH	24
Otros	56 (34%)
Sexo	
Masculino / femenino	100 / 65
Regimen de acondicionamiento	
Mieloablativo / AIR	56 / 109 (66%)
Profilaxis de EICH	
CsA + MTX	165
CD34 infundidas x 10^6 / kg	5.2 (0.8 – 13.2)
EICHc	
Sí / No	86(52%) / 79
Tipo de EICHc	
Limitado / extenso	23 / 63 (73,2%)
Grado de severidad de EICHc (critérios del NIH)	
Leve	27 (31,4%)
Moderado	40
Severo	19
Tipo de inicio	
De novo/ quiescente	49 / 37 (43%)
Órganos afectados	
Boca	
Leve / moderado	61 / 5
Piel	
Leve / Moderado / Severo	37 / 10 / 3
Hígado	
Leve / Moderado / Severo	31 / 9 / 7
Ojos	
Leve / Moderado / Severo	33 / 3 / 1
Tracto gastrointestinal	
Leve / Moderado / Severo	2 / 6 / 1
Pulmón	
Leve / Moderado / Severo	4 / 7 / 1
Músculos / articulaciones	
Leve / Moderado	4 / 2
Genitales	
Leve / Moderado	5 / 2
Riñon	
Leve / Moderado	2 / 2
Pacientes con EICHc bajo tratamiento inmunosupresor sistémico	73,2%
Cifras al diagnóstico de la EICHc	
Plaquetas: Mediana (rango) x 10^9 /L	169 (18-482)
Pacientes con plaquetas $< 100 \times 10^9$ /L	32 (19,4%)
Eosinofilos: Mediana (rango) x 10^9 /L	0,2 (0-3,550)

3.1.3 Pacientes en el estudio titulado "Oral beclomethasone dipropionate for the treatment of gastrointestinal chronic graft-versus-host disease"

Características de los pacientes al trasplante

	(N=33)
Edad: mediana (rango)	33 (18-56 años)
CD34 infundidas x 10^6 / kg:	
mediana (rango)	5.35 (1-6.26)
Diagnostico:	
LAM	11
LAL	3
LMC	2
LLC	3
SMD	4
LNH	6
EH	1
MM	2
Otras	1
***Status* de la enfermedad al trasplante**	
1ª RC	15
≥2ª RC	5
Fase crónica	2
Respuesta parcial	6
Enfermedad refractária / progresiva	2
Sin tratamiento previo	2
Otros	1
Sexo:	
Masculino / femenino	20 / 13
Tipo de donante	
Emparentado	21
No emparentado	11
Cordón umbilical	1
Regimen de acondicionamiento	
Mieloablative	10
Intensidad reducida	23
Profilaxis de la EICH	
CsA + MTX	25
ATG o CAMPATH 1H	4
CsA + MMF	4

3.1.4 Pacientes en el estudio titulado "Effect of vitamin D in the treatment of chronic graft-versus-host disease"

Caracteristícas de los pacientes al trasplante

	Numero de pacientes (N)=12
Diagnóstico	
LMA	3
LLC	3
LMC	1
SMD	3
LNH	1
EH	1
***Status* de la enfermedad al transplante**	
1ª RC	4
≥ 2ª RC	3
RP	2
Progresión	3
Acondicionamiento	
Intensidad reducida	8
Mieloablativo	4
Tipo de donante	
Emparentado	9
No emparentado	3
CD34 infundidas x 10^6 / kg	
mediana (rango)	5,8 (3,5 – 11,3)
Profilaxis de la EICH	
CsA + MTX	11
CsA + MMF	1
Edad: mediana (rango)	46 (23 – 68 años)
Sexo: masculino / femenino	1 /11
Tratamiento IS al inicio de vitD	
CsA	2
CsA/Fk 506 + tópico	8
Fk 506 + Prd + tópico	1
MMF + Prd	1

3.2 Profilaxis de la EICH

Como profilaxis de EICH la mayoría de los pacientes recibió ciclosporina-A (CsA) 0,5 mg/kg cada 12 horas desde el día -7 al -2 y 1,5 mg/kg cada 12 horas a partir del día -1, mas metotrexate(MTX) 15 mg/m^2/día en el día -1 y 10 mg/m^2 en los días +3, +6 y +11, seguido de rescate con acido folinico. El descenso de la CsA se iniciaba alrededor del día +50 y se suspendía en el día +180 en ausencia de manifestaciones de EICH. En los pacientes con enfermedad activa

o enfermedad mínima residual positiva se iniciaba un esquema de descenso rápido de inmunosupresión.

3.3 Evaluación de la EICHc

El día + 100 postrasplante se procedió a la evaluación de los pacientes incluyendo:

*1- Anamnesis y examen físico

Piel, mucosa oral y zona genital, cabello, pelo, uñas, sistema músculo-esquelético, ECOG/Karnofsky. Síntomas y calidad de vida.

*2- Signos vitales / peso / altura

*3- Hemograma

*4-Bioquímica incluyendo pruebas de función hepática y renal, ClCr, magnésio, calcio, fósforo, CPK, aldolasa (sí se sospecha miositis), hierro sérico, ferritina, Ac. antinucleares, proteinograma, galactomanano e antigenemia/PCR de CMV y EBV (esto no se hace sistemáticamente)

5- Serología para virus: VHS, VVZ, CMV, EBV, VHA, VHB, VHC, VIH, toxoplasma

*6- Niveles séricos de CsA/Tacrolimus

7- Cuantificación serica de Inmunoglobulinas

8- Sangre periférica

Frótis (si anomalías en el hemograma)

EMR (citometria de flujo, biologia molecular o citogenetica)

Quimerismo de línea si acondicionamiento de intensidad reducida

9- Médula ósea

Morfologia

EMR (citometria de fluxo, biologia molecular dependiendo de la enfermedad de base)

Quimerismo

10- Reevaluación de la enfermedad de base con TC si linfoma

*11- Biopsias cuando justificado: cutánea, mucosa labial, gastrointestinal, hígado, etc

*12- Test de Schirmer

*13- Pruebas de función respiratória con DLCO
14- Tratamento inmunosupressor actual y respectiva dosis
15- EICHa
Fecha del diagnóstico, órganos afectados, evolución y tratamiento
16- EICHc
Fecha de inicio, órganos afectados, evolución y tratamiento.

***** Indican las exploraciones complementarias que se realizan en caso de sospecha de EICHc para valorar inicio de tratamiento. No es necesaria la realización de ninguna prueba invasiva en la reevaluación del día +100 postrasplante sí no hay justificación clínica dado que hasta el momento no se ha demostrado su valor predictivo.

En los pacientes que desarrollaron EICHc se realizaron reevaluaciones trimestrales. En cada reevaluación se especifica la evolución de los órganos afectados, de acuerdo con los formularios recomendados por el Consensus Project que incluyen ítems para valorar signos y síntomas de los diferentes órganos afectados por la EICHc. Los criterios de respuesta al tratamiento de EICHc son los recomendados por el *Response Criteria Working Group* especificados en el apartado 1.2.3 de esta tesis doctoral.

Todos los pacientes recibieron profilaxis antiinfecciosa de acuerdo con el protocolo del servicio:

1- Profilaxis de las infecciones bacterianas:

Cuando el paciente sometido a trasplante alogénico presenta menos de 500 granulocitos / mm^3:

- Meropenem 1 gr / 8 horas, intravenoso que se mantiene hasta que se recupere la cifra de > 500 granulocitos / mm^3 si el paciente se encuentra afebril.

2. Profilaxis de la infección por Pneumocistis Jiroveci

- Trimetropim (TMP) +Sulfametoxazol (SMX) a dosis de 160 mg de TMP + 800 mg de SMX / 12 horas via oral.

- Pretrasplante: Se inicia el tratamiento en la primera consulta pretrasplante y se suspende 48 horas antes de la infusión de las CPH.

- Postrasplante: cuando la cifra de granulocitos > 1000 / mm^3 x 3 dias reiniciar TMP/SMX a las dosis mencionadas anteriormente 2 días a la semana. El tratamiento se mantendrá hasta los 6 meses postrasplante o bien hasta finalizar el tratamiento inmunosupresor.

En los casos en que no se puede iniciar el tratamiento antes del día +30 y en aquellos en los que el TMP+SMX esta absolutamente contraindicado se inicia tratamiento alternativo con Pentamidina inhalada: 300 mg / 28 días.

3. Profilaxis de las infecciones víricas

- En todos los pacientes seropositivos para virus herpes simplex (VHS) y/ó virus varicela zoster (VVZ): Aciclovir: 800 mg / 12 horas vía oral a partir del primer día del acondicionamiento hasta día +365 postrasplante.

4. Profilaxis antifungica

Inicialmente los pacientes recibian Fluconazol a dosis de 400 mg / 24 horas por vía oral; posteriormente (desde 2001) se inició la profilaxis con Itraconazol suspensión a dosis de 2,5 mg / kg / 12 horas por vía oral. Recientemente se ha utilizado ambisome inhalado o Posaconazol.

3.4 Tratamiento de la EICHc

Mientras que los tipos moderado y grave requieren tratamiento inmunosupresor sistémico el leve puede manejarse con tratamiento tópico y sintomático[3].

Como tratamiento de primera línea para EICHc extensa o moderada/severa todos los pacientes recibieron inhibidor de calcineurina (CsA y en caso de intolerancia tacrolimus) asociado a prednisona a dosis de 1 mg/kg/día. La respuesta al tratamiento se evaluó 4 semanas tras el inicio del tratamiento con corticoides y después cada 3 meses hasta finalizar el tratamiento inmunosupresor. Todos los pacientes recibieron profilaxis antiinfecciosa de acuerdo con el protocolo del servicio.

3.4.1 Beclometasona

El dipropionato de beclometasona (BDP) es un corticoesteroide que es metabolizado en la mucosa intestinal y en hígado por lo que su absorción desde la mucosa intestinal hacia la circulación sistémica es muy reducida. Además, ejerce un efecto antiinflamatorio sobre la mucosa intestinal y tiene escasos efectos secundarios por lo que se ha utilizado en el tratamiento de la EICH digestiva[92-94,96].

En el presente estudio[96] hemos utilizado la beclometasona en pacientes con EICHc con afectación de tubo digestivo sin respuesta al tratamiento de primera línea y/o en aquellos pacientes con EICHc extensa que afecte el tubo digestivo en los que, debido a persistencia de enfermedad mínima residual positiva deba limitarse la administración de tratamiento sistémico. Previamente se descartó la presencia de infecciones bacterianas, viricas, fungicas o por parásitos en el tubo digestivo. La pauta de tratamiento utilizada consistió en la mezcla de la BDP con aceite de oliva a una concentración final de 0,5 mg/ml y se administró en dosis de 4 ml (2 mg) 4 veces al día (8 mg/día) durante 16 semanas con descenso progresivo en otras 4 semanas. Dicho compuesto en todos los casos fue preparado en la farmacia del hospital.

3.4.2 Vitamina D

La vitamina D (vit D) tiene un potente efecto inmunomodulador demostrado en estudios in vitro y en modelos animales. Casi todos los tejidos y células incluyendo las células mononucleadas de la sangre periférica poseen receptores de vitamina D (VDR) y muchas de ellas tienen la capacidad de convertir la 25-hidroxivitamina D en 1,25 hidroxivitamina D que es su forma biológicamente activa. Estudios in vitro indican que la vitamina D inhibe la activación y proliferación de las células T dependientes de células dendríticas y disminuye la producción de citocinas producidas por las células Th1 como IL-2, IFNγ and TNFα lo que explica su efecto inmunomodulador.

La vitamina D en asociación con calcio en el contexto del TPH esta indicado en la prevención y tratamiento de la osteoporosis y osteopenia fundamentalmente relacionada en este contexto con el uso de corticoides.

Al año postrasplante esta indicada la realización de densitometría ósea. En caso de osteoporosis u osteopenia se prescribe vitamina D 1000 IU más calcio 1250 mg 1 vez al día por vía oral durante por lo menos 6 meses.

3.5 Análisis estadístico

La creación de las bases de datos empleadas en los diferentes estudios así como el análisis estadístico de los parámetros demográficos, clínicos y de laboratorio se llevó a cabo mediante el paquete estadístico *SPSS Software 10.0* (SPSS Inc, Chicago Ill, USA).

Para las variables continuas se calcularon los valores de media y mediana con sus respectivos intervalos de confianza (IC) del 95%. Los tests de *t Student* e x^2 *de Pearson* se utilizaron para comparar variables continuas y cualitativas. En los casos en que fue necesario el uso de testes no paramétricos se utilizo el de *Mann-Whitney* o el *exacto de Fisher*. Los valores de *p* fueron reportados mediante two-tailed *p*-values.

Los eventos analizados se calcularon a partir de la fecha de infusión de las células progenitoras mediante el método del producto de Kaplan–Meyer. Se definió la mortalidad relacionada con el trasplante como la ocurrida por causas ajenas a la enfermedad de base. Los pacientes que recayeron fueron censurados a partir del momento de la recaída.

Se consideró mortalidad relacionada con EICH como la muerte debido a causas directamente relacionadas con la EICH, incluyendo las atribuidas al tratamiento inmunosupresor en los pacientes con EICH bajo tratamiento sistémico. La EICHa e EICHc fueron calculadas a partir de la fecha de infusión de las CPH hasta la fecha de diagnóstico de cada una de ellas. La supervivencia global (SG) se calculo a partir de la fecha del trasplante hasta la muerte ocurrida por cualquier causa. Para la supervivencia libre de enfermedad (SLE) se considero el periodo desde la infusión de las CPH hasta la progresión o muerte.

La evaluación de variables en el análisis univariante se realizo mediante la comparación de curvas de supervivencia de acuerdo con el método de Kaplan-Meyer, usando el test de Log-Rank para estimar la significación estadística de

las diferencias. Todas las variables que pudieran tener influencia en los parámetros en estudio de manera significativa o marginal ($p<0.1$) se incluyeron en el análisis multivariante empleando el modelo de regresión de Cox. Las diferencias fueron consideradas estadísticamente significativas cuando el valor de p era <0.05.

RESULTADOS

4. Resultados

En este aparatado se presentarán las publicaciones que han dado lugar a la presente Tesis Doctoral. Para su presentación se ha dividido en dos partes: 4.1- Factores pronósticos en la EICHc y 4.2- Nuevas estrategias terapéuticas encaminadas a evitar el tratamiento sistémico con esteroides en la EICHc. Cada uno de los artículos producidos irá precedido de un breve resumen en español.

4.1 Factores pronósticos en la EICHc

4.1.1 Valor pronóstico de la clasificación del NIH y otros factores pronósticos

Prognostic factors of chronic graft-versus-host disease following allogeneic peripheral blood stem cell transplantation: The National Institutes of Health scale plus the type of onset can predict survival rates and the duration of immunosuppressive therapy. Biol Blood Marrow Transplant 2008; 14: 1163-1171.

Diversos modelos pronósticos en pacientes con EICHc han sido desarrollados en el contexto del trasplante de progenitores hematopoyéticos de médula ósea. En este estudio evaluamos el valor pronóstico de la clasificación del NIH y hemos analizado el impacto de factores pronósticos adicionales en una serie de 171 pacientes sometidos a trasplante alogénico de progenitores hematopoyéticos de sangre periférica de donante emparentado. La incidencia global de EICHc fue de 70% siendo leve, moderada y severa en el 29%, 42% y 28% respectivamente. A los 5 años postrasplante el 68% de los pacientes estaba libre de tratamiento inmunosupresor sistémico. La ausencia de EICHa previa (HR=2; *p*=0.004) y la EICHc leve (HR=4,2; *p*=0.007) son factores que aumentan la probabilidad de estar libre de tratamiento inmunosupresor sistémico. La supervivencia global (SG) a los 5 años fue de 52%. La EICHc severa (HR=13,27; *p*=0.001) de acuerdo con la clasificación del NIH fue el factor con peor impacto en la SG, mientras que la EICHc de novo (HR=0,094;

p=0.003) fue la variable asociada a mejor SG. La combinación de ambas variables (grado de severidad de la EICHc según la clasificación del NIH y el tipo de comienzo) permitieron identificar 4 subgrupos de pacientes con diferentes pronósticos. Así, los pacientes con EICHc moderada y el tipo de comienzo *de novo*, EICHc moderada y tipos de comienzo quiescente o progresivo, EICHc severa y tipo de comienzo *de novo* y EICHc severa y tipos de comienzo quiescente o progresivo tienen una SG a los 5 años de 82%, 70%, 50% y 25%, respectivamente. Es Importante destacar que los pacientes con EICHc leve presentan una SG similar (80 – 87% a los 5 años) independientemente del tipo de comienzo. Estos resultados indican que la clasificación del NIH tiene valor pronóstico en los pacientes sometidos a trasplante alogénico de PHSP y junto al tipo de comienzo son variables pronósticas importantes en los pacientes con EICHc.

ASBMT
American Society for Blood and Marrow Transplantation

Prognostic Factors of Chronic Graft-versus-Host Disease Following Allogeneic Peripheral Blood Stem Cell Transplantation: The National Institutes Health Scale Plus the Type of Onset Can Predict Survival Rates and the Duration of Immunosuppressive Therapy

José A. Pérez-Simón, Cristina Encinas, Fernando Silva, Maria José Arcos, María Díez-Campelo, Fermín M. Sánchez-Guijo, Enrique Colado, Jesús Martín, Lourdes Vazquez, Consuelo del Cañizo, Dolores Caballero, Jesús San Miguel

Several grading systems have been developed in the bone marrow transplantation setting in attempts to predict survival in patients with chronic graft-versus-host disease (cGVHD). In this study, we evaluated the prognostic value of the National Institutes of Health (NIH) scoring system and investigated for any additional prognostic factors in a series of 171 patients undergoing peripheral blood stem cell transplantation (PBSCT) from matched related donors. The cumulative incidence of cGVHD was 70%; cumulative incidences of mild, moderate, and severe cGVHD were 29%, 42% and 28%, respectively. Overall, 68% of patients were free from immunosuppression 5 years after transplantation. Absence of previous acute GVHD (aGVHD; hazard ratio [HR] = 2; $P = .004$) and mild cGVHD (HR = 4.2; $P = .007$) increased the probability of being off immunosuppressive treatment by the last follow-up. Overall survival (OS) at 5 years was 52%. Severe cGVHD, according to the NIH scoring system (HR = 13.27; $P = .001$) adversely influenced outcome, whereas de novo onset (HR = 0.094; $P = .003$) had a more favorable impact on survival. The combination of both variables allowed us to identify 4 different subgroups of patients with OS of 82%, 70%, 50%, and 25%. Our findings indicate that the NIH scoring system has some prognostic value in patients undergoing PBSCT and, together with the type of onset, must be considered to predict the possible outcome of patients who develop cGVHD.

Biol Blood Marrow Transplant 14: 1163-1171 (2008)

KEY WORDS: Chronic graft-versus-host disease, scoring system, peripheral blood stem cell transplantation

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) is a major complication after allogeneic hematopoietic stem cell transplantation (HSCT) that impairs quality of life and functional status and adversely affects long-term survival [1-4]. Historically, cGVHD has been classified as "limited" or "extensive" on the basis of the results of a small retrospective study [5]. This classification system was developed primarily to distinguish patients requiring systemic immunosuppression from those for whom local care might suffice. Nevertheless, most patients experience extensive-stage cGVHD. This constitutes an extremely heterogeneous population. Furthermore, although such a classification system can be easily used in many centers [6], it fails to stratify patients according to outcome. For this reason, several grading systems have been developed to predict survival and late treatment-related mortality in patients diagnosed with cGVHD. Along these lines, Akpek et al. [7] developed a prognostic model based on the presence of extensive skin involvement, thrombocytopenia, and progressive-type onset in a series of patients undergoing allogeneic bone marrow transplantation (BMT). According to the Karnofky performance score,

From the Department of Hematology, University Hospital of Salamanca Cancer Research Center (CIC/CSIC) of Salamanca and Center of Regenerative Medicine and Cellular Therapy of Castilla y Leon, Spain

Correspondence and reprint requests: José A Pérez-Simón, MD, PhD, Servicio de Hematología, Hospital Clínico Universitario de Salamanca, Paseo de San Vicente s/n, 37007 Salamanca, Spain (e-mail: pesimo@usal.es).

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Table 1. Characteristics of the patients at transplantation (n =171)

Tr	Tr
Age, years, median (range)	45 (14 to 69)
Diagnosis	
AML, n	44
ALL, n	24
MM, n	21
NHL, n	20
MDS, n	19
CML, n	14
CLL, n	10
HD, n	9
MPD, n	6
Aplasia, n	2
Others, n	2
Disease status at transplantation*	
Low risk, n (%)	63 (37%)
Intermediate risk, n (%)	75 (43%)
High risk, n (%)	33 (20%)
Sex†	
Male, n	100
Female, n	71
Conditioning regimen	
Myeloablative, n	68
Reduced-intensity conditioning, n	103
Charlson comorbidity index	
Low, n	113
Intermediate low, n	37
Intermediate high, n	10
High, n	11
CD34 infused $\times$ 10^6/kg, median (range)	5.1 (1.9 to 13.2)

*Low risk: first complete remission or chronic phase; high risk: relapse or progressive disease, blast crisis; intermediate risk: all others.
†A total of 39 male patients received PBSCT from a female donor.

diarrhea, weight loss, and skin or oral involvement also have been identified as prognostic factors in a large registry-based analysis of patients receiving BMT almost exclusively [8].

More recently, the National Institutes of Health (NIH) Consensus Development Project proposed a new clinical scoring system for the global assessment of cGVHD severity based on the number of organs involved and the degree of functional impairment in affected organs (mild, moderate, or severe). This allows the identification of patients requiring a purely topical approach or no immunosuppression, and also facilitates decision making regarding the timing and intensity of therapy. Nevertheless, this scoring system requires validation with a large series of patients to demonstrate its prognostic impact [9].

Previous studies have identified the risk factors for cGVHD after HSCT, including previous acute GVHD (aGVHD), advanced age, use of female donors for male recipients, and use of unrelated or HLA-mismatched donors [10,11]. Compared with BMT, in peripheral blood stem cell transplantation (PBSCT), the incidence of cGVHD is higher [12], and patients require more successive treatments to achieve control [13], leading to a longer duration of immunsuppressive therapy. Accordingly, the prognostic models of cGVHD in the BMT setting may not necessarily apply to patients undergoing PBSCT. With regard to this, Pavletic et al. [14] reported that a platelet count $<$ 100 $\times$ 10^9/L and a history of aGVHD point to a poor outcome in patients undergoing PBSCT who develop cGVHD.

In this current study, we evaluated the prognostic impact of the new clinical scoring system proposed by the NIH Consensus Development Project and investigated for additional prognostic factors in a series of patients undergoing PBSCT from a matched related donor.

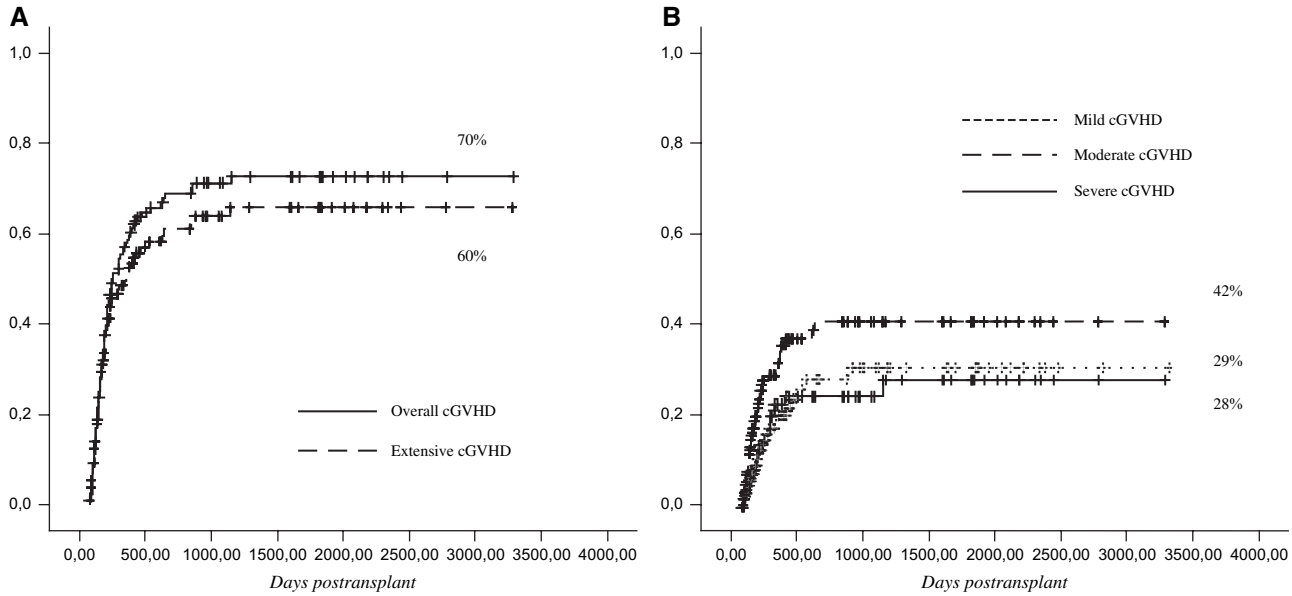

Figure 1. Cumulative incidence of cGVHD according to standard criteria (A) and to NIH-based criteria (B).

Table 2. Actuarial incidence of cGVHD and organ involvement (patients with > 100 days of follow-up; n = 150)

CGVHD	
Yes	91
No	59
Type of cGVHD	
Limited	22
Extensive	69
Severity of cGVHD	
Mild	24
Moderate	42
Severe	25
Type of onset	
De novo	45
Quiescent	38
Progressive	8
Organ involvement	
Skin	
Mild	38
Moderate	9
Severe	5
Mouth	
Mild	58
Moderate	4
Severe	
Eyes	
Mild	35
Moderate	3
Severe	1
Gut	
Mild	17
Moderate	9
Severe	2
Liver	
Mild	33
Moderate	7
Severe	7
Lung	
Mild	5
Moderate	9
Severe	1
Kidney	
Mild	1
Moderate	1
Severe	
Muscle/joints	
Mild	3
Moderate	2
Severe	
Counts at the time of cGVHD diagnosis	
Platelets: Median (range) $\times 10^6$/L	179 (18 to 482)
Eosinophils: Median (range) $\times 10^6$/L	198 (0 to 3.550)

cGVHD indicates chronic graft-versus-host disease.

METHODS

Patient Characteristics

A total of 171 patients consecutively undergoing non–T cell–depleted PBSCT at our institution between January 1998 and March 2007 were included in the analysis. Patients receiving a bone marrow transplantation, an allogeneic transplant from an unrelated donor, or GVHD prophylaxis other than cyclosporin (CsA) and methotrexate (MTX) were not included in the analysis. Patients were retrospectively categorized according to the NIH scoring system based on the data obtained from the medical history, which specified organ involvement and graded according to the classical limited vs extensive classification. The patient characteristics are summarized in Table 1.

Myeloablative conditioning consisted of cyclophosphamide (Cy) 60 mg/kg ×2 days intravenously and fractionated total body irradiation (TBI; total 12 Gy) or busulfan (Bu) 1 mg/kg 4 times daily over 4 days. Patients receiving reduced-intensity conditioning (RIC) were treated with fludarabine (Flu) 30 mg/m^2/day on days -9 to -5, followed by either busulfan (Bu) 1 mg/kg every 6 hours on days -6 and -5 and 1 mg/kg every 12 hours on day -4 or melphalan 70 mg/m^2/day on days -3 and -2 in patients diagnosed with myelogenous or lymphoid malignancies, respectively.

GVHD Prophylaxis and Treatment

For GVHD prophylaxis, patients received CsA 0.5 mg/kg every 12 hours from day -7 to -2 and 1.5 mg/kg every 12 hours from day –1, plus MTX 15 mg/m^2/day on day -1 and 10 mg/m^2 on days +3, +6, and +11, followed by folinic acid rescue. CsA taper was started on approximately day +50 and stopped on day +180 if GVHD did not flare. A faster taper could have been scheduled, had active disease or minimal residual disease been detected. Tacrolimus was used instead of CsA to avoid CsA-related toxicity.

The first-line treatment for aGVHD was based on the administration of 6-methyl-prednisonole 2 mg/kg/day in the event that grade ≥ 2 aGVHD developed. Second-line treatment was administered if there was progression at day +3, no response at day +7, and no complete remission at day +14 after the beginning of the treatment.

The first-line treatment for extensive cGVHD was based on CsA or tacrolimus plus prednisone at 1 mg/kg/day, which was switched to alternating days after 4 weeks of treatment. The disease response was evaluated 5 weeks after the introduction of steroids and every 3 months thereafter until the end of treatment. All patients received antibacterial, antifungal, and antiviral prophylaxis according to standard protocols [15].

Definitions

Based on to the NIH scoring system [9], mild cGVHD was diagnosed when only 1 or 2 organs or sites (except the lung; see below) were involved, with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). Moderate cGVHD involved at least 1 organ or site with clinically significant impairment but no major disability (maximum score of 2 in any affected organ or site) or 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 also was considered moderate cGVHD. Severe cGVHD was defined as a major disability caused by cGVHD

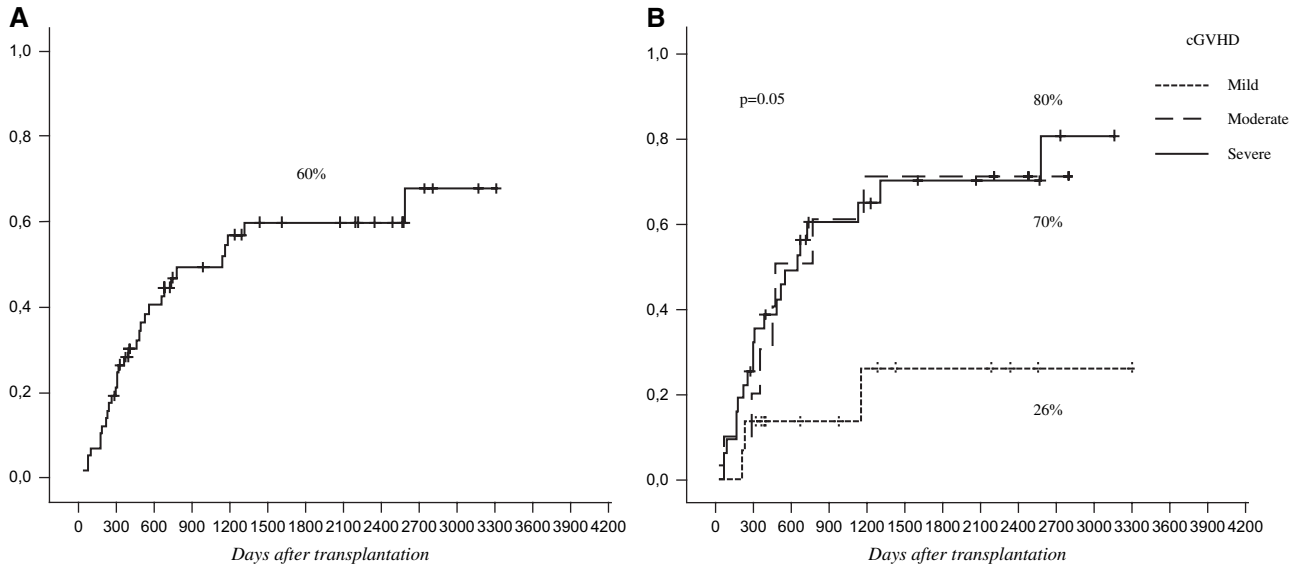

Figure 2. Relapse of cGVHD in patients who responded to first-line treatment (A) and according to the NIH scale (B).

(score of 3 in any organ or site). A lung score of ≥ 2 also was considered severe cGVHD.

Patients who were receiving prednisone or were still receiving a therapeutic dose of CsA to treat previous aGVHD that had evolved into cGVHD without the resolution of symptoms were considered to have "progressive" cGVHD. Patients who were on CsA taper with resolution of symptoms or who were free from immunosuppression at the time of diagnosis were categorized as "quiescent," whereas those with no previous history of aGVHD were classified as having "de novo" cGVHD. Otherwise, aGVHD and limited GVHD versus extensive cGVHD were graded based on established criteria [5].

Statistical Analysis

Mean and median values along with 95% confidence intervals (CI) and ranges were calculated for each continuous variable. The Student *t* test and Pearson's χ^2 test were used to compare continuous and qualitative variables. In comparisons in which the number of cases precluded the use of parametric tests, the Mann-Whitney test and Fisher's exact tests for 2×2 tables were used. All *P* values for these tests are reported as 2-tailed *P* values.

The events analyzed were calculated from the time of transplantation using Kaplan-Meier product-limit estimates. Treatment-related mortality (TRM) was defined as death due to causes unrelated to the underlying disease, and relapsing patients were censored at the time of relapse. GVHD-related mortality was defined as death due to causes directly related to GVHD. Deaths attributed to immunosuppression in patients requiring treatment for GVHD also were considered GVHD-related mortalities. Event-free survival (EFS) was calculated from the time of transplantation until disease progression or death. Patients who did not achieve disease response (complete remission [CR] or partial response [PR]) at any time after transplantation were considered events on day 100, because this was the first date of complete disease evaluation. Overall survival (OS) was calculated from transplantation until death from any cause, and surviving patients were censored at the last follow-up.

Patients who demonstrated evidence of engraftment were evaluable for aGVHD, whereas patients who engrafted and survived for more than 100 days were evaluable for cGVHD. The contraction of aGVHD or cGVHD was calculated from the time of transplantation until diagnosis of aGVHD or cGVHD in an evaluable patient. The cumulative incidence estimates for GVHD were performed taking into account death as a competing risk. To calculate the percentage of patients receiving immunosuppressive therapy at any time after transplantation, only those patients at risk at that specific time point were included in the analysis. All of the factors that significantly or marginally ($P < .10$) influenced the incidence or outcome of cGVHD in the univariate analysis were included in multivariate analysis using a forward-step Cox regression model.

SPSS version 10 (SPSS Inc, Chicago, IL) was used for most of the statistical analyses. Computations and testing of cumulative incidences were performed with the cmprsk package R 1.9.1. Differences were considered statistically significant at a *P* value $< .05$.

RESULTS

GVHD Incidence and Characteristics

The cumulative incidence of aGVHD was 40% for grade II-IV and 10% for grade III-IV. cGVHD

Table 3. Factors associated with response or relapse to immunosuppressive treatment

Response to treatment CR plus PR/CR	Actuarial incidence (%)	Univariate *P* value	HR (95% CI)	Multivariate *P* value
Grade of cGVHD		.4		
Mild	95/68			
Moderate	92/60			
Severe	82/41			
Type of onset		.04		
De novo	90/67			
Quiescent	90/53			
Progressive	60/20			

Relapse to immunosuppressive treatment among responders	Cumulative incidence			
Patient sex		.05		
Male	70%			
Female	47%			
Grade of cGVHD		.05		
Mild	26%		12.5 (2.6 to 60)	.002
Moderate	70%		20.9 (2.3 to 183)	.006
Severe	80%			
cGVHD		.07		
limited	38%			
extensive	74%			
Performance status		.03		
ECOG < 2	54%			
ECOG ≥ 2	82%			
Ocular involvement		.002		
No	63%			
Grade 1	76%			
Grade 2 to 3	100%			
Gut involvement		< .001		
No	60%			
Grade 1	70%			
Grade 2	96%			
Grade 3	100%			
Liver involvement		.011		
No	59%			
Grade 1	64%			
Grade 2	80%			
Grade 3	100%			
Lung involvement		.007		
No	65%			
Grade 1	60%			
Grade 2	100%			

flared at a median of 182 days posttransplantation (range, 90 to 1150 days). The cumulative incidence of cGVHD was 70% in patients surviving > 100 days after transplantation, with 60% classified with extensive cGVHD. The cumulative incidences of mild, moderate, and severe cGVHD were 29%, 42%, and 28%, respectively (Figure 1). Among the 69 patients diagnosed with extensive cGVHD, 6 had mild cGVHD, 39 had moderate cGVHD, and 24 had severe cGVHD. Organ involvement in the 6 patients with extensive cGVHD (retrospectively classified as mild GVHD) included involvement of the skin and gut (n = 1), liver and gut (n = 1), kidney (n = 1), gut (n = 1), and liver and mouth (n = 2). In all of these cases, the severity of organ involvement was considered mild. Table 2 summarizes the incidences of cGVHD and organ involvement.

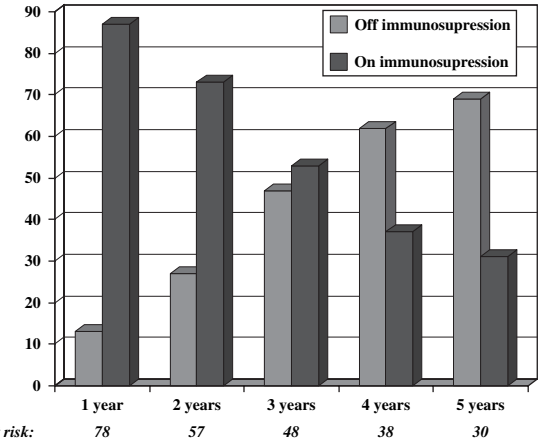

Figure 3. Probability of being off immunosuppression at last follow-up.

Response to Immunosuppressive Treatment and Relapse of GVHD

To evaluate the response to or relapse after immunosuppressive therapy, only patients who received first-line treatment (ie, patients diagnosed with extensive cGVHD) were evaluated. Overall, 90% of patients reached at least PR after first-line treatment, with 58% achieving CR. Only the type of onset significantly influenced the probability of responding to treatment; the CR rate was 67% in patients with cGVHD, 53% in those with quiescent cGVHD, and 20% in those with progressive cGVHD.

Table 4. Variables associated with immunosuppression withdrawal

Off immunosuppression at last follow-up*	Cumulative incidence	Univariate *P* value	HR (95% CI)	Multivariate *P* value
Age		.081		
<p50	81%			
>p50	47%			
Prior aGVHD		.04		
Yes	72%			
No	49%		2.7 (1.3 to 6)	.004
Grade of cGVHD		< .001		
Mild	71%		4.2 (1.4 to 12.12)	.007
Moderate	58%			
Severe	57%			
Onset		.02		
De novo	71%			
Quiescent	56%			
Progressive	13%			
Performance status		.015		
ECOG < 2	66%			
ECOG ≥ 2	44%			
Ocular involvement		.015		
No	73%			
Grade 1	50%			
Grade 2	0%			
Grade 3	0%			

*Univariate and multivariate analysis included those patients requiring systemic immunosuppression any time after transplantation.

Table 5. GVHD-related mortality and survival

cGVHD-related mortality	Cumulative incidence	Univariate *P* value	HR (95% CI)	Multivariate *P* value
Age		.02		
< p75 (55 years)	5%			
> p75 (55 years)	23%			
cGVHD		.24		
Limited	0%			
Extensive	13%			
Type of onset		.004		
De novo	3%			
Quiescent	12%			
Progressive	38%			
Grade of severity		.004		
Mild	0%			
Moderate	9%			
Severe	30%			
Performance status		< .001		
ECOG < 2	4%			
ECOG ≥ 2	30%			
Lung involvement		< .001		
No	5%			
Mild	20%			
Moderate	24%			
Severe	100%			
Liver involvement		.008		
No	3%			
Mild	11%			
Moderate	15%			
Severe	38%			
OS	**5-year OS**			
cGVHD		.04		
Limited	87%			
Extensive	64%			
Grade of cGVHD		< .001		
Mild	83%		13.27 (2.81 to 62.5)	.001
Moderate	77%			
Severe	46%			
Type of onset		.03		
De novo	77%		0.094 (0.02 to 0.43)	.003
Quiescent	64%			
Progressive	57%			
Performance status		< .001		
ECOG < 2	79%			
ECOG ≥ 2	47%			
Liver involvement		< .001		
No	85%			
Grade 1	76%			
Grade 2	63%			
Grade 3	28%			
Lung involvement		< .001		
No	80%			
Grade 1	71%			
Grade 2	66%			
Grade 3	0%			

The cumulative incidence of relapse in patients who responded to initial treatment was 61% (Figure 2A). Table 3 summarizes the variables that had a significant influence on relapse after first-line treatment. In multivariate analysis, only the severity of cGVHD according to the NIH scoring system significantly influenced the risk of relapse (hazard ratio [HR] = 12.5; 95% confidence interval [CI] = 2.6-60; P = .002 for moderate cGVHD and HR = 20.9; 95% CI = 2.3-183; P = .006 for severe cGVHD) (Figure 2B).

Overall, 68% of patients were free from immunosuppressive treatment 5 years after transplantation (Figure 3). Table 4 presents the variables that influenced the probability of being free from immunosuppression by the last follow-up. According to multivariate analysis, the absence of previous aGVHD (HR = 2.7; 95% CI = 1.3-6; P = .004) and mild cGVHD (HR = 4.2; 95% CI = 1.4-12.12; P = .007) significantly increased the probability of being off immunosuppressive treatment by the last follow-up.

cGVHD-Related Mortality and Outcome

TRM was 19% by 5 years for the entire series of patients and 12% for those patients surviving for more than 100 days after transplantation. In this latter subset of patients, cGVHD-related mortality was 10%. Causes of cGVHD-related death included fungal and/or bacterial infection in 9 patients, respiratory failure related to cGVHD in 4 patients, and both in 1 patient. Table 5 summarizes the variables that significantly affected cGVHD-related mortality. Interestingly, no significant differences were observed between patients diagnosed with limited cGVHD and those with extensive cGVHD (0% vs 13%; P = .24), whereas both the NIH scoring system and the type of onset significantly affected cGVHD-related mortality. In terms of organ involvement, the severity of liver and lung involvement, as well as performance status, significantly influenced the mortality of cGVHD-relapsed cases.

At 5 years, OS was 52% and event-free survival (EFS) was 48%. Table 5 also summarizes the variables that significantly influenced OS. In multivariate analysis, severe cGVHD adversely influenced outcome (HR = 13.27; 95% CI = 2.81-62.5; P = .001), whereas de novo onset had a more favorable impact on survival (HR = 0.094; 95% CI = 0.02-0.43; P = .003) (Figure 4).

Interestingly, patients categorized as mild cGVHD had similar survival regards of the type of onset, ranging from 80% to 87% at 5 years. In contrast, in patients with moderate cGVHD, de novo onset allowed us to differentiate a subgroup of patients with more favorable outcome, similar to those diagnosed with mild cGVHD (82% at 5 years) and significantly better than those with quiescent or progressive onset (70% at 5 years). Finally, within the severe cGVHD subgroup, de novo onset versus moderate or severe cGVHD also allowed us to differentiate 2 subgroups in terms of survival (50% OS at 5 years for patients with de novo vs 25% OS at 5 years for those with quiescent or progressive onset). Thus, the combination of both variables allowed us to identify different subgroups of patients in terms of outcome (Figure 5).

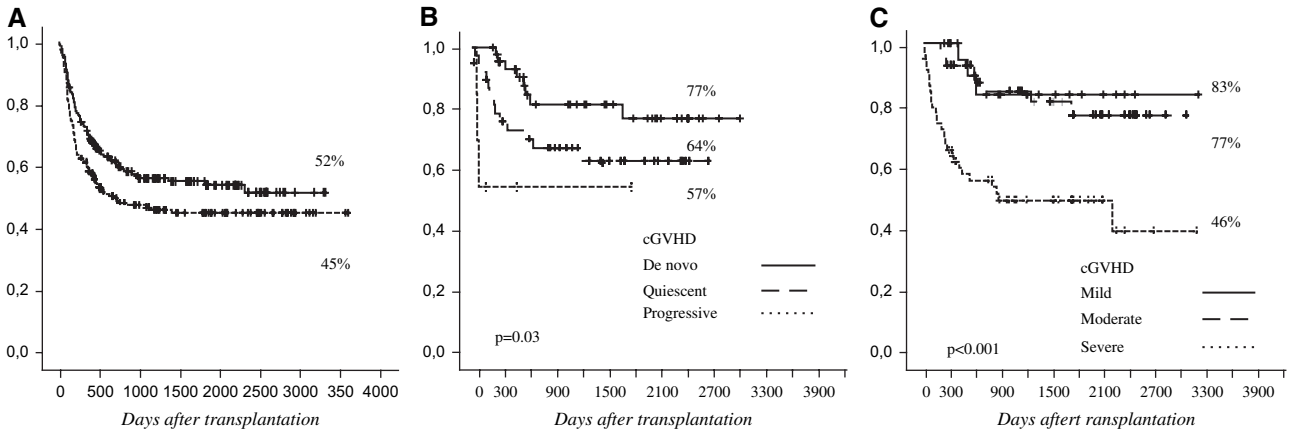

Figure 4. OS and EFS for the whole series of patients (A); OS in patients with de novo, quiescent, or progressive-onset cGVHD (B); and OS in patients with mild, moderate, or severe cGVHD (C).

To evaluate which severity grade had a greater impact on outcome, we carried out a multivariate analysis excluding the overall severity grade according to the NIH scale. In this analysis, performance status < 2 (HR = 0.26; 95% CI = 0.1-0.62; $P = .003$) and liver involvement = 3 (HR = 14.3; 95% CI = 3.4-60.32; $P < .001$) significantly affected the outcome.

DISCUSSION

Several models have been developed to identify the clinical and biological features with prognostic significance in patients who develop cGVHD [7,8]. Most previous studies have been conducted in patients undergoing BMT, not taking into account the fact that characteristics of cGVHD differ between PBSCT and BMT. With regard to this, PBSCT is associated with a higher incidence of cGVHD compared with BMT [12]. Furthermore, the number of successive lines of treatment needed to control cGVHD is higher after PBSCT, meaning that these patients require a longer duration of immunosuppressive therapy [13,16]. In this regard, Pavletic et al. [14] have reported that some prognostic factors may be unique to recipients of PBSCT and do not apply to those undergoing BMT. Accordingly, in the PBSCT setting, specific models are needed to establish different prognostic subgroups to allow identification of patients who can be treated with topical or mild immunosuppression, in contrast to those requiring a more aggressive approach. This is especially pertinent because although cGVHD can lead to severe complications adversely affecting quality of life and survival, it also is related to a graft-versus-leukemia effect, which significantly decreases the risk of relapse after allogeneic transplantation [15,17-19]. Accordingly, the development of accurate models with prognostic significance in the PBSCT setting will help individualize therapeutic strategies.

The NIH has proposed a new scoring system to establish standard criteria for the diagnosis of cGVHD [9]. This system attempts to do this by describing the extent and severity of cGVHD for each organ or site involved at any given time. In doing so, it seeks to establish new guidelines for the global assessment of cGVHD and to propose indications for topical and systemic therapies. Nevertheless, this scoring system requires validation to define the prognostic impact of the subgroups that it identifies as mild, moderate, and severe cGVHD. In the current study, we confirmed that most of the patients who developed cGVHD were classified as having extensive cGVHD, with only a minority having limited cGVHD according to the

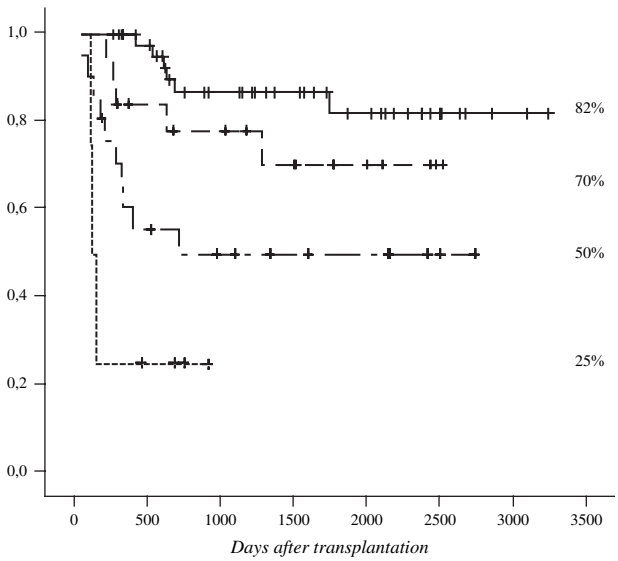

Figure 5. OS in patients with cGVHD according to NIH score plus type of onset. OS, depending on grade of severity according to the NIH scoring system plus type of onset, was 82% for patients with mild cGVHD regardless of the type of onset and patients with moderate cGVHD with de novo onset, 70% for patients with moderate cGVHD and quiescent or progressive onset, 50% for patients with severe cGVHD and de novo onset, and 25% for patients with severe cGVHD and quiescent or progressive onset.

standard criteria. This contrasts with the NIH scoring system, because all 3 categories had a similar number of patients, thus allowing better stratification of the patients for both therapeutic and prognostic purposes. In addition, some of the patients diagnosed with extensive cGVHD were retrospectively classified as having mild cGVHD. Based on the superior outcome of this small subset of patients, it can be speculated that they could have benefited from avoiding systemic immunosuppression, as suggested by the NIH scoring system.

Concerning cGVHD-related mortality, a good performance status at the time of cGVHD diagnosis, according to the NIH scoring system and the type of onset, significantly influenced the outcome in univariate analysis. Regarding specific organs, the severity of liver and lung involvement significantly influenced the outcome of patients who developed cGVHD. These variables have been identified as independent prognostic factors in previous studies [16]. In contrast, we did not identify platelet count as a prognostic factor, which may be explained by the high median number of platelets (79×10^9/L) found at the time of cGVHD diagnosis [7,8,14]. The same variables also influenced OS in univariate analysis, whereas in multivariate analysis, both the type of onset and NIH scoring significantly affected outcome. In this regard, de novo onset of GVHD was associated with a favorable prognosis, whereas severe cGVHD had an adverse impact on survival. Based on multivariate analysis, we developed a scoring system that considers both type of onset and grade of severity, which allowed us to differentiate 4 subgroups that clearly differed in terms of outcome, with OS of 82%, 70%, 50%, and 25%.

Previous studies have shown that 30% to 70% of patients surviving beyond 100 days after transplantation require immunosuppressive treatment for more than 2 years [1,16,20]. In the current study, we confirmed, in a series of homogeneously treated patients undergoing PBSCT, that the NIH scoring system, besides its impact on outcome, is the most important prognostic factor in predicting the risk of relapse after first-line cGVHD treatment. When considered along with previous development of aGVHD, this system allows us to identify those patients receiving immunosuppressive therapy at the last follow-up. In this regard, patients with mild cGVHD had a significantly higher probability of being free from immunosuppressive therapy at last follow-up compared with those with moderate or severe cGVHD.

In conclusion, the NIH scoring system is of prognostic value in patients undergoing PBSCT and, together with the type of onset, must be considered to predict the outcome of patients who develop cGVHD. These parameters should be taken into account to adapt immunosuppressive strategies and decrease the risk to patients.

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4.1.2 Valor predictivo de los tests de *screening* del día + 100 en el contexto de la EICHc

Liver function tests and absolute lymphocyte count at day +100 are predictive factors for extensive and severe chronic graft versus host disease after allogeneic peripheral blood stem cell transplant. American J Hematology 2010; 85: 290-293.

En el presente trabajo se ha analizado el valor predictivo de las pruebas de reevaluación no invasivas del día + 100 postrasplante en el desarrollo de EICHc extensa o severa en una serie de 165 pacientes sometidos a trasplante alogénico de progenitores hematopoyéticos de sangre periférica de donantes emparentados HLA idénticos. En los pacientes con más de 100 días de supervivencia postrasplante la incidencia global de EICHc fue de 67% siendo extensa y severa en el 56% y 23% respectivamente. En el análisis univariante los pacientes con pruebas de función hepática alteradas (bilirrubina total, fosfatasa alcalina y GGT 2 veces por encima del limite superior de lo normal) y cifras de linfocitos en sangre periférica (< percentil 25: 0.750 x 10^9/L) presentan un mayor riesgo de desarrollar EICHc extensa o severa. En el análisis multivariante la combinación de pruebas de función hepática anormales y el número de linfocitos permiten predecir el riesgo del desarrollo de EICHc [HR = 3.35 (95% IC =1.65 – 6.83) p<0.001], EICHc extensa [HR = 4.22 (95% IC =1.96 – 9.12) p<0.001] y severa [HR = 8.17 (95% IC =2.55 – 26.17) p=0.002].

En resumen, en los pacientes con pruebas de función hepáticas alteradas y cifras bajas de linfocitos en sangre periférica en el día + 100 el riesgo de desarrollar EICHc severo es 3 veces superior que en los pacientes sin ningún factor de riesgo (58 % vs 16%).

ment of LIC via biopsy or noninvasive imaging methods, with iron chelation therapy being initiated in patients with LIC levels indicating liver iron overload.

[1]Hematology-Oncology Division, Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon
[2]Division of Pediatric Cardiology, Children's Hospital Los Angeles and Keck School of Medicine, University of Southern California, Los Angeles, California
[3]Division of Radiology, Children's Hospital Los Angeles and Keck School of Medicine, University of Southern California, Los Angeles, California
[4]Department of Internal Medicine, Universitá di Milano, Policlinico Foundation IRCCS, Milan, Italy
Grant Sponsor: Novartis Pharmaceuticals
*Correspondence to: Ali T. Taher, Professor of Medicine, Hematology and Oncology, Department of Internal Medicine, American University of Beirut Medical Center, P.O. Box 11-0236, Riad El Solh 1107 2020, Beirut, Lebanon.
E-mail: ataher@aub.edu.lb
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Liver function tests and absolute lymphocyte count at day +100 are predictive factors for extensive and severe chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplant

Fernando Silva, José A. Pérez-Simón,* Teresa Caballero Velazquez, Cristina Encinas, Fermín M. Sánchez-Guijo, María Díez-Campelo, Enrique Colado, Jesús Martín, Fernanda Villanueva-Gomez, Lourdes Vazquez, Consuelo del Cañizo, Dolores Caballero, and Jesús San Miguel

Chronic graft-versus-host disease (cGVHD) is the major late complication after allogeneic hematopoietic stem cell transplant [1,2]. **In this article, we have analyzed the value of noninvasive day +100 tests as predictors of severe cGVHD development in 165 patients undergoing allogeneic peripheral blood stem cell transplant (allo-PBSCT) from a matched related donor. The cumulative incidence of overall, extensive, and severe cGVHD was 67, 56, and 23%, respectively, among patients surviving >100 days after transplant. In univariate analysis, patients displaying an abnormal liver function tests (LFTs) (total bilirubin, alkaline phosphatase, and GGT > 2 times above the upper normal limit) and a low absolute lymphocyte count (ALC) (<percentile 25: 0.750 × 10^9/L) had a significantly higher risk of overall, extensive, and severe cGVHD. In multivariate analysis, the combination of abnormal LFT and low ALC allowed to predict the risk of overall [HR = 3.35 (95% CI: 1.65–6.83), *P* < 0.001], extensive [HR = 4.22 (95% CI: 1.96–9.12), *P* < 0.001], and severe cGVHD [HR = 8.17 (95% CI: 2.55–26.17), *P* = 0.002]. Our findings show that an increased total bilirubin, alkaline phosphatase, and GGT levels together with the ALC at day +100 are noninvasive, simple, fast, and efficient predictors of severe cGVHD development after allogeneic PBSCT.**

Patient characteristics. Data were collected from 165 patients who received allogeneic PBSCT at the University Hospital of Salamanca from January 1998 to March 2008. Patients who received bone marrow transplants, allogeneic transplantation from unrelated donor or agents other than cyclosporin A (CsA), and methotrexate (MTX) for GVHD prophylaxis or had clinical signs of active GVHD or were receiving treatment with steroids at day +100 were excluded from this analysis.

The criteria for grading chronic GVHD was the classical limited versus extensive classification [3] and the NIH scoring system [4] based on the data collected from the patients' medical records. The minimal time of follow-up for the occurrence of extensive chronic GVHD among patients who were alive and without relapse was 1 year. Patients' characteristics are shown in Table I.

TABLE I. Characteristics of the Patients at Transplant and Incidence of cGVHD

	$n = 165$
Age: median (range)	49 (14–69)
Diagnosis, AML/ALL/MM/NHL/Others	41/19/25/24/56
Sex, Male/female	100/65
Conditioning regimen, Myeloablative/RIC	56/109
CD34 infused $\times 10^6$/kg	5.2 (0.8–13.2)
cGVHD, Yes/No	86/79
Type of cGVHD, Limited/extensive	23/63
Severity of cGVHD according to NIH criteria, Mild/Moderate/Severe	27/40/19
Type of onset, De novo/quiescent	49/37
Organ involvement	
Mouth, Mild/moderate	61/5
Skin, Mild/moderate/severe	37/10/3
Liver, Mild/moderate/severe	31/9/7
Eyes, Mild/moderate/severe	33/3/1
GI tract, Mild/moderate/severe	2/6/1
Lung, Mild/moderate/severe	4/7/1
Muscle/joints, Mild/moderate	4/2
Genitalia, Mild/moderate	5/2
Kidney, Mild/moderate	2/2
cGVHD patients required systemic therapy	73.2%
Counts at the time of cGVHD diagnosis	
Platelets: median (range) $\times 10^9$/L	169 (18–482)
Patients with platelets count $< 100 \times 10^9$/L	32 (19.4%)
Eosinophils: median (range) $\times 10^9$/L	0.2 (0–3.550)

Low risk, first complete remission or chronic phase; high risk, relapse or progressive disease, blast crisis; intermediate risk, remaining cases; RIC, reduced intensity conditioning.

TABLE II. Prognostic Factors for the Development of Extensive and Severe cGVHD ($n = 165$)

	Cumulative incidence (%)	*P* univariate	HR (95% CI)	*P* multivariate
Variables at transplant				
Conditioning regimens		0.57		
RIC/myeloablative	70/68			
Sex mismatched (F → M)		0.28		
Yes/No	74/63			
Age (p75 = 57 years old)		0.83		
<p75/>p75	69/69			
CD34+ (p75 = 7.5×10^6/kg)		0.66		
<75/>75	74/67			
Variables at day +100				
Prior aGVHD		0.51		
Yes/no	77/68			
Platelets (p25 = 113×10^9/L)		0.68		
<p25/>p25	76/72			
ALC (p25 = 750/mm^3)				
$<0.750 \times 10^9$/L/$>0.750 \times 10^9$/L	83/65	0.05	2.26 (1.43–4.5)	0.021
Eosinophils (p75 = 176/mm^3)				
$<0.176 \times 10^9$/L/$>0.176 \times 10^9$/L	86/90	0.23		
LFT				
Bil + AP + GGT > 2 UNL	100	0.036	3.05 (1.37–6.7)	0.005
AP + GGT + TA > 2 UNL	88			
TA > 2 UNL	86			
<2 UNL	66			
Extensive cGVHD				
CD34+ (p75 = 7.5×10^6/kg)		0.70		
<75/>75	58/65			
Eosinophils (p75 = 176/mm^3)		0.44		
$<0.176 \times 10^9$/L/$>0.176 \times 10^9$/L	58/58			
Platelets (p25 = 113×10^9/L)		0.56		
<p25/>p25	66/56			
ACL (p25 = 0.750×10^9/L)				
$<0.750 \times 10^9$/L/$>0.750 \times 10^9$/L	77/51	0.06	3.06 (1.27–7.34)	0.012
LFT				
Bil + AP + GGT > 2 × normal	100	0.002	4.3 (1.9–9.73)	<0.001
AP + GGT + TA > 2 × normal	88			
TA > 2 × normal	72			
<2 × normal	50			
Severe cGVHD				
CD34+ (p75 = 7.5×10^6/kg)		0.33		
<75/>75	28/12			
Eosinophils (p75 = 176/mm^3)		0.88		
$<0.176 \times 10^9$/L/$>0.176 \times 10^9$/L	25/19			
Platelets (p25 = 113×10^9/L)		0.13		
<p25/>p25	36/19			
ALC (p25 = 0.750×10^9/L)				
$<0.750 \times 10^9$/L/$>0.750 \times 10^9$/L	60/18	0.034	7.22 (0.99–55)	0.053
LFT				
Bil + AP + GGT > 2 UNL	32	0.001	6.26 (1.7–23)	0.006
AP + GGT + TA > 2 UNL	50			
TA > 2 UNL	20			
<2 UNL	22			

LFT, liver function tests; Bil, total bilirubin; TA, transaminases; ALC, absolute lymphocyte count; UNL, upper normal limit; RIC, reduced intensity conditioning.

Myeloablative conditionings regimens consisted of cyclophosphamide 60 mg/kg × 2 days intravenously and fractionated total body irradiation (total 12 Gy) or busulfan 1 mg/kg four times daily for 4 days. Patients receiving reduced intensity conditionings were treated with fludarabine 30 mg/m^2/day on days −9 to −5 followed by either busulfan 1 mg/kg every 6 hr on days −6 and −5 and 1 mg/kg every 12 hr on day −4 or melphalan 70 mg/m^2/day on days −3 and −2 in patients diagnosed with myeloid or lymphoid malignancies, respectively. For GVHD prophylaxis, all patients received CsA 0.5 mg/kg every 12 hr from day −7 to −2 and 1.5 mg/kg every 12 hr from day −1 plus MTX 15 mg/m^2/day on day −1 and 10 mg/m^2 on days +3, +6, and +11, followed by folinic acid rescue. CsA taper was started around day +50 and stopped on day +180 in case no GVHD flared. Tacrolimus was used instead of CsA in case of cyclosporine-related toxicity.

The first line treatment for acute GVHD was based on the administration of 6-methyl-prednisonole at 2 mg/kg/day in case ≥ Grade II acute GVHD developed. The first line treatment for extensive chronic GVHD was CsA or tacrolimus plus prednisone at 1 mg/kg/day, which was switched to alternating days after 4 weeks of treatment.

Screening studies. Screening studies performed around day +100 post-transplant included physical examination; complete blood counts; and LFTs: total bilirubin (TBil), serum alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (GGT). The normal ranges (NR) for LFT were: TBil (0.1–1.2 mg/dl), AP (35–104 U/L), AST (1–32 U/L), ALT (1–31 U/L), and GGT (10–36 U/L). Pulmonary function testing and Schirmer's test were not included in the study because not all patients had data available. Skin and lip mucosal biopsies, which were performed in a subset of patients were excluded for this analysis since we focused this study on the evaluation of noninvasive techniques.

Statistical analysis. Data were analyzed from patients alive for more than 100 days after transplantation without relapse, and until the time of hematologic relapse, death, or last visit. In addition to day +100 screening studies, other factors analyzed were patient age, sex mismatched, conditioning regimens, CD34+ cell dose, histories, and grade of acute GVHD based on previous reports. Percentiles were calculated for complete blood count (platelets, lymphocytes, and eosinophils). The events analyzed were calculated from the time of transplantation using Kaplan–Meier product-limit estimates. The predictive value of the screening studies for the development of extensive and severe chronic GVHD was examined using univariate and multivariate time to an event analyses. Tests of significance were reported as two-tailed *P*-values. All the factors which significantly or marginally ($P < 0.1$) influenced the incidence of chronic GVHD in the univariate analysis were included in a multivariate analysis using a forward step Cox-regression model. Differences were considered to be statistically significant when *P*-values were <0.05. Statistical analysis was performed using SPSS software program (SPSS 15.0, Chicago, IL).

The cumulative incidence of acute GVHD was 35% for Grades II–IV and 9% for Grades III–IV. Chronic GVHD was diagnosed at a median day of 182 post-transplant (range: 90–1,150). The cumulative incidence of chronic GVHD was 67% among patients surviving more than 100 days after transplantation, with 56% categorized as having extensive cGVHD. The cumulative incidences of mild, moderate, and severe cGVHD were 34, 39, and

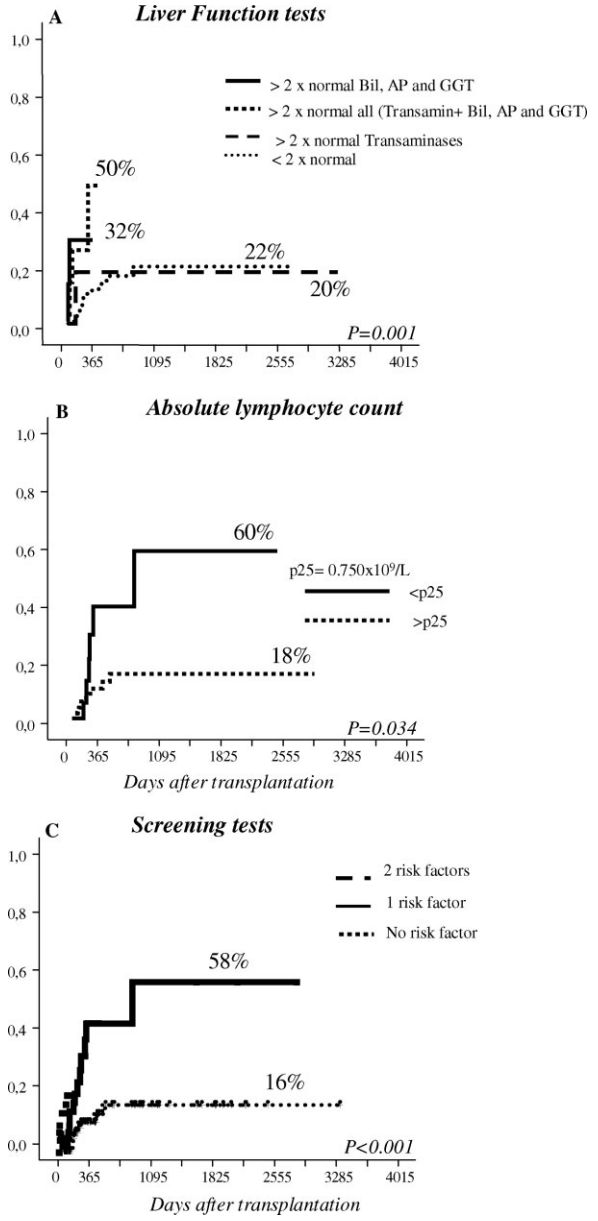

Figure 1. Cumulative incidence of severe chronic GVHD according to liver function tests (A), absolute lymphocyte count (B), and the combination of LFT and ALC [Bil, AP, and GGT > 2 upper normal limit (UNL) plus absolute lymphocyte count (ALC) < 0.750 × 10^9/L versus those patients with Bil, AP, and GGT <2 UNL and/or ALC >0.750 × 10^9/L] (C).

23%, respectively. In our experience, the overall survival (OS) at 5 years for patients displaying severe cGVHD and progressive type of onset after allo-PBSCT was 25% [5]. Table I shows the incidence and the characteristics of chronic GVHD. As shown in Table II, none of the variables at the time of transplantation significantly increased the risk of overall cGVHD. Among day +100 screening tests, in univariate analysis patients displaying abnormal LFT (total bilirubin, alkaline phosphatase, and GGT > 2 times above the upper normal limit with or without increased transaminases) and a low ALC (<percentile 25: 0.750 × 10^9/L) had a significantly higher risk of overall, extensive, and severe cGVHD. In this regard, the cumulative incidence of extensive cGVHD among patients displaying ALC < 0.750 × 10^9/L was 77% versus 51% for the rest of the patients ($P = 0.06$), whereas the incidence of severe cGVHD was 60% versus 18% ($P = 0.034$), respectively (Fig. 1B). Regarding LFTs, among patients with abnormal LFT, the cumulative incidence of extensive cGVHD was 100% as compared with 50% for the rest of the patients ($P = 0.002$), whereas the incidence of severe cGVHD was 50% versus 20% ($P = 0.001$), respectively (Fig. 1A). Other factors such as conditioning regimen, sex mismatched, patient age, CD34+ dose cell, eosinophils, or platelet count did not influence on the incidence of extensive or severe cGVHD. In multivariate analysis, as shown in Table II, ALC and LFT significantly influenced on the risk of overall, extensive, and severe cGVHD. To avoid the effect of acute GVHD on these parameters, a multivariate analysis was performed excluding patients who had developed acute GVHD or had active cGVHD at the time of screening or were receiving treatment with corticosteroids at day +100. Again, ALC and LFT did predict for the risk of extensive and severe cGVHD. On combining these two variables, the resulting score allowed to better estimate the risk of cGVHD: the cumulative incidence of extensive cGVHD in patients with two risk factors (TBil, AP, and GGT 2 × upper normal limit plus ALC < 0.750 × 10^9/L) was 100% as compared with 85% with one risk factor and 48% for patients without any risk factor ($P < 0.001$), whereas the incidence of severe cGVHD was 58% among patients displaying one or two risk factors versus 16% for patients without risk factors ($P < 0.001$) (Fig. 1C). When we included the combined variable in the multivariate analysis, it was the only prognostic parameter predicting overall [HR = 3.35 (95% CI: 1.65–6.83), $P < 0.001$], extensive [HR = 4.22 (95% CI: 1.96–9.12), $P < 0.001$], and severe cGVHD [HR = 8.17 (95% CI: 2.55–26.17), $P = 0.002$], respectively.

Previous studies have identified a chronic myeloid leukemia diagnosis, sex mismatched, age [6], and an early complete donor hematopoietic chimerism in peripheral blood [7] as risk factors for the development of extensive cGVHD. Despite these few studies, information on the value of noninvasive screening tests to predict the risk of extensive or severe cGVHD development after PBSCT is lacking, and considering its wide use as the preferred cell source and the higher incidence of cGVHD in this setting as compared with bone marrow transplant [8–10], it would be desirable to identify noninvasive parameters, which predict the risk of extensive or severe cGVHD development to allow early intervention before clinical deterioration GVHD onset. In this study, abnormal LFTs (TBil, alkaline phosphatase, and GGT > 2 times above the normal value with or without increased transaminases) and a low-absolute lymphocytes count (<0.750 × 10^9/L) did predict for the risk of cGVHD and its severity. Although liver dysfunction by itself is not usually the ultimate cause of GVHD-related mortality, some authors [11] have reported that acute liver GVHD increased the risk of cGVHD-related death in both the allo-PBSCT and allo-BMT settings. Nevertheless, in this study, TBil, AP, and GGT abnormalities did predict for a higher risk of extensive and severe cGVHD development both in patients with or without prior aGVHD irrespective of transaminase values. Considering that it is a very simple, fast, and costless test, TBil, AP, and GGT values at day +100 should represent a most helpful screening test to be considered in all patients after allo-HSCT.

Contrary to previous reports, we did not identify aGVHD as a predictive factor for extensive or severe cGVHD. Some of these studies did not exclude patients with active GVHD and or on steroids what could impact the results of such analysis [12–15]. In this regard, Atkinson et al. [16] have reported that the 3 years risk of the development of cGVHD was 28% ± 3%, 49% ± 5%, 59% ± 6%, 80% ± 9%, and 85% ± 15% for patients with Grades 0, I, II, III, and IV aGVHD, respectively ($P < 0.0001$), while among patients with no or Grade I aGVHD, prior aGVHD did not predict the subsequent development of cGVHD. It is worth mentioning that in the current series of patients 50% had Grade I or no aGVHD and only 9% developed Grades III–IV aGVHD. This could explain the lack of correlation between acute and chronic GVHD in our work.

We did not identify a low-platelet count as a risk factor for extensive or severe cGVHD development. Nevertheless, thrombocytopenia (<100 × 10^9/L) has been widely described as a surrogate marker of cGVHD severity after allogeneic transplantation [11,15,17]. By contrast, we identified low ALC (<0.750 × 10^9/L) as a risk factor for predicting the development of extensive and severe cGVHD, which has not been described to date. To rule out the potential confounding effect of aGVHD-related therapy [18], the analysis was performed excluding patients which were on steroids at day +100 or had prior aGVHD. Several reports have pointed out that patients with a slower lymphocyte recovery after transplantation had a poorer outcome [19–24] and, in this regard, Pavletic et al. [20] have previously shown that a faster lymphocyte recovery did correlate with better survival after allo-PBSCT, although these studies did not focus on the development of extensive or severe cGVHD. The correlation between low-lymphocyte counts and subse-

quent development of extensive or severe cGVHD could reflect the fact that alloreactive T-cell clones induce a cytotoxic effect not only on cGVHD target organs but also on the T-cell clones, which may react against pathogens, explaining both the immunosuppression induced by cGVHD and the narrow TCR repertoire observed among patients with cGVHD. Thus, the patients who developed chronic GVHD have a lower average score of TCR-Vβ complexity than that of patients without cGVHD [25]. In this regard, it has recently been reported that a high natural killer cell reconstitution at day +60 after transplantation is associated with reduced relapse and death after reduced intensity conditioning without an increased incidence of GVHD [26]. These studies indicate that an early immune reconstitution have prognostic implications after allogeneic transplantation. Unfortunately, the retrospective nature of our study precludes a detailed analysis of lymphocyte subsets, which would have been more informative in understanding some of the mechanisms behind this observation.

In summary, in this article, we have shown that an increased total bilirubin, alkaline phosphatase, and gamma glutamyltransferase levels together with the low ALC at day +100 are a noninvasive simple, fast, and accurate tests to predict the risk of extensive and severe cGVHD after allogeneic peripheral blood stem cell transplantation from matched related donor. Further studies are required to evaluate its prognostic value in other patients' populations such as those undergoing unrelated donor transplant or bone marrow transplant.

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Servicio de Hematología, Hospital Clínico Universitario y Centro de Investigación del Cáncer (CIC/CSIC) de Salamanca, Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León, Salamanca, Spain
**Correspondence to: Jose A. Pérez-Simón, Servicio de Hematología, Hospital Universitario de Salamanca, Paseo de San Vicente s/n, 37007 Salamanca, Spain. E-mail: pesimo@usal.es*
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4.2 Nuevas opciones terapéuticas encaminadas a evitar el tratamiento sistémico con esteroides en la EICHc

4.2.1 Tratamiento de la EICHc digestiva con beclometsona

Oral beclomethasone dipropionate for the treatment of gastrointestinal chronic graft-versus-host disease. Biol Blood Marrow Transplant 2009; 15: 1331-1336.

El tratamiento estandar de la EICHc se basa en el uso de inmunosupresores asociados a corticoesteroides sistémicos durante largos periodos. La beclometasona es un corticoesteroide con una escasa absorción sitémica y que ejerce su acción a nivel local en la mucosa gastrointestinal. Ha sido utilizado con buenos resultados en el tratamiento de la EICH aguda sin embargo su uso en la EICHc es limitado. En este trabajo se describe el efecto de la beclometasona en el tratamiento de la EICH crónica digestiva en un grupo de 33 pacientes sometidos a trasplante de progenitores hematopoyéicos que presentaban EICHc digestiva comprobada por biopsia gastrointestinal. De acuerdo con la clasificación del NIH 12, 17 y 4 pacientes presentaban EICHc leve, moderda y severa respectivamente. En 26 pacientes fue tratamiento de primera línea y en 7 de segunda o tercera línea. Todos los pacientes recibieron beclometasona y un inhibidor de calcineurina excepto 1 paciente que además, estaba recibibiendo mofetil micofenolato. En los pacientes con EICHc moderada y severa los motivos de administrar la beclometasona como tratamiento de primera línea en lugar de un tratamiento estándar con prednisona sistémica fueron: riesgo elevado de recaída de la enfermedad de base (enfermedad activa incluyendo enfermedad minima residual positiva ó quimerismo mixto n=14), historia previa de infección fungica (n=1), reactivación del virus de la hepatitis B (n=1), historia previa de toxicidad relacionada a corticoides (n=2) ó por preferencia del clínico asistente (n=3). Se administró por un mínimo de 16 semanas seguida de reducción hasta suspender durante 4 semanas más. Entre los pacientes que recibieron como primera línea de tratamiento 22 (84,6%) presentaron remisión completa de la EICHc gastrointestinal, 2 (7,7%) tuvieron respuesta parcial y 2 (7,7%) no respondieron

o progresaron. El tiempo medio para la obtención de respuesta fue de 28 días. En el momento del último seguimiento 7 (27%) pacientes tenían respuesta mantenida y 19 (73%) presentaron recaída o progresión de la EICHc digestiva. El tiempo medio para la recaida al finalizar el tratamiento con beclometasona fue de 147 días. Entre los pacientes que la recibieron como 2ª o 3ª línea de tratamiento, 3 (42,9%) alcanzaron remisión completa y 2 (28,6%) respuesta parcial. Globalmente en 13 (39,4%) pacientes se pudo suspender el tratamiento inmunosupresor. Respecto a las complicaciones infecciosas, 4 pacientes presentaron reactivación del citomegalovirus y por tal motivo recibieron terapéutica antivirica con éxito. Ningún paciente desarrolló infección fungica durante el periodo de tratamiento. Conclusión: la beclometasona es eficaz como terapéutica inicial de la EICHc digestiva evitando el uso de corticoesteroides sistémicos.

BRIEF ARTICLES

Oral Beclomethasone Dipropionate for the Treatment of Gastrointestinal Chronic Graft-versus-Host Disease

Fernanda N. Villanueva, Jose Antonio Pérez-Simón, Fernando F. Silva, Teresa T. Caballero-Velázquez, Fermin F. Sánchez-Guijo, Conzuelo C. Cañizo, Lourdes L. Vázquez, Dolores D. Caballero, Jesús F. San Miguel

The most common approach for the treatment of chronic graft-versus-host disease (cGVHD) has been the long-term use of systemic steroids. Beclomethasone dipropionate (BDP) is a topically active corticosteroid with relatively low absorption from the gastrointestinal mucosa. It has been successfully used to treat acute GVHD (aGVHD), but its use in the cGVHD setting is far more limited. In the current study, BDP was administered to 33 patients who underwent allogeneic transplantation and had biopsy-proven gastrointestinal cGVHD (GI cGVHD). Twenty-six patients with GI cGVHD received BDP as first-line and 7 as either second- or third-line treatment. All patients received BDP together with a calcineurin inhibitor, except for 1 patient who was also receiving mycophenolate mofetil (MMF). BDP was administered for a minimum of 16 weeks and was tapered during 4 additional weeks. Of those patients receiving BDP as the first line of treatment, 22 (84.6%) achieved complete remission (CR) of GI cGVHD, 2 (7.7%) achieved a partial response (PR) and 2 (7.7%) did not respond or progressed. Median time to response was 28 days. Nevertheless, only 7 (27%) patients had maintained the response at last follow-up, whereas 19 (73%) finally relapsed or progressed. Median time to relapse was 147 days after the end of BDP. In the case of the patients who received BDP as a second- or third-line treatment, 3 (42.9%) achieved CR and 2 (28.6%) PR. For the whole series of patients, 13 patients (39.4%) were not receiving immunosuppressive treatment at final follow-up. Only 4 patients developed cytomegalovirus (CMV) reactivation, which was successfully treated with antiviral drugs. No fungal infection was observed during the treatment period. In conclusion, the current study shows that BDP, in the absence of systemic steroids, is a highly effective initial therapeutic approach for GI cGVHD, which helps to avoid complications related to systemic steroids.

Biol Blood Marrow Transplant 15: 1331-1336 (2009)

KEY WORDS: Gastrointestinal chronic graft-versus-hot disease, cGVHD, Beclomethasone dipropionate, BDP, Allogeneic stem cell transplantation

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) is a major complication after allogeneic hematopoietic stem cell transplantation (HSCT) [1]. Its incidence has increased over the past few years because of the older age of the patients, the use of peripheral blood as a source of progenitor cells, and the use of alternative donors [2].

Gastrointestinal (GI) GVHD affects up to 60% of patients after HSCT [3]. In the cGVHD setting, diagnostic features for the GI tract include esophageal web, stricture, or concentric rings documented by endoscopy or a barium contrast radiograph [4]. Symptoms of anorexia, nausea, vomiting, and diarrhea are not considered diagnostic of cGVHD, but are common symptoms in patients with the condition. Wasting syndrome can be a manifestation of cGVHD, but is often multifactorial and may result from decreased caloric intake, poor absorption, increased resting energy expenditures, and hypercatabolism, for example [5]. Intestinal involvement is usually more severe and difficult to treat compared with other target organs. In this regard, the Karnofsky score, presence of chronic diarrhea, weight loss, and skin involvement, allowed 3 subgroups of patients to be distinguished with respect to

From the Servicio de Hematología, Hospital Clínico Universitario de Salamanca y Centro de Investigación del Cáncer, Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y Leon, Salamanca, Spain.

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Correspondence and reprint requests: Jose A. Pérez-Simón, MD, PhD, Servicio de Hematología, Hospital Clínico Universitario, Paseo de San Vicente s/n, 37007 Salamanca Spain (e-mail: pesimo@usal.es).

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different survival in an International Bone and Marrow Transplant Research (IBMTR) study [6].

The most common approach for the treatment of cGVHD has been the use of prednisone. When used as a single agent, 3-year survival reported among high-risk patients [7], identified as those with extensive cGVHD plus thrombocytopenia, reached 26%. In this subset of patients, the addition of cyclosporine A (CsA) increased survival to 52% [8]. By contrast, the combination therapy did not improve the results of prednisone as a single agent among patients undergoing bone marrow transplantation (BMT) who developed standard-risk cGVHD [9]. The risks of this prolonged immunosuppression include viral and fungal infections, hypothalamic-pituitary-adrenal (HPA) axis suppression, myopathy, glucose intolerance, neuropsychiatric disease, and bone demineralization [10].

Beclomethasone dipropionate (BDP) is a topically active corticosteroid with relatively low absorption from the GI mucosa into systemic circulation compared with oral prednisone. BDP is metabolized in the intestinal mucosa and the liver. The active metabolite, 17-BMP, has an approximately 25-fold greater glucocorticoid-receptor binding activity than BDP [11,12]. In fact, BDP does not appear in the systemic circulation because of its metabolism in the intestinal mucosa and the liver, although 17-BMP can be detected in the blood stream [13,14]. Accordingly, adverse systemic effects are limited by incomplete absorption and intestinal hydrolysis of the propionate residues and by rapid clearance from the circulation [14,15]. Oral BDP has demonstrated activity in GI acute GVHD (aGVHD) [16,17] either alone [18] or in combination with prednisone at 1 mg/kg. In this patient population, BDP reduced the exposure to systemic corticosteroids, was associated with fewer infections and, possibly, preserved graft-versus-tumor (GVT) effects, yielding a statistically significant improvement in survival in a randomized, multicenter clinical trial [19].

Despite the deleterious effect of long-term exposure to systemic steroids in the cGVHD setting, the information available in the literature on the effectiveness of BDP in gastrointestinal cGVHD is limited to 13 patients. In this series of patients, BDP was shown to be safe and effective, although multiple courses might have been necessary to achieve or maintain response in some patients [20]. In the present report we describe the safety and efficacy of BDP as a treatment in a series of patients diagnosed with GI cGVHD.

MATERIALS AND METHODS

Patient Characteristics

BDP was administered to 33 patients who underwent allogeneic peripheral blood stem cell transplantation (PBSCT) and had biopsy-proven GI GVHD and clinical symptoms of cGVHD that developed after 100 days following transplantation. Patients were able to swallow medication and had confirmed negative stool cultures. Patient characteristics are summarized in Table 1.

Patients were classified according to National Institutes of Health (NIH) consensus criteria [21]. Diagnostic criteria were based on the clinical features, although confirmatory biopsies were available for all patients evaluated. No patient had esophageal involvement.

Twenty-six patients with GI cGVHD received BDP as first-line and 7 as either second- or third-line treatment. As before, symptoms consisted of nausea/vomiting in 13 patients, diarrhea in 12, anorexia and/or malabsorption plus weight loss in 9, and abdominal pain in 6 patients. As shown in Table 2, skin or mucosal involvement was also observed and, for these patients,

Table 1. Patients and Transplant Characteristics

	Patient characteristics (N = 33)
Age	
median (range)	33 (18-56)
CD34 cell dose	
median (range)	5.35 (1-6.26)
Diagnosis:	
AML	11
ALL	3
CML	2
CLL	3
MDS	4
NHL	6
HD	1
MM	2
Others	1
Disease status at transplant	
First CR	15
≥2nd CR	5
Chronic phase	2
Partial response	6
Refractory/progressive disease	2
Untreated	2
Others	1
Sex	
Male/female	20 / 13
Type of donor	
Related	21
Unrelated	11
Cord blood	1
Conditioning	
Myeloablative	10
Reduced intensity	23
GVHD prophylaxis	
CsA plus MTX	25
ATG or CAMPATH	4
CsA plus MMF	4

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndromes; NHL, nonHodgkin lymphoma, HD, Hodgkin disease; MM, multiple myeloma; CR, complete remission; GVHD, graft-versus-host disease; ATG, antithymocyte globulin; CsA, cyclosporine; MMF, mycophenolate motefil; MTX, methotrexate.

The infectious prophylaxis consisted of Trimethoprim-Sulfamethoxazole and Acyclovir.

a topical treatment was administered. In addition, 9 patients had liver function test abnormalities without biopsy-proven GVHD, for whom ursodeoxycholic acid was administered. Patients receiving BDP as second- or third-line therapy had already received systemic steroids.

BDP Treatment and Response Assessment

BDP was prepared as an emulsion of 250 mg of BDP in 500 mL olive oil. It was administered at a dose of 4 mL (2 mg) every 6 hours for a minimum of 16 weeks, with tapering during 4 additional weeks. Disease response was assessed at 4, 10, and 20 weeks after the beginning of the treatment. cGVHD response was assessed as recommended by the NIH Consensus Development Project [21]: no response or progression was defined as >25% worsening of cGVHD, partial response was defined as >50% improvement, and complete remission (CR) was recognized as the resolution of all signs and symptoms. Relapse was defined as recurrence of symptoms after a CR once treatment had been stopped.

Statistical Analysis

Events analyzed were calculated from the time of transplantation using Kaplan-Meier product-limit estimates. Treatment-realated mortality (TRM) was defined as death because of causes unrelated to the underlying disease and relapsing patients were censored at the time of relapse. GVHD-related mortality was defined as death because of causes directly related to GVHD, and deaths attributed to immunosuppression in patients requiring treatment for GVHD were also considered as GVHD-related mortality. Overall survival (OS) was calculated from transplantation until death from any cause, and surviving patients were censored at last follow-up. The day of cGVHD was calculated from transplantation to a diagnosis of cGVHD. For statistical analyses, SPSS 10.0 (SPSS Inc, Chicago, IL) was used. Differences were considered to be statistically significant for values of $P < .05$.

RESULTS

Of the 33 patients included in the study, cGVHD flared at a median of 157 days (range: 95-1145 days). All patients were considered as having extensive cGVHD. Twenty-three patients had de novo, 6 patients had quiescent, and 4 progressive onset types of cGVHD.

Five patients were considered as having an overlapping syndrome. The degree of severity according to the NIH scale was mild in 12 patients, moderate in 17 patients, and severe in 4 patients. Patients with moderate or severe cGVHD received BDP because of a high risk of relapse, considered as either active disease including minimal residual disease (MRD) or mixed chimerism at the time of treatment (n = 14), prior history of fungal infection (n = 1), hepatitis B virus (HBV) reactivation at the time of treatment (n = 1), prior history of steroid-related toxicity (n = 2) or as the preference of the attending physician (n = 3). Twenty-six patients received BDP as a first-line treatment, 3 patients as a second-line, and 4 patients as a third-line treatment. All patients received BDP in conjunction with a calcineurin inhibitor plus topical treatment on the skin, oral mucosa, or eyes when required, except for 1 patient who was also receiving mycophenolate mofetil (MMF). Organ involvement is specified in Table 2.

Of those patients receiving BDP as first-line treatment 22 (84.6%) achieved CR of the GI cGVHD, 2 (7.7%) achieved a partial response (PR), and 2 (7.7%) did not respond or progressed. Median time to response was 28 days (range: 7-137 days). Nevertheless, only 7 (27%) patients maintained the response at

Table 2. Organ Involvement at the Time of BDP Treatment

	Patients: N (%)
Organ involvement among patients receiving BDP as first-line treatment	
Skin	
Mild	6%
Moderate	1%
Oral mucosa	
Mild	11%
Eyes	
Mild	3%
Moderate	1%
Gut	
Mild	22%
Moderate	4%
Gastrointestinal symptoms	
Nausea/vomiting	13%
Diarrhea	12%
Anorexia/malabsortion/weight loss	9%
Abdominal pain	6%
Liver	
Mild	5%
Organ involvement among patients receiving BDP as greater than first-line treatment	
Skin	
Mild	3%
Moderate	1%
Severe	1%
Oral mucosa	
Mild	7%
Eyes	
Mild	7%
Moderate	1%
Gut	
Mild	7%
Moderate	3%
Severe	1%
Gastrointestinal symptoms	
Nausea/vomiting	7%
Diarrhea	4%
Anorexia/malabsorption/weight loss	7%
Abdominal pain	4%
Liver	
Mild	4%

BDP indicates beclomethasone dipropionate.

final follow-up, whereas 19 (73%) finally relapsed or progressed. Median time to relapse was 147 days after the end of BDP (range: 35-736 days). At the time of relapse 2 patients were categorized as having limited and 17 extensive cGVHD.

In the case of the patients who received BDP as a second- or third-line treatment, 3 (42.9%) obtained CR and 2 (28.6%) PR, whereas 2 patients (28.6%) did not respond or progressed. Median time to response was 45 days (range: 11-107 days). All patients eventually relapsed at a median time of 231 days after the end of BDP (range: 11-311 days). At the time of relapse, 1 patient was considered to have limited and 6 had extensive cGVHD. At the time of relapse, 4 had moderate and 3 had severe cGVHD. Of the entire series of patients 13 (39.4%) were not receiving immunosuppressive treatment.

No differences in response were found between the use of tacrolimus or CsA in combination with BDP. Among patients who relapsed after first-line treatment, 7 patients received BDP plus calcineurin inhibitor and 3 received BDP as a single agent. In the first group, 6 patients achieved a CR in the gut and 1 could not be evaluated, whereas in the latter group, 2 achieved a CR and 1 a PR.

No significant differences in response were observed upon comparing patients categorized as having mild or moderate cGVHD. Thus, 83% of patients with mild compared with 76% of those with moderate cGVHD achieved complete remission ($P = .39$).

Toxicity and Outcome

With respect to treatment-related toxicity, 2 of the 33 patients included in the study developed Cushing-like syndrome, 1 patient developed hyperglycemia, 2 patients developed musculoskeletal pain associated with the BDP taper, and 1 patient developed nausea that was probably related to the drug. During the treatment period, only 4 patients developed CMV reactivation, which was successfully treated with antiviral drugs. No fungal infection was observed and galactomannan assays were negative.

With a median follow-up of 950 days (range: 158-1554 days) OS and event-free survival (EFS) were 78% and 55% at 5 years, respectively (Figure 1).

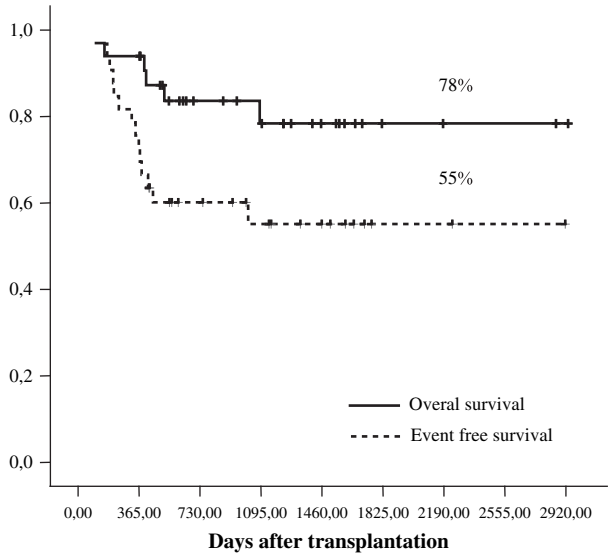

Figure 1. Overall and event free survival.

DISCUSSION

Several studies have addressed the role of BDP in treating aGVHD. In a randomized trial, the use of BDP in combination with prednisone at 1 mg/kg reduced GVHD treatment failures from 65% in the placebo arm to 39% in the BDP group ($P = .003$). During the 80-day study period, there was additional evidence of clinical benefit in the BDP arm, largely as a result of the decreased need for protracted prednisone dosing [14].

In our own experience [18], the use of BDP without systemic steroids yielded a 77% response rate in a series of 26 patients diagnosed with GI aGVHD, with 65.5% of patients achieving CR. At final follow-up, 50% of the 26 patients did not require systemic steroids to treat GI aGVHD.

In the cGVHD setting, the use of drugs with topic effect in target organs should be of great importance for avoiding systemic exposure to steroids, which remain the gold standard of care for these patients. Indeed, many patients diagnosed with cGVHD finally die, not because of cGVHD itself, but to infectious complications secondary to the immunosuppressive effect of drugs administered to control it [2,14,22]. Moreover, toxicity associated with long-term treatment steroids, such as myopathy, osteoporosis, hyperglycemia, weight gain with the characteristic redistribution of body fat, growth retardation in children, psychiatric disorders, or avascular hip necrosis, also hamper the quality of life of the patients. To complicate its management further, many studies have shown cGVHD to be a favorable prognostic factor in terms of survival because of a powerful graft-versus-leukemia (GVL) effect that contributes to the lower relapse rate observed in patients who develop it [23-25]. For this reason, immunosuppressive treatment must be carefully administered, not only on the basis of the severity of cGVHD, but also taking into account the risk of relapse and the disease status at the time of treatment [26]. Under these conditions, it would be desirable to develop strategies that allow cGVHD to be controlled, but avoid the long-term exposure to steroids. BDP therefore represents an interesting alternative.

Only 1 study reported by Iyer et al. [20] has so far evaluated the use of BDP in the GI cGVHD. In this study, 13 patients with GI cGVHD and 2 with aGVHD were analyzed. All patients but 1 had received methylprednisolone at 2 mg/kg/day as prior therapy for GI cGVHD and had no symptom relief. Nine (60%) patients responded to BDP as measured by improvement or complete resolution of symptoms and the ability to taper steroids. There were 20% complete and 40% partial responses.

In the current study, we observed 84.6% CRs and 7.7% PRs among patients receiving BDP as first-line treatment, whereas these figures were 42.9% and 28.6%, respectively, among patients receiving it as more than first-line treatment. Our results illustrate the efficacy of BDP as a first-line treatment, with an impressive 84.6% CRs in this subset of patients. For those patients who received BDP as a second- or third-line treatment, which is a population more similar to the series previously reported [20], 42.9% of the patients achieved CR. It is worth mentioning that, unlike the patients analyzed by Iyer et al. [20], who had no symptom relief after receiving 2 mg/kg/day methylprednisolone, patients included in the current study were already off systemic steroids at the time of cGVHD relapse, thus representing a population with a better prognosis.

Despite this high response rate, a high relapse rate was observed in the current study. Nevertheless, most relapses occurred after BDP discontinuation and, considering that standard therapy is usually maintained for at least 9 months, the use of BDP for 16 weeks with an additional 4 weeks of tapering could have been too short a period to ensure the maintenance of responses. Moreover, all patients in the current series had received peripheral blood as a source of progenitor cells and, according to previous studies, cGVHD relapses occur at a high frequency in this subset of patients [9,22]. In this context, Flowers et al. [27] reported a relapse rate ranging from 61% to 84% among patients diagnosed with GVHD after peripheral blood allogeneic transplantation. Despite the high rate of recurrence, in our series of patients, 39.4% of our patients were finally free of immunosuppressive treatment.

In addition, our study documents a low toxicity profile, with only 2 cases of Cushing's syndrome, 1 case of hyperglycemia, and 2 cases of musculoskeletal pain during the period of BDP taper, which suggests some degree of absorption of the drug. Corticosteroid activity studies evaluating treatment with BDP have not revealed any important secondary effects related to infectious disease, although partial HPA axis suppression is possible [14,18]. Metabolites of BDP are systemically bioavailable, resulting in decreased adrenal responsiveness during the period of drug exposure [20,28]. Recent studies of long-term use of oral, topically active corticosteroids have demonstrated little evidence of clinical adrenal insufficiency [20,29]. Nevertheless, 3 published series [14,18,19] did not produce any evidence of HPA axis suppression in patients with oral BDP treatment for GI GVHD, and clinical responses to this treatment suggest that absorption is not necessary for efficacy.

In conclusion, the current study shows that BDP, in the absence of systemic steroids, is a highly effective initial therapeutic approach for GI cGVHD. This helps to avoid complications related to systemic steroids, although the final duration of treatment remains to be determined.

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4.2.2 Papel de la vitamina D en el control de la EICHc

Effect of vitamin D treatment in the chronic graft-versus-host disease. Aceptado para publicación en Bone Marrow Transplantation

Estudios in vitro y en modelos animales han demostrado que la vitamina D (vit D) tiene un potente efecto inmunomodulador. En el contexto del TPH se usa asociada al calcio en la prevención y tratamiento de la osteoporosis y osteopenia relacionada con el uso de inmunosupresores y corticoesteroides sistémicos sin embargo no hay información con relación a su uso en el tratamiento de la EICHc. En este trabajo hemos evaluado de forma retrospectiva, el efecto del tratamiento con vitamina D en la EICHc en un grupo de 12 pacientes que estaban recibiendo dicho tratamiento por vía oral debido a osteoporosis u osteopenia y que presentaban EICHc refractaria o en recaída. A los tres y seis meses tras el inicio de la vit D sin haber añadido fármacos inmunosupresores, 3 y 5 pacientes obtuvieron respuesta completa, respectivamente. Además, a los seis meses tras el inicio de la vit D 5 pacientes estaban libres de cualquier tratamiento inmunosupresor sistémico. Finalmente en 11 de los 12 pacientes fue posible reducir o suspender el tratamiento inmunosupresor durante este periodo; no se encontraron efectos adversos relacionados con el uso de la vit D. Por tanto, el presente estudio sugiere que la vit D mediante su acción inmunomoduladora puede ser útil en el tratamiento de la EICHc.

Title:

Effect of vitamin D treatment in the chronic graft-versus-host disease

Authors:

Fernando Silva[1], José A Pérez-Simón[1,2], Teresa Caballero-Velazquez[1], Fermín M Sánchez-Guijo[1], Fernanda Villanueva-Gomez[1], Lourdes Vazquez[1], Consuelo del Cañizo[1], Dolores Caballero[1], Jesús San Miguel[1]

Institution:

(1) Servicio de Hematología, Hospital Clínico Universitario y Centro de Investigación del Cáncer (CIC/CSIC) de Salamanca, Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León, Spain; (2) current address: Servicio de Hematología; Instituto de Biomedicina de Sevilla (IBIS) Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla

Corresponding author:

Jose A Pérez-Simón, MD, PhD

Department of Hematology

Instituto de Biomedicina de Sevilla (IBIS) Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla

e.mail: josea.perez.simon.sspa@juntadeandalucia.es

Key words: vitamin D, chronic graft -versus-host disease

Short title: VitD and cGVHD

Currently there is no standard approach for patients with chronic graft versus host disease (cGVHD) who do not respond or relapse after first line treatment and rescue therapy is based on immunosuppressive drugs and

glucocorticoids which are responsible for the development of severe complications[1]. Vitamin D (VitD) has a potent immunomodulatory effect as shown in vitro and in animal models, nevertheless there is no information about its use in the cGVHD setting. We evaluated retrospectively the outcome of cGVHD in a series of 12 patients receiving vitD due to proved osteopenia or osteoporosis by bone densitometry after allo-HSCT. These patients also had active cGVHD at the time when vitD was started. We observed a marked improvement in cGVHD for most of these patients without appreciable secondary effects.

Chronic GVHD was classified as limited versus extensive and also the National Institutes of Health (NIH) scoring system[2] was used based on the data collected from patients medical files. The first line treatment for cGVHD was CsA or tacrolimus plus prednisone at 1 mg/kg/day which was switched to alternating days after 4 weeks of treatment. Patients characteristics are summarized in table 1.

According to our standard procedures patients undergoing allo-HSCT have a bone densitometry performed between 6 months and 1 year after transplantation in order to rule out osteopenia or osteoporosis. In case it is detected, vitamin D 1000 IU per oral daily plus calcium carbonate 1250 mg 1 pill per oral daily are prescribed for at least 6 months. Chronic GVHD response was assessed according with NIH response criteria[3] at 3 and 6 months after the beginning of vitD plus calcium treatment. For skin involvement a partial response(PR) was considered to have occurred when at least 50% of the skin involvement appeared to be non-inflammatory or fixed and for complete response(CR) as either the disappearance of all lesions or the presence of fixed and pigmented lesions.

Chronic GVHD was diagnosed at a median day of 147 post-transplant (range: 119 to 491 days). At the time when vitD was started six patients had received one line of immunosuppressive treatment, three patient two lines, two patients three lines and one patient four lines. Seven patients had mild, two moderate and three severe cGVHD; organ involvement is summarized in table

2. Three months after vitD treatment 3 patients obtained CR, 6 PR and 1 had no response with six patients displaying mild and one severe cGVHD. Finally, 6 months after treatment five patients obtained CR, six PR and one had no response with six displaying mild and one moderate cGVHD. No immunosuppressive drugs were added to the treatment during this period. Interestingly, at the beginning of vitD treatment 10 patients were receiving calcineurin inhibitors, 1 patient was receiving calcineurin inhibitor plus prednisone and 1 patient was receiving mofetil mycophenolate plus prednisone. After 6 months of vitD treatment five patients were not receiving immunosuppressive drugs while seven patients were receiving immunosuppressive treatment based on CsA or tacrolimus (with or without topic treatment in five patients and with other systemic immunosuppressive drug in two patients). We compared patients who received vitD and had not previously relapsed (n=6) to a cohort of 24 patients transplanted during the same period of time who had not received vitD and were on first line treatment for cGVHD and had similar characteristics concerning GVHD severity and extension. Interestingly 50% of the patients receiving vitD were off immunosuppresion 6 months after the beginning of treatment as compared to 20% among those who did not receive vitD (p=0.1).

Remarkably, six patients had a history of previous relapses of cGVHD prior to vitD treatment (two of them relapsed after the end of immunosuppression while four relapsed during taper of CsA or tacrolimus). After vitD treatment only three out of these patients had cGVHD relapse, one of them occurring during the taper of CsA.

VitD is a fat-soluble prohormone, the two major forms being ergocalciferol ($vitD_2$) and cholecalciferol ($vitD_3$). The most important sources of vitD in humans are the sunshine, foods and supplementation. The active form of vitD is 1,25-hydroxyvitamin D. VitD is essential for optimal skeletal development, maintenance of bone health and neuromuscular function. It is used in conjunction with calcium in the management and prevention of primary or corticosteroid-induced osteoporosis. Most tissues and cells including peripheral blood mononuclear cells[4,5] possess a vitD receptor (VDR), and many have the ability to convert 25-hydroxyvitamin D to 1,25 hydroxyvitamin D. In

vitro data indicate that vitD inhibits dendritic cell-dependent T cell activation, T-cell proliferation and decreases the production of type-1 helper cells (Th1) cytokines IL-2, IFNγ and TNFα, thus displaying a very potent immunomodulatory effect[6,7].

Previous reports describe the efficacy of a vitD analog MC1288 in preventing acute GVHD (aGVHD) in a rat bone marrow transplant model[8] as well as the relationship between VDR gene polymorphism and aGVHD and cGVHD[9]. The effects of vitD are mediated by the nuclear VDR. It is constitutively expressed in monocytes, and in both B and T activated lymphocytes. The effect of vitD on dendritic and T cells were evaluated by Rosenblatt et al which demonstrated the inhibitory effect of vitD on T cells proliferation or in the production of Th2 cytokines[10]. In spite of these data, there is a lack of information regarding the use of vitD in the GVHD setting. For this reason, we analyzed patients who were receiving immunosuppressive treatment and required vitD due to osteoporosis or osteopenia and compared their outcomes prior to and after this treatment. No other immunosuppressive drugs were added during the whole period. Interestingly, we found an important improvement in the severity of cGVHD so that at 6 months after vitD treatment no patients displayed severe cGVHD versus 3 at the beginning. Moreover, at that time 5 patients had complete remission and were not receiving immunosuppressive treatment. In addition we observed a remarkable reduction of cGVHD relapses or progressions. Accordingly, 9 out of 12 patients had no relapse/progression(table 2).

In conclusion, treatment with vitD appears to be effective, safe and inexpensive for the management of patients with cGVHD. The current study establishes the basis for further studies with a larger number of patients to better assess the potential immune-modulatory effect of vitD on the cGVHD setting.

Table 1. Patients characteristics at transplant

	Number of patients (N)=12
Diagnosis	
AML	3
CLL	3
CML	1
MDS	3
NHL	1
HD	1
Disease status at transplant	
1st CR	4
≥ 2nd CR	3
PR	2
Progresive disease	3
Conditioning	
Reduced intensity	8
Myeloablative	4
Type of donor	
Related donor	9
Unrelated donor	3
Age: median (range)	46 (23 – 68 years)
Sex: male / female	1 /11

AML Acute Myeloid Leukemia**; CLL** Chronic Lymphocytic Leukemia; **CML** Chronic Myeloid Leukemia; **CR** Complete Response; **HD** Hodgkyn Disease; **MDS** Myelodisplastic Síndrome; **NHL** Non-Hodgkyn Lymphoma; **PR** Partial Response

Table 2. Patients outcome prior to and after vitamin D treatment

Patient number	Relapse prior to vitD (Nº)	cGVHD extension		NIH		cGVHD status		Organ involvement		IS treatment		Relapse after vitD treatment (Nº)
		At the time of vitD start	*At 6 months*	*At the time of vitD start*	*At 6 months*	*At the time of vitD start*	*At 6 months*	*At the time of vitD start*	*At 6 months*	*At the time of vitD start*	*At 6 months*	
1	Yes (4)	Ext	Lim	Mod	Mild	SD	PR	E+S	S	CyA+ topic	CyA+ topic	No
2	No	Lim	-	Mild	-	SD	CR	E+O	-	CyA+ topic	-	No
3	Yes (3)	Lim	Lim	Mild	Mild	SD	PR	Eo+H+O	O	CyA+ topic	CyA	No
4	No	Lim	Ext	Mild	Mod	PR	PR	O+S	L+O	CyA+ topic	CyA+ MMF	No
5	Yes (1)	Ext	-	Sev	-	PR	CR	E+L	-	Fk506+ Prd+topic	Fk506	Yes(1)
6	No	Lim	-	Mild	-	SD	CR	S	-	Fk-506+ topic	-	No
7	Yes (3)	Lim	-	Mild	-	PR	CR	Eo+H	-	CyA	-	Yes(1)
8	Yes (2)	Lim	-	Mild	-	PR	CR	GI	-	CyA	-	No
9	No	Lim	Lim	Mod	Mild	SD	PR	O	O	CyA+ topic	-	No
10	No	Ext	Lim	Sev	Mild	PR	PR	K	K	MMF+Prd	CyA+ Prd	No
11	No	Ext	Lim	Sev	Mild	SD	PR	E+H+O+S	E+O	Fk-506+ topic	Fk506+ topic	No
12	Yes (3)	Lim	Lim	Mild	Mild	SD	SD	E+O+S	E+O	Fk-506+ topic	Fk506+ topic	Yes(1)

cGVHD chronic graft-versus-host disease; **CyA** cyclosporin A; **CR** complete response; **Ext** extensive cGVHD; **E** eyes; **Eo** Eosinophilia; **GI** gastrointestinal; **H** hepatic; **IS** immunosupressive treatment; **K** kidney (nefrotic síndrome); **L** lung; **Lim** limited cGVHD**; Mod** moderate cGVHD; **MMF** mofetil micofenolate**; Nº** number of cGVHD relapse prior to and after vit D treatment**; NR** no response; **O** oral**; PR** partial response; **Prd** prednisone; **S** skin **; SD** stable disease; **Sev** severe cGVHD.

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DISCUSIÓN

5. Discusión

El trasplante alogénico de progenitores hematopoyéticos permite la curación de varias enfermedades hematológicas y no hematológicas malignas y no malignas adquiridas o hereditarias. Una de sus principales complicaciones es la EICHc que constituye la principal causa tardia de morbilidad y mortalidad. Su incidencia ha aumentado significativamente debido sobre todo al incremento de la utilización de donantes no emparentados y de progenitores de sangre periférica así como a la edad más avanzada de los pacientes al trasplante.

Durante muchos años la clasificación de la EICHc en limitada ó extensa ha sido la más utilizada, sin embargo tiene varias limitaciones, fundamentalmente su escaso valor pronóstico. Además, la mayoría de los pacientes quedan finalmente incluidos dentro del grupo de EICHc extenso. Recientemente el NIH ha propuesto una nueva clasificación según la cuál son las manifestaciones clínicas más que el tiempo de aparición las que permiten establecer el diagnóstico diferencial entre EICHa y EICHc, definir criterios mínimos para el diagnóstico de la EICHc y establecer grupos pronósticos que permiten perfilar el tratamiento en función del riesgo del paciente.

En el presente trabajo nos planteamos evaluar el valor pronóstico de la clasificación del NIH así como identificar nuevos factores predictivos para el desarrollo de EICHc severa o extensa; otro objetivo del presente trabajo ha sido evaluar el posible papel de nuevas opciones terapéuticas como son el tratamiento tópico con beclometasona en la EICH digestiva y la utilización de fármacos no inmunosupresores como la vitamina D. A continuación serán discutidos los resultados obtenidos en los diferentes objetivos planteados.

5.1 Factores pronósticos

Diversos estudios en el contexto de la EICHc han intentado identificar variables clínicas y biológicas con valor pronóstico[5,6,52-55]. La mayoria de estos estudios se han realizado en pacientes sometidos a TPHMO, sin embargo, el aumento del uso de PHSP ha modificado las características y la incidencia de EICHc extensa en pacientes que reciben progenitores hematopoyéticos de sangre periférica[4].

Además, la EICHc puede ser más resistente al tratamiento en comparación con el TPHMO requiriendo varias líneas de tratamiento para su control y una mayor duración del tratamiento inmunosupresor. En este sentido, Pavletic y col.[55] identifican en una serie de pacientes sometidos a TPHSP la cifra de plaquetas < 100 x 10^9/L y el antecedente de EICHa hepático como factores adversos tras el diagnóstico de EICHc, de manera que las variables que condicionan una mayor morbimortalidad entre ambos tipos de trasplante pueden ser diferentes. Por tanto, en el TPHSP se requieren modelos pronósticos especificos para establecer subgrupos que permitirán la identificación de pacientes que podrian recibir tratamiento tópico en contraste con aquellos que requieren una actitud terapéutica más agresiva. Aunque la EICHc se asocia a complicaciones que pueden afectar considerablemente la calidad de vida y la supervivencia de los pacientes también tiene una clara relación con el efecto injerto contra leucemia lo que contribuye significativamente a disminuir el riesgo de recaída tras trasplante alogénico[65,69,70,97,98]. Por tanto, el desarrollo de modelos con valor pronóstico en el contexto de trasplante alogénico de PHSP permitiría la individualización de la estrategia terapéutica.

El NIH ha propuesto un nuevo sistema en que establece una serie de criterios para el diagnóstico de la EICHc[3]. En este sistema se describe la extensión y el grado de afectación de la EICHc en cada organo ó tejido afectado independientemente del tiempo transcurrido desde el trasplante. Además, establece nuevas guías para la evaluación global de la EICHc y define las indicaciones para el tratamiento tópico y sistémico. Sin embargo, esta clasificación requiere validación para definir su impacto pronóstico.

En nuestra experiencia[56] en una serie de 171 pacientes sometidos a alo-TPHSP a partir de donante emparentado HLA idéntico la incidencia global de EICHc fue de 70% y la de EICHc extensa fue de 60%; al reclasificar estos pacientes mediante la clasificación del NIH la incidencia acumulada de EICHc leve, moderada o severa es del 29%, 42% y 28%, respectivamente, permitiendo una mejor estratificación pronóstica y terapéutica. Además, algunos pacientes que inicialmente presentaban el diagnóstico de EICHc extensa fueron retrospectivamente clasificados como EICHc leve por tanto,

basándose en el mejor pronóstico de este subgrupo de pacientes, de acuerdo con las recomendaciones del NIH, se podria haber evitado la terapéutica inmunosupresora sistémica en estos pacientes.

Con relación a la mortalidad relacionada con la EICHc no se encontraron diferencias significativas entre los pacientes que desarrollaban formas limitada ó extensa (0% vs 13%; *P* = 0,24, respectivamente), mientras que el estado general < 2 de acuerdo con el *score* del NIH al diagnostico de la EICHc y el tipo de inicio *de novo* son factores pronósticos favorables. En este estudio se confirma que el grado de severidad de la afectación hepática y pulmonar de acuerdo con el sistema del NIH son factores pronósticos importantes en cuanto a la supervivencia. Estudios previos han identificado la gravedad de la afectación hepática y pulmonar como factores pronosticos independientes[99]. Contrariamente a otros estudios, no identificamos la cifra de plaquetas como factor pronóstico en nuestra serie de pacientes, lo que puede estar en relación con el valor relativamente alto de la mediana de la cifra de plaquetas (79 x 10^9/L) al diagnóstico de la EICHc[6,54,55]. En el análisis univariante las variables anteriormente mencionadas influyen en la supervivencia global mientras que en el análisis multivariante el tipo de inicio y la clasificación del NIH son las variables con impacto en la SG de forma que el tipo de comienzo de *novo* tiene un valor pronostico favorable (HR = 0,094; *P* = 0,003) mientras que la EICHc severa tiene un efecto desfavorable (HR = 13,27; *P* = 0,001). Basado en el análisis multivariante desarrollamos un modelo pronóstico que combina ambas variables (el tipo de comienzo y grado de severidad según el NIH) que permite identificar 4 subgrupos de pacientes con diferentes supervivencias: 1. pacientes con EICHc moderada y el tipo de comienzo *de novo* (SG a los 5 años de 82%); 2. pacientes con EICHc moderada y tipos de comienzo quiescente o progresivo (SG de 70%); 3. pacientes con EICHc severa y tipo de comienzo *de novo* (SG de 50%) y 4. pacientes con EICHc severa y tipos de comienzo quiescente o progresivo (SG de 25%). Los pacientes con EICHc leve presentan una SG similar (80 – 87% a los 5 años) independientemente del tipo de comienzo.

Estudios previos han demostrado que 30% a 70% de los pacientes que sobreviven más de 100 días postrasplante requieren tratamiento

inmunosupresor por más de dos años[35,99,100]. En el presente estudio, en una serie homogénea de pacientes sometidos a trasplante de PHSP, se confirma que la clasificación del NIH además de su impacto en la supervivencia, es el factor pronóstico más importante para evaluar el riesgo de recaida de la EICHc en pacientes que responden al tratamiento inmunosupresor de primera línea. Al considerar esta clasificación junto al desarrollo de EICH aguda previa, este sistema nos permite identificar aquellos pacientes que reciben tratamiento inmunosupresor en el momento del último seguimiento de manera que la ausencia de EICHa previa (HR = 2; *P* = 0,004) y la presencia de EICHc leve (HR = 4,2; *P* = 0,007) permiten identificar a los pacientes en los que, con mayor probabilidad, se podra suspender el tratamiento inmunosupresor.

En conclusión, la clasificación del NIH y el tipo de comienzo de la EICHc en pacientes sometidos a trasplante alogénico de PHSP son factores que influyen en la probabilidad de respuesta al tratamiento inicial de la EICHc, identifican a los pacientes en los que con mayor probabilidad se podra suspender el tratamiento inmunosupresor, identifican a los pacientes con mayor riesgo de mortalidad relacionada con la EICHc y finalmente permiten la identificación de subgrupos de pacientes con diferente supervivencia. Estos parámetros deben de tenerse en cuenta para adaptar la estrategia de la terapéutica inmunosupresora.

Sin tratamiento, menos del 20% de los pacientes con EICHc extensa sobrevive con un karnofsky ≥ 70[71]. El tratamiento inmunosupresor disminuye la morbilidad y la mortalidad[101] por lo que la identificación de factores predictivos para el desarrollo de EICHc especialmente en su forma extensa o severa antes del deterioro clinico es importante, además de permitir adaptar la estrategia del tratamiento inmunosupresor y de este modo reducir la morbimortalidad. En este sentido, Remberger y col.[102] en una serie de 679 pacientes, la mayoria de ellos sometidos a TPHMO, identificaron la EICHa grado II-IV (HR = 2,30; *P* = 0,005), la LMC (HR = 2,37; *P* = 0.002) y la incompatibilidad de sexo [donante mujer inmunizada y receptor hombre] (HR = 2,16; *P* = 0.02) como factores de riesgo independientes para el desarrollo de EICHc moderada y severa; la edad del paciente (> 60 años) al trasplante también presentó valor pronostico (HR =

2,42; *P* = 0.01), de manera que a los 5 años la probabilidad de desarrollar EICHc moderada o severa en los pacientes con 0, 1, 2 y 3 factores de riesgo es de 5%, 13%, 23% y 45%, respectivamente. Entre los pacientes con EICHc (n=279); el antecedente de EICH aguda grado II-IV (HR = 2,18; *P* = <0.01) fue la única variable con valor pronóstico para el desarrollo de EICHc moderada o severa. En otro estudio con 54 pacientes que habian recibido TPH (46 pacientes TPHSP y 8 TPHMO) se identificó la obtención de quimerismo completo precoz (80 - 100 días postrasplante) en sangre periférica como un factor de riesgo para el desarrollo de EICHc extensa[103].

Algunos estudios previos han evaluado el valor predictivo de los tests de reevaluación del día +100 postrasplante para el desarrollo de EICHc. Loughran y col.[104] identifican como factores prnósticos para el desarrollo de EICHc la historia previa de EICHa, la biopsia cutánea positiva y biopsia de mucosa oral positiva para células plasmaticas IgA. Sin embargo, varios estudios no han confirmado el valor pronóstico de las biopsias cutáneas y de mucosas realizadas en el día +100 postrasplante para el desarrollo de EICH en pacientes asintomáticos que siguen bajo tratamiento inmunosupresor[105-107]. Por otra parte, Wagner y col.[108] describen que el uso de corticoides en el día + 100 aumenta el riesgo de desarrollar EICHc y que los demás estudios de *screening* no tienen valor pronóstico para el desarrollo de EICHc. First y col. reportan que los pacientes con plaquetas < 100 x 10^9 / L en el día +120 tras trasplante presentan una mayor incidencia de EICHc y menor supervivencia[109]. La mayoria de estos estudios se realizaron en pacientes sometidos a TPHMO mientras que hay poca información sobre el valor predictivo de los estudios de *screening* tras TPHSP lo que, unido al mayor riesgo de EICHc de estos pacientes, hace especialmente necesario llevar a cabo estudios que permitan predecir precozmente el riresgo de desarrollar dicha complicación[4,61]. En nuestra experiencia[110], al analizar el valor predictivo de las pruebas no invasivas del día +100 en una serie de 165 pacientes que recibieron TPHSP de donante emparentado, verificamos que las pruebas de función hepática (PFH) alteradas [bilirrubina total (BT), fosfatasa alcalina (FA), gammaglutamil transferasa (GGT) 2 veces por encima del limite superior de lo normal] y la cifra

de linfocitos en sangre periférica < 0.750 x 10^9/L permiten identificar pacientes con mayor riesgo de desarrollar EICHc severa o extensa. A pesar de que la disfunción hepática *per se* no suele ser causa de muerte por EICHc, algunos autores[55] han descrito que la EICH hepática aumenta el riesgo de mortalidad en pacientes sometidos a TPHSP y TPHMO. En nuestro estudio, las alteraciones de la BT, FA y GGT se relacionan con un riesgo mayor de desarrollar EICHc extensa ó severa en los pacientes independientemente del valor de las transaminasas. Considerando el carácter sencillo, rápido y no costoso de las PFH en el día 100, deberían realizarse de manera rutinaria en todos los pacientes sometidos a TPH.

Contrariamente a estudios previos, en el presente estudio no hemos identificado la EICH aguda como factor predictivo para el desarrollo de EICHc extensa ó severa. Algunos de estos trabajos no excluyeron a pacientes con EICH activa ó a los pacientes bajo tratamiento con corticoides lo que podria influir en el resultado de dichos análisis[104,108, 111,112]. En este sentido, Atkinson y col. [113] describen que el riesgo de desarrollar EICHc a los 3 años fue de 28% ± 3%, 49% ± 5%, 59% ± 6%, 80% ± 9% y 85% ± 15% en pacientes con EICH aguda grado 0, I, II, III y IV respectivamente (*P < 0.0001*), mientras que en los pacientes sin EICH aguda ó con EICH aguda grado I, el antecedente de EICH aguda previa no tenia valor predictivo para el desarrollo de EICHc. Es importante mencionar que en nuestra serie el 50% de los pacientes tenia EICH aguda grado I o no presentaba EICH aguda y únicamente el 9% desarrolló EICH aguda grado III-IV. Este hecho puede explicar la falta de correlación entre la EICH aguda y la EICHc en nuestro trabajo.

En cuanto a los datos hematimétricos, en este estudio no identificamos la cifra de plaquetas como factor de riesgo para el desarrollo de EICHc extensa ó severa si bien la trombocitopenia (< 100 x 10^9/L) sí ha sido descrita previamente como un marcador de gravedad de la EICHc[6,55,112]. Por el contrario identificamos la cifra baja de linfocitos en sangre periférica (< 0.750 x 10^9/L) como factor de riesgo para el desarrollo de EICHc extensa ó severa, dato que no se había descrito previamente.

En un intento de excluir el "efecto confusión" de la EICHa previa, realizamos un análisis multivariante excluyendo los pacientes que habian desarrollado EICHa que tenían EICHc activa en el momento del *screening* o que estuvieran recibiendo tratamiento con corticoesteroides en el día + 100[113] y de nuevo ambas variables mantenian su valor predictivo. En cuanto a la cifra de linfocitos, estudios previos indican que los pacientes con una recuperación lenta de linfocitos pos-trasplante tienen peor pronóstico[114-119]. En este sentido Pavletic y col han demostrado, que una recuperación rápida de los linfocitos se correlaciona con una mejor supervivencia tras trasplante alogenico de PHSP[125]. La correlación entre la cifra baja de linfocitos y el desarrollo de EICHc extensa ó severa podria reflejar el hecho de que los linfocitos T aloreactivos inducen un efecto citotóxico no solamente en los órganos afectados por la EICHc sino que también actúan sobre clones de células T que reaccionan contra los patógenos, lo que explicaria la inmunosupresión inducida por la EICHc y el escaso repertorio de receptores de células T (TCR) observados entre los pacientes con EICHc. Así, el promedio de complejos TCR-Vβ en los pacientes con EICHc es menor que en los que no presentan EICHc[120]. Por otro lado, ha sido publicado recientemente que la recuperación de las células NK en el día + 60 postrasplante se asocia a una reducción de las recaidas y muerte tras trasplante con AIR[121]. En resumen, en este artículo demostramos que el aumento de la bilirrubina total, fosfatasa alcalina y GGT junto a una cifra baja de linfocitos en sangre periferico en el día + 100 postrasplante son tests no invasivos, sencillos, rapidos, y económicos que tienen valor predictivo para el desarrollo de EICHc extensa ó severa en pacientes sometidos a trasplante alogenico de PHSP de donante emparentado. Se requieren estudios posteriores para evaluar su valor pronóstico en otras situaciones tales como en pacientes sometidos a TPHMO y de donante no emparentado.

5.2 Nuevas opciones terapéuticas encaminadas a evitar el tratamiento sistémico con esteroides

El tratamiento de primera línea para la EICHc siguen siendo los inhibidores de calcineurina asociado a la prednisona. En los pacientes que no responden al

tratamiento inicial no existe una opción terapéutica estándar. El tratamiento prolongado con estos fármacos, principalmente con corticoesteroides, puede producir efectos adversos graves tales como: infecciones, hipertensión arterial, supresión del eje hipotalamo-pituitaria-adrenal, miopatia, hiperglicemia, cataratas, redistribución del tejido adiposo, edemas, retraso del crecimiento en niños, atrofia cutánea y estrías, trastornos neuropsiquiatricos, gastritis, necrosis avascular, osteoporosis, osteopenia, por lo que la reducción de la exposición de estos pacientes al tratamiento inmnosupresor sistémico es importante para reducir la morbimortalidad relacionada con el tratamiento. Por otra parte, la EICHc es un factor pronóstico importante para la supervivencia, en cuanto que se asocia con un efecto injerto contra leucemia, por lo que seria útil desarrollar estrategias terapéuticas que permitan el control de la EICHc evitando la exposición prolongada a los corticoesteroides. En este sentido, la beclometasona por su acción tópica sobre la mucosa intestinal y la vitamina D por su efecto inmunomodulador pueden desempeñar un papel importante.

Varios autores han descrito el uso de la beclometasona en el tratamiento de la EICH aguda. En un estudio randomizado, el uso de beclometasona en combinación con la prednisona a dosis de 1 mg/kg disminuyó la tasa de fracaso del tratamiento de la EICHa de 65% en la rama del placebo hasta 39% en la rama de los pacientes que recibian beclometasona (*P = 0.003*). Durante el periodo del estudio se verificó beneficios adicionales en la rama de la beclometasona debido fundamentalmente a la reducción de la necesidad del tratamiento prolongado con prednisona[104]. En nuestra experiencia, en una serie de 26 pacientes con EICH aguda gastrointestinal, el uso de beclometasona sin corticoides sistémicos permitó obtener un 77% de respuestas globales con 65,5% de remisiones completas. Finalmente el 50% de los pacientes no necesitó tratamiento sistemico con corticoides[92]. En el contexto de la EICHc, el uso de fármacos con efecto tópico sobre los órganos afectados permitiría evitar la exposición a corticoides sistémicos. En este sentido, muchos pacientes con EICHc fallecen no debido propriamente a la EICHc sino a las complicaciones infecciosas secundarias al tratamiento inmunosupresor que reciben para su control[56,94,122]. Por otro lado, los corticoides podrían inhibir el efecto

antileucémico asociado al EICL[70,97,123]. Por todo ello, el tratamiento inmunosupresor debe ser administrado no solamente en base a la gravedad de la EICHc sino también teniendo en cuenta el riesgo de recaida y el estado de la enfermedad[124].

Unicamente 1 estudio ha evaluado el uso de la beclometasona para el tratamiento de la EICHc gastrointestinal[125]. En este estudio se analizaron 13 pacientes con EICHc gastrointestinal y 2 con EICH aguda. Todos los pacientes excepto en 1 habian recibido metilprednisolona a dosis de 2 mg/kg/día como tratamiento previo sin respuesta. Nueve (60%) pacientes presentaron respuesta a la beclometasona con mejoria o desaparición de los síntomas.

En el presente estudio[96] en una serie de 33 pacientes sometidos a trasplante de progenitores hematopoyéicos que presentaban EICHc digestiva comprobada por biopsia gastrointestinal, observamos un 84,6% de remisiones completas y 7,7% de respuestas parciales entre los pacientes que recibieron beclometasona como tratamiento de primera línea, mientras que entre los que la recibieron como segunda ó tercera línea de tratamiento se constató un 42,9% y 28,6% de remisiones completas y respuestas parciales respectivamente.

Es Importante mencionar que a diferencia de los pacientes analizados por Iyer y col[125] que no habían respondido al tratamiento con metilprednisolona a dosis de 2 mg/kg/día, nuestros pacientes estaban sin tratamiento con corticoides en el momento de la recaída de la EICHc por lo que representan una población con mejor pronóstico.

A pesar de la alta tasa de respuesta inicial, observamos una elevada tasa de recaídas la mayoría tras suspender la beclometasona. Sin embargo, considerando que la duración estándar del tratamiento para la EICHc se mantiene durante por lo menos 9 meses y que la beclometasona se administró durante un periodo de 16 semanas con 4 semanas adicionales de reducción progresiva hasta suspender dicha tasa de recaídas podría estar en relación con el corto periodo de tratamiento. Además, todos los pacientes en este estudio habían recibido un trasplante de PHSP lo que incrementa el riesgo de recaída de la EICHc tras tratamiento inicial[56,72] tal y como describen Flowers y col.[61]

con tasas de recaídas del 61% al 84% entre los pacientes con EICH que habían recibido un trasplante de PHSP. En nuestra serie, a pesar de la alta tasa de recaídas, el 39,4% de los pacientes finalmente estaban sin tratamiento inmunosupresor.

Adicionalmente se verificó un bajo perfil de toxicidad con síndrome de Cushing en 2 pacientes, hiperglicemia en 1 paciente y dolores músculo esqueléticos en 2 pacientes que ocurrieron en el periodo de reducción de la beclometasona lo que sugiere un grado ligero de absorción sistémica del fármaco. Estudios previos sobre la actividad de la beclometasona no revelaron importantes efectos secundarios relacionados con enfermedades infecciosas, aunque es posible la aparición de supresión del eje hipotalamo adreno hipofisário[92,94]. En este sentido, los metabolitos de la beclometasona tienen una biodisponibilidad sistemática resultando en una reducción de la respuesta adrenal durante el periodo de exposición a la droga[125,126]. Estudios recentes sobre el uso a largo plazo de corticoesteroides con actividad tópica administrados por vía oral han demostrado escasas evidencias de insuficiencia adrenal clínica[125,127]. Sin embargo, en 3 series publicadas no describen evidencias de supresión del eje hipotalomo adreno hipofisarioen pacientes bajo tratamiento con beclometasona por vía oral[92,94,128] y la respuesta clínica a este tratamiento sugiere que la absorción no es necesaria para que sea eficaz.

En conclusión, el presente estudio demuestra que la beclometasona en ausencia de corticoides sistémicos es eficaz como terapéutica inicial en los pacientes con EICHc gastrointestinal lo que permite evitar las complicaciones relacionadas con los corticoides sistémicos aunque la duración completa del tiempo de tratamiento esta por determinar.

Estudios previos describen la eficacia del MC1288, un analogo de la vitamina D (vit D) en la la prevención de la EICHa en ratones sometidos a TPHMO[129]. Middleton y col. han demostrado la relación existente entre polimorfismos del gen VDR y la presencia de EICHa severa[130]. También se ha demostrado la presencia de receptores de vit D (VDR) en las células mononucleadas de sangre periférica así como la capacidad de la vit D de inhibir la activación y proliferación de las células T y disminuir la producción de

citocinas producidas por las células Th1, explicando su efecto inmunomodulador[88-90]. Recientemente Rosenblatt y col[111] demostraron el efecto inhibidor de la vit D sobre la proliferación de las células T y sobre la producción de citocinas Th2.

Sin embargo, existe escasa información sobre el uso de la vit D en el tratamiento de la EICHc, por lo que hemos analizado el efecto del tratamiento con vit D antes y después de su inicio en la EICHc en un grupo de 12 pacientes que estaban recibiendo dicho tratamiento por vía oral debido a osteoporosis u osteopenia y que presentaban EICHc refractaria o en recaída[132].

A los 6 meses tras el inicio de la vitamina D ningún paciente presentaba EICHc severa versus 3 al inicio. Además, 5 pacientes alcanzaron remisión completa y estaban sin tratamiento inmunosupresor. Durante este periodo no fue necesario añadir otros fármacos inmunosupresores a ningún paciente. También se verifico una importante reducción en las recaídas o progresión de la EICHc de forma que al inicio del tratamiento con vit D 6 pacientes habían presentado recaídas o progresión mientras que a los 6 meses tras su inicio unicamente 3 pacientes presentaron recaídas. No se apreciaron efectos secundarios relacionados con la vit D. Por tanto, la asociación de vit D con otros inmunosupresores seria una opción interesante, segura y no tan costosa para el tratamiento de la EICHc. El presente estudio establece las bases para posteriores estudios prospectivos con un mayor número de pacientes.

CONCLUSIONES

6. CONCLUSIONES

6.1 Con relación a los factores pronósticos de la EICHc

- La clasificación del NIH de la EICHc tiene valor pronóstico en pacientes sometidos a trasplante alogénico de progenitores hematopoyéticos de sangre periférica.
- El tipo de comienzo (*de novo*, quiescente y progresivo) y el grado de afectación según la clasificación del NIH (leve, moderada y severa) son factores que influyen significativamente en la respuesta al tratamiento de la EICHc y que predicen la supervivencia.
- La suma de los dos factores anteriores permite obtener cuatro grupos de pacientes con supervivencia diferente.
- La alteración de las pruebas de función hepática (Bil total, FA, GGT > 2 x normal) y la cifra de linfocitos en sangre periférica (< 0.750 x 10^9/L) en el día + 100 postrasplante son factores predictivos para el desarrollo de EICHc extenso o severo tras trasplante alogénico de progenitores hematopoyéticos de sangre periférica.

6.2 Con relación a las opciones terapéuticas encaminadas a evitar el tratamiento sistémico con esteroides

- El dipropionato de beclometasona por vía oral es eficaz y seguro en el tratamiento inicial de la EICHc gastrointestinal evitando así la exposición a la corticoterapia sistémica.
- La vitamina D asociada a los inmunosupresores puede ser una opción terapéutica eficaz, segura y de bajo coste en los pacientes con EICHc.

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SUMMARY

8. Summary

The major later complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is chronic graft versus host disease (cGVHD) that may lead to severe morbidity and mortality and occurs in 20-77% of patients at long-term follow-up with higher frequencies occurring when the patient is older, when the donor is other than HLA matched sibling and when peripheral blood stem cells are utilized as the hematopoietic stem cell source[1]. The higher non-relapse mortality among these patients, the higher incidence of secondary malignancies and the impairment of quality of life explains why cGVHD remains the most severe complication among patients surviving > 100 days after allogeneic stem cell transplantation[2,3]. Classically, cGVHD has been classified as “limited” or “extensive” to distinguish patients requiring systemic immunosupression from those for whom local care might suffice[4]. Although this classification system can be easily used in many centers, it fails to stratify patients according to outcome[5]. Furthermore, most patients experience extensive-stage cGVHD. For this reason, several grading systems have been developed to predict survival and late treatment–related mortality in patients diagnosed with cGVHD[6-8]. Akpek et al[7] analysed a series of 151 patients who developed cGVHD after allogeneic bone marrow stem cell transplantation (allo-BMSCT). The probability of survival at 10 years after the diagnosis of cGVHD was 51%. Three factors were identified that predicted the outcome of the patients: extensive skin involvement (>50% of body surface area), thrombocytopenia (less than 100 x 10^9/L) and progressive-type of onset. At the time of primary treatment failure, the previously mentioned risk factors, in addition to a Karnovsky score <50%, were identified as independent predictors for a poor outcome. Lee et al[8] in a large registry-based analysis of patients from the International Bone Marrow Transplant Registry (IBMTR) and the National Marrow Donor Program (NMDP) identified three subgroups of patients (low, intermediate and high risk) with different survivals according with the Karnovsky performance score, presence of chronic diarrhea, weight loss and skin involvement. The majority of these patients were receiving BMSCT.

Recently, the National Institutes of Health (NIH) Consensus Development Project proposed a new scoring system for the global assessment of cGVHD based on the number of organs involved and the degree of functional impairment in affected organs (mild, moderate, or severe)[9]. This allows the identification of patients requiring only topical approach or no immunosupression, and also facilitates decision making regarding the timing and intensity of therapy.

Peripheral blood is increasingly as stem cell source in allogeneic transplantation and particularly for patients with haematological malignancies[10,11]. Patients undergoing allogeneic peripheral blood stem cell transplantation (allo-PBSCT) require a higher number of successive treatments to control cGVHD as compared to allo-BMSCT, which leads to a longer duration of immunesuppressive therapy[12,13]. Previous studies have identified prognostic factors for cGVHD after BMSCT[6-8] nevertheless, these prognostic models may not necessarily apply to patients undergoing PBSCT. With regard to this, Pavletic et al[14] reported that a platelet count < 100 x 10^9/L and history of acute GVHD (aGVHD) point to a poor outcome in patients undergoing allo-PBSCT who develop cGVHD. Accordingly, In PBSCT further studies are required to identify prognostic factors for cGVHD development and severity.

The standard treatment for cGVHD remains on calcineurin inhibitors and corticosteroids. Furthermore, there is no standard approach for patients with cGVHD who do not respond or relapse after first line treatment and rescue therapy is based on immunosuppressive drugs and corticosteroids which are responsible for most of the complications. For this reason a new therapeutic options without systemic immnusupressive effect are necessary.

In this thesis, we aim to evaluate the prognostic value of the NIH cGVHD scoring system, identified new prognostic and risk factors for the development of severe or extensive cGVHD and non immunosuppressive treatments in order to avoid systemic steroids.

AIMS

The NIH classification together with other prognostic factors at cGVHD diagnosis allow to identify patients with different outcomes. Along these lines, the day +100 screening tests can be useful to identify patients at high risk for development of extensive or severe cGVHD. Finally, in patients with mild or moderate cGVHD the use of non immunosuppressive and / or topical drugs would be desirable to control symptoms. According with these premises we consider the following objectives:

A. Identification of prognostic factors in the cGVHD setting

1. To evaluate retrospectively the prognostic impact of NIH classification and searching for additional prognostic factors

2. To evaluate retrospectively the predictive value of day +100 screening tests

B. Identification of new therapeutic approach for cGVHD

1. The rol of beclomethasone in gastrointestinal cGVHD

2. Effect of vitamin D in cGVHD

RESULTS

The results of this doctoral thesis have been published in scientific journals. Papers have been attached previously.

The order of its presentation were divided in two parts: 1. Prognostic factors for cGVHD and 2. New therapeutic strategies for cGVHD.

1. Prognostic factors for cGVHD

Several grading systems have been developed in the bone marrow setting in attempts to predict survival in patients with chronic graft-versus-host disease (cGVHD). We evaluated the prognostic value of the National Institutes of Health (NIH) scoring system and investigated for any additional prognostic factors in a series of 171 patients undergoing non-T cell-depleted allo-PBSCT from matched related donors. The cumulative incidence of cGVHD was 70%; cumulative incidences of mild, moderate, and severe cGVHD were 29%, 42% and 28%, respectively. Overall, 68% of patients were free from immunosuppression 5 years after transplantation. Absence of previous acute GVHD (aGVHD; hazard ratio [HR] = 2; *P = 0.004*) and mild cGVHD (HR = 4.2; *P = 0.007*) increased the probability of being off immunosuppressive treatment by the last follow-up.Overall survival (OS) at 5 years was 52%. Severe cGVHD, according to the NIH scoring system (HR = 13.27; *P = 0.001*) adversely influenced outcome, whereas de novo onset (HR = 0.094; *P = 0.003*) had a more favourable impact on survival. The combination of both variables allowed us to identify 4 different subgroups of patients in terms of OS: **1**. those patients with mild cGVHD regardless of the type of onset and patients with moderate cGVHD with de novo onset the OS was 82%; **2**. those patients with moderate cGVHD and quiescent or progressive onset the OS was 70%; **3**. those patients with severe cGVHD and de novo onset the OS was 50%; **4**. those patients with severe cGVHD and quiescent or progressive onset the OS was 25%. Our study indicate that the NIH scoring system has some prognostic value in patients undergoing PBSCT and, together with the type of onset, must be considered to predict the possible outcome of patients who develop cGVHD.

This work was published in Biology of Blood and Marrow Transplantation Journal: "*Prognostic Factors of Chronic Graft-versus-Host Disease Following*

Allogeneic Peripheral Blood Stem Cell Transplantation: The National Institutes Health Scale Plus the Type of Onset Can Predict Survival Rates and the Duration of Immunosuppressive Therapy" . Biol Blood Marrow Transplant. 2008; 14: 1163-1171.

In the second paper we analyzed the value of non invasive day +100 screening tests as predictors of severe or extensive cGVHD development in a series of 165 patients undergoing allo-PBSCT from a matched related donor. The cumulative incidence of overall, extensive, and severe cGVHD was 67, 56 and 23%, respectively, among patients surviving > 100 days after transplant. In univariate analysis, patients displaying an abnormal liver function tests (LFTs) (total bilirubin, alkaline phosphatase, and GGT > 2 times above the upper normal limit) and a low absolute lymphocyte count (ALC) (< percentil 25: 0.750 x 10^9/L) had a significantly higher risk of overall, extensive, and severe cGVHD. In multivariate analysis, the combination of abnormal LFTs and low ALC allowed to predict the risk of overall [HR = 3.35 (95% CI: 1.65-6.83); *P < 0.001],* extensive [HR = 4.22 (95% CI: 1.96-9.12); *P < 0.001],* and severe cGVHD [HR = 8.17 (95% CI: 2.55-9.26.17); *P= 0.002]*. Our findings show that an increased total bilirubin, alkaline phosphatase, and GGT levels together with the ALC at day +100 are non invasive, simple, fast and efficient predictors of severe cGVHD development after allogeneic PBSCT.

This manuscript was published in American Journal of Hematology: "*Liver function tests and absolute lymphocyte count at day +100 are predictive factors for extensive and severe chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplant".* American J Hematology. 2010: 85; 290-293.

2. New therapeutic strategies for cGVHD

The most common approach for the treatment of cGVHD has been the long-term use of systemic steroids. Beclomethasone dipropionate (BDP) is a topically active corticosteroid with low absorption from the gastrointestinal mucosa and has been succesfully used to treat acute GVHD, but its use in the cGVHD setting is far more limited. We evaluated the safety and efficacy of BDP as treatment in a series of 33 patients who underwent allogeneic transplantation and had biopsy-proven gastrointestinal cGVHD (GI cGVHD). Twenty-six

patients with GI cGVHD received BDP as first-line and 7 as either second or third-line treatment. All patients received BDP together with calcineurin inhibitor, except for 1 patient who was also receiving mycophenolate mofetil. BDP was administered for a minimum of 16 weeks and was tapered during 4 additional weeks. Of those patients receiving BDP as first line of treatment, 22 (84,6%) achivied complete remission (CR) of GI cGVHD, 2 (7,7%) achivied partial response (PR) and 2 (7,7%) did not respond or progressed. Median time to response was 28 days. Nevertheless, only 4 patients developed cytomegalovirus (CMV) reactivation, which was successfully treated with antiviral drugs. No fungal infection was observed during the treatment period. In conclusion, this study shows that BDP, in the absence of systemic steroids, is a highly effective initial therapeutic approach for GI cGVHD, which helps to avoid complication related to systemic steroids.

The results regarding this manuscript was published in Biology of Blood and Marrow Transplantation Journal: *"Oral Beclomethasone Dipropionate for the Treatment of Gastrointestinal Chronic Graft-versus-Host Disease".* Biol Blood Marrow Transplant. 2009; 15: 1331-1336.

Vitamin D (vit D) has a potent immunomodulatory effect as shown in vitro and in animal models, nevertheless there is no information about its use in the cGVHD setting. We evaluated the outcome of cGVHD in 12 patients with refractory or relapsed cGVHD receiving vit D due to osteopenia or osteoporosis after allogeneic transplantation. After six months of treatment 5 patients obtained complete response and were free from immnusupressive treatment, 5 partial response and 2 patients had no response. No immunosuppressive drugs were added during this period and no adverse effects were described in association with vitamin D treatment. We found an important improvement in the severity of cGVHD so that at six months after vitD treatment no patients displayed severe cGVHD versus 3 at the beginning. In addition we observed a remarkable reduction of cGVHD relapses or progressions. Accordingly, 9 out of 12 patients had no relapse/progression.

This paper has been accepted for publication in Bone Marrow Transplantation Journal.

DISCUSSION

Several models have been described in patients with cGVHD in order to identify features with prognostic significance[6,7]. Most of these previous studies were performed in patients undergoing BMSCT. But, there are some differences in cGVHD characteristics between PBSCT and BMSCT recipients. With regard to this, PBSCT is associated with a higher incidence of cGVHD compared with BMSCT[11]. Furthermore, the number of successive lines of treatment needed to control the cGVHD is higher after PBSCT, meaning that these patients require a longer duration of immunosuppressive treatment[12-13]. Accordingly, the prognostic models of cGVHD in the BMSCT setting may not necessarily apply to patients undergoing PBSCT. In this regard, a platelet count < 100 x 10^9/L and a history of aGVHD have been associated with a poor outcome in patients undergoing PBSCT who develop cGVHD[14]. Although cGVHD is associated with an important morbidity and mortality, it also relates to graft-versus-leukemia effect, which decreases the risk of relapse after allogeneic transplantation[15-17]. In these lines, specific prognostic models in PBSCT setting will help individualize therapeutic strategies in order to allow identification of patients who can be treated with topical or mild immunosupression, in contrast to those requiring a more aggressive approach.

Recently the NIH has proposed a new scoring system to establish standard criteria for the diagnosis of cGVHD[9]. This system attempts to do this by describing the extent and severity of cGVHD for each organ or site involved at any given time. In doing so, it seeks to establish new guidelines for the global assessment of cGVHD and to propose indications for topical and systemic therapies. Nevertheless, this scoring system requires validation to define the prognostic impact of the subgroups that it identifies as mild, moderate, and severe cGVHD. In the current study[19], we confirmed that most of the patients who developed cGVHD were classified as having extensive cGVHD, with only a minority having limited cGVHD according to the standard criteria. This contrasts with the NIH scoring system, because all 3 categories had similar number of patients, thus allowing better stratification of the patients for both therapeutic and prognostic purposes. In addition, some of the patients diagnosed with extensive cGVHD were retrospectively classified as having mild cGVHD. Based on the superior outcome of this small subset of patients, it can be speculated

that they could have benefited from avoiding systemic immunosupression, as suggested by the NIH scoring system. Concerning cGVHD-related mortality, a good performance status at the time of cGVHD diagnosis, according to the NIH scoring system and the type of onset, significantly influenced the outcome in univariate analysis. Regarding specific organs, the severity of liver and lung involvement significantly influenced the outcome of patients who developed cGVHD. These variables have been identified as independent prognostic factors in previous studies[15]. In contrast, we did not identify platelet count as a prognostic factor, which may be explained by the high median number of platelets (79 x 10^9/L) found at the time of cGVHD diagnosis[7,8,14]. The same variables also influenced overall survival (OS) in univariate analysis, whereas in multivariate analysis, both the type of onset and NIH scoring system significantly affected outcome. In this regard, de novo onset of cGVHD was associated with a favourable prognosis, whereas severe cGVHD had an adverse impact on survival. Based on multivariate analysis, we developed a scoring system that considers both type of onset and grade of severity, which allowed us to differentiate 4 subgroups that clearly differed in terms of outcome, with OS at five years of 82%, 70%, 50% and 25% as it shown in figure 1.

Previous studies have shown that 30% to 70% of long-term survivors after transplantation require immunosuppressive treatment for more than 2 years[19,20,21]. In the current study, we confirmed in a series of homogeneously treated patients undergoing PBSCT, that the NIH scoring system, besides its impact on outcome, is the most important prognostic factor in predicting the risk of relapse after firs-line cGVHD treatment. When considered along with previous development of aGVHD, this system allows us to identify those patients receiving immunosuppressive therapy at the last follow-up. In this regard, patients with mild cGVHD had a significantly higher probability of being off free from immunosuppressive therapy at last follow-up compared with those with moderate or severe cGVHD. In conclusion, the NIH scoring system is of prognostic value in patients undergoing PBSCT and, together with the type of onset, must be considered to predict the outcome of patients who develop cGVHD. These parameters should be taken into account to adapt immunosuppressive strategies and decrease the risk to patients.

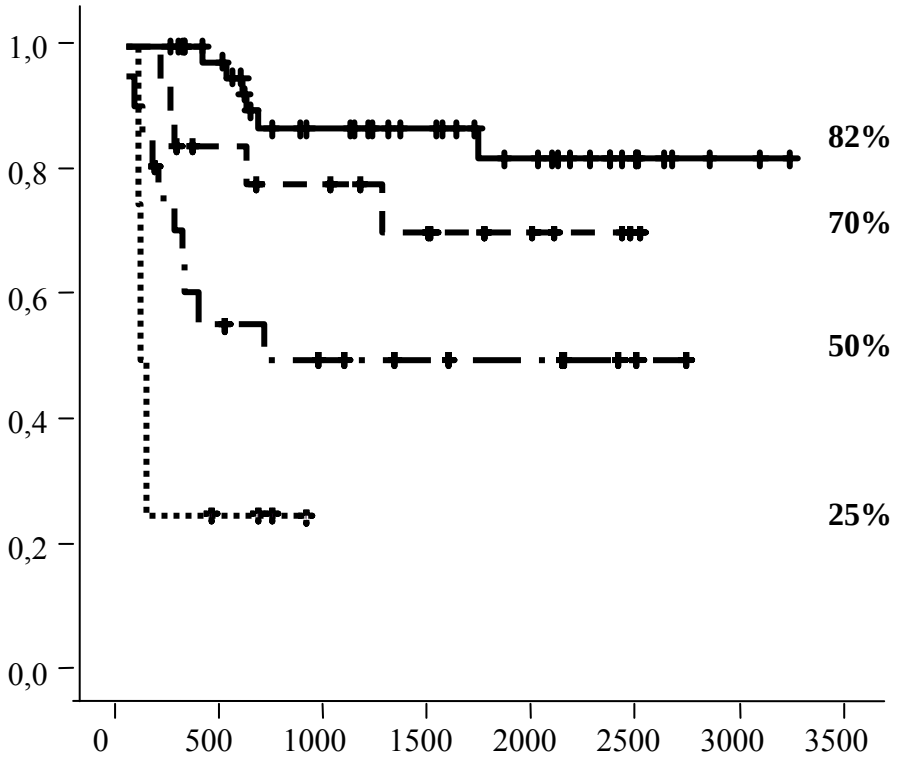

Figure 1. OS in patients with cGVHD according to NIH score plus type of onset. OS, depending on grade of severity according to the NIH scoring system plus type of onset, was 82% for patients with mild cGVHD regardless of the type of onset and patients with moderate cGVHD with *de novo* onset, 70% for patients with moderate cGVHD and quiescent or progressive onset, 50% for patients with severe cGVHD and *de novo* onset, and 25% for patients with severe cGVHD and quiescent or progressive onset.

Our next aim was the identification of predictive factors for the development of severe or extensive cGVHD. Untreated, less than 20% of patients with extensive cGVHD survive with a Karnovsky score ≥70. Indeed, the clinical management of patients with extensive cGVHD is difficult because of the wide variability of disease manifestations, clinical course, infectious complications, and treatment related toxicity[13]. Because immunosupressive treatment decrease morbidity and mortality predicting the onset of cGVHD before clinical deterioration is important[21,22]. Previous studies have identified a chronic myeloid leukaemia diagnosis, sex mismatched, age[23] and an early

complete donor hematopoietic chimerism in peripheral blood[24] as risk factors for development of extensive cGVHD in patients undergoing allo-BMSCT.

Concerning the predictive value of day 100 screening studies for development of cGVHD a few studies in patients undergoing allogeneic bone marrow transplantation have been published. In regard to this, Loughran and colleagues[25] reported that a positive skin biopsy, history of acute GVHD and oral biopsies staining for IgA plasma cells predicting the development of cGVHD. By contrary, JL Wagner and colleagues[26] reported that day 100 screening tests may not be of value in predicting cGVHD development and the use of corticosteroids for acute GVHD (aGVHD) at day 100 had a predictive effect on cGVHD development. Some studies have been performed to assess the predictive value of skin and mucosa biopsies. These studies indicated that mucocutaneous biopsies have been of limited value to confirm or predict the development of systemic GVHD that requires steroid treatment[26,27]. Thus, skin biopsies taken on day +100 have no predictive value with regard to the development of cGVHD later according to Hedal et al[28].

Despite these few studies, information on the value of non-invasive screening tests to predict the risk of extensive or severe cGVHD development after PBSCT is lacking and, considering its wide use as the preferred cell source and the higher incidence of cGVHD in this setting as compared to bone marrow transplant[11-12,29], it would be desirable to identify non-invasive parameters which predict the risk of extensive or severe cGVHD development to allow early intervention prior to clinical deterioration. In our study[30], abnormal liver function tests (LFT) [total bilirubin(Tbil), alkaline phosphatase(AP) and GGT > 2 times above the upper normal limit with or without increased transaminases] and a low absolute lymphocyte count (ALC) [< percentil 25: 0.750x10^9/L] (figure 2) did predict for the risk of cGVHD and its severity. Although liver dysfunction by itself is not usually the ultimate cause of GVHD-related mortality, some authors[14] have reported that acute liver GVHD increased the risk of cGVHD-related death in both the allo-PBSCT and allo-BMSCT settings. Nevertheless, in our study, Tbil, AP, and GGT abnormalities did predict for a higher risk of extensive and severe cGVHD development both in patients with or without prior acute GVHD irrespective of transaminases values. Considering that it is a very simple, fast and costless test, Tbil, AP, and GGT values at day +100 should represent a

most helpfull non invasive screening test to be considered in all patients after allogeneic hematopoietic stem cell transplantation.

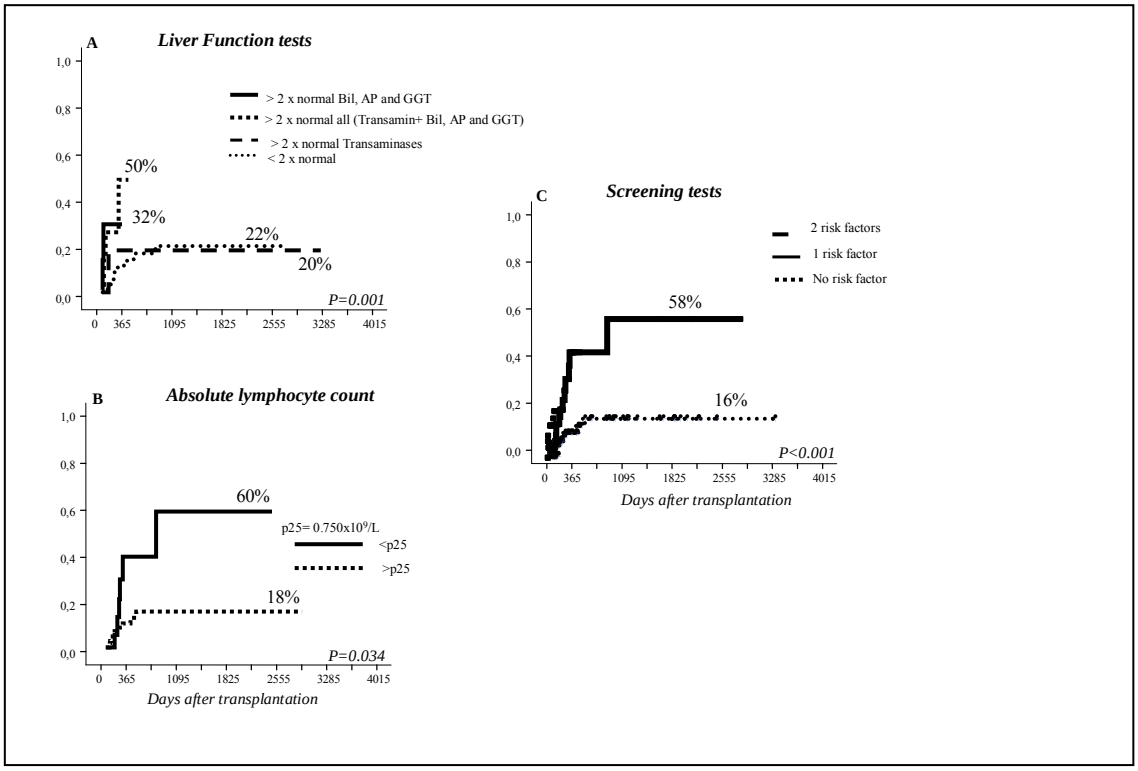

Figure 2 : Cumulative incidence of severe chronic GVHD according to liver function tests (**A**), absolute lymphocyte count (**B**) and the combination of LFT and ALC [Bil, AP and GGT > 2 upper normal limit (UNL) plus absolute lymphocyte count (ALC) < 0.750 x 10^9 / L versus those patients with Bil, AP and GGT < 2 UNL and / or ALC > 0.750 x 10^9 / L] (**C**).

Contrary to previous reports, we did not identify aGVHD as a predictive factor for extensive or severe cGVHD. Some of these studies did not exclude patients with active GVHD and or on steroids what coul impact the results of such analysis[25,26,31,32]. In this regard Atkinson et al.[33] have reported that the 3 years risk of the development of cGVHD was 28% ±3%, 49% ±5%, 59% ±6%, 80% ±9%, and 85% ±15% for patients with grades 0, I, II, III, and IV aGVHD, respectively (*P < 0.0001*), while among patients with no or grade I aGVHD, prior aGVHD did not predict the subsequent development of cGVHD. It is worth mentioning that in our series of patients 50% had grade I or no aGVHD and only 9% developed grades III-IV aGVHD. This could explain the lack of correlation between acute and chronic GVHD in our work.

We did not identify a low-platelet count as a risk factor for extensive or severe cGVHD development. Nevertheless, thrombocytopenia (< 100 x 10^9/L) has been widely described as a surrogate marker of cGVHD severity after

allogeneic transplantation[7,14,32]. In our serie the median platelet count was 169 x 10^9/L. However, the risk factor "thrombocytopenia" has been identified mainly in cohorts receiving a myeloablative conditioning regimen and bone marrow as a graft source. Therefore, it remains to be shown whether low platelets remain as a risk factor in patients receiving nonmyeloablative regimens and PBSC as a graft source. By contrast, we identified low ALC (<percentil 25: 0.750x10^9/L) as a risk factor for predicting the development of extensive and severe cGVHD, which has not been described to date. To rule out the potential confounding effect of aGVHD–related therapy[34], the analisys was performed excluding patients which were on steroids at day +100 or had prior aGVHD. Several reports have pointed out that patients with a slower lymphocyte recovery after transplantation had a poorer outcome[35-40] and, in this regard, Pavletic et al.[36] have previously shown that a faster lymphocyte recovery did correlate with better survival after alloPBSCT, although these studies did not focus on the development of extensive or severe cGVHD. The correlation between low lymphocyte counts and subsequent development of extensive or severe cGVHD could reflect the fact that alloreactive T cell clones induce a cytotoxic effect not only on cGVHD target organs but also on the T cell clones which may react against pathogens, explaining both the immunosuppression induced by cGVHD and the narrow TCR repertoire observed among patients with cGVHD. Thus, the patients who developed chronic GVHD have a lower average score of TCR-Vβ complexity than that of patients without cGVHD.[41] In this regard, it has recently been reported that a high natural killer cell reconstitution at day +60 after transplantation is associated with reduced relapse and death after reduced intensity conditioning without an increased incidence of GVHD.[42] These studies indicate that an early immune reconstitution have prognostic implications after allogeneic transplantation. Unfortunately, the retrospective nature of our study precludes a detailed analysis of lymphocyte subsets, which would have been more informative in understanding some of the mechanisms behind this observation.

In summary, in the current study we have shown that an increased total bilirubin, alkaline phosphatase and gammaglutamil transferase levels together with the low absolute lymphocyte count at day +100 are a non invasive simple, fast and accurate tests to predict the risk of extensive and severe cGVHD after

allogeneic peripheral blood stem cell transplantation from matched related donor. Further studies are required to evaluate its prognostic value in other patients populations such as those undergoing unrelated donor transplant or bone marrow transplant.

Treatment for cGVHD remain on the basis of systemic immunosuppressive and corticosteroid therapy. However, these drugs are associated with important toxicity. Indeed, many patients diagnosed with cGVHD finally die, not because of cGVHD itself, but to infectious complications secondary to the immunosuppressive effect of drugs administered to control it. The risks of prolonged immunosupression include hypertension, glucose intolerance, osteoporosis, osteopenia, myopathy, weight gain with characteristic redistribution of body fat, neuropsychiatric disorders, avascular hip necrosis, growth retardation in children, cataract, also hamper the quality of life of the patients. Furthermore, cGVHD has been associated with the powerful graft-versus-leukemia effect that contributes to the lower relapse rate observed in patients who develop it[15-18]. For this reason, systemic immunosuppressive treatment must be carefully administered on the basis of the severity of cGVHD and also taking into account the risk of relapse and the disease status at the time of treatment[43]. Along these lines, in the cGVHD setting the use of drugs with topic or non systemic immunosupressive effect should be helpfull to avoiding systemic exposure to corticosteroids. Beclomethasone dipropionate (BDP) represents an interesting therapeutic option. BDP is a potent topically active steroid with limited systemic adverse effects by incomplete absorption and intestinal hydrolysis of the propionate residues[44] and has demonstrated efficacy in the treatment of gastrointestinal acute GVHD (GI aGVHD) either alone or in combination with prednisone[45-47]. In a randomized trial, the use of BDP in combination with prednisone at 1 mg/kg/ reduced GVHD treatment failures from 65% in the placebo arm to 39% in the BDP group ($P = 0.003$). During the 80-day study period, there was additional evidence of clinical benefit in the BDP arm, largely as a result of the decreased need for protracted prednisone dosing[48]. In our own experience[46], the use of BDP without systemic steroids yelded a 77% response rate in a series of 26 patients diagnosed with GI aGVHD, with 65,5% of patients achivieng complete response (CR). At final

follow-up, 50% of the 26 patients did not require systemic steroids to treat GI aGVHD.

Only 1 study reported by Iyer et al.[49] has so far evaluated the use of BDP in the GI cGVHD. They evaluated the efficacy of BDP in 13 patients with GI cGVHD and 2 patients with aGVHD. All patients but 1 had received methylprednisolone at 2 mg/kg/day as prior therapy for GI cGVHD and had no symptom relief. Nine (60%) patients responded to BDP as measured by improvement or complete resolution of symptoms and the ability to taper steroids. There were 20% complete and 40% partial responses (PRs).

We described the efficacy and safety of BDP in a series of 33 patients with GI cGVHD[50]. We observed 84,6% CRs and 7,7% PRs among patients receving BDP as first-line treatment, whereas these figures were 42,9% and 28,6%, respectively, among patients receiving it as more than first-line treatment. Our results illustrate the efficacy of BDP as a first-line treatment, with an impressive 84,6% of CRs in this subset of patients. For those patients who received BDP as a second – or third-line treatment, which is a population more similar to the series previously reported[49], 42,9% of the patients achivied CR. It is worth mentioning that, unlike the patients analyzed by Iyer et al.[49], who had no symptom relief after receiving 2 mg/kg/day methylprednisolone, patients included in the current study were already off systemic steroids at the time of cGVHD relapse, thus representing a population with a better prognosis.

Despite this high initial response rate, a high relapse rate was observed in the current study. Nevertheless, most relapses occurred after BDP discontinuation and, considering that standard therapy is usually maintained for at least 9 months, the use of BDP for 16 weeks with an additional 4 weeks of tapering could have been too short a period to ensure the mantainance of responses. Moreover, all patients in this study had received peripheral blood as a progenitor stem cell source and, according to previous studies, cGVHD relapses occur at a high frequency in this subset of patients[19,51]. In this context, Flowers et al.[52] reported a relapse rate ranging from 61% to 84% among patients diagnosed with cGVHD after peripheral blood allogeneic transplantation. Despite the high rate of recurrence, 39,4% of our patients were finally free of systemic immunosuppressive treatment. In addition our study documents a low toxicity profile, with only 2 cases of Cushing`s syndrome, 1

case of hyperglicemia, and 2 cases of musculoskeletal pain during the period of BDP taper, which suggests some degree of absorption of the drug. Corticosteroid activity studies evaluating treatment with BDP have not revealed any important secondary effects related to infectious disease, although partial HPA axis suppression is possible[46,48]. Metabolites of BDP are systemically bioavailable, resulting in a decreased adrenal responsiveness during the period of drug exposure[49,53]. Recent studies of long-term use of oral, topically active corticosteroids have demonstrated little evidence of clinical adrenal insufficiency[49,54]. Nevertheless , 3 published series[45,46,48], did not produce any evidence of HPA axis suppression in patients with oral BDP treatment for GI cGVHD, and clinical responses to this treatment suggest that absorption is not necessary for efficacy. In conclusion, the current study shows that BDP, in the absence of systemic steroids, is a highly effective initial therapeutic approach for GI cGVHD. This helps to avoid complications related to systemic steroids, although the final duration of treatment remains to be determined.

Chronic GVHD treatment is based on the use of immunosupressive drugs plus corticosteroids. Although a large number of patients obtain response after first line treatment, the incidence of relapse is rather high, specially among patients receiving peripheral blood progenitors stem cells, so that one third of these patients required immunosupresive treatment five years after transplantation[19]. Moreover, the morbility and mortality in allo-HSCT long term survivors have a close relationship with the use of immunosupressive drugs and corticosteroids for cGVHD treatment. Accordingly, it would be desirable to develop strategies which allow the control of cGVHD but avoiding the long-term exposure to these drugs and in this regard vitamin D (vit D) represents an interesting alternative due to its immunomodulatory effect.

Previous reports describe the efficacy of a vit D analog MC1288 in preventing clinical and histological signs and symptoms of acute GVHD in a rat bone marrow transplant model[55]. In other study Middleton et al.[56] demonstrated the relationship between VDR gene polymorphism in recipients and severe aGVHD, and also between VDR gene polymorphism in donors and cGVHD and survival. The effects of vitamin D are mediated by the nuclear vitamin D receptor (VDR). It is constitutively expressed in monocytes, and in both B and T activated lymphocytes. More recently, the effect of vit D on the phenotypic and

functional characteristics of dendritc cells and T cells were evaluated by Rosenblatt et al.[57] In this study the authors demonstrated the inhibitory effect of vit D on T cells proliferative response, the production of Th2 cytokines for T cells stimulated by alloreactive dendritic cells and the absence of FOXP3 expressing regulatory T cells populations in the presence of vit D.

There is a lack of information regarding the use of vit D treatment for cGVHD and to the best of our knowledge this is the first study describing the clinical impact of the treatment with vit D on the outcome of cGVHD. For this purpose we analyzed patients who were receiving immunosuppressive treatment and require vit D due to osteoporosis or osteopenia and compared for each patient the outcome of cGVHD prior to and after the beginning of this treatment[58]. No other immunosuppressive drugs were added during the whole period. Interestingly, we found an important improvement in the severity of cGVHD so that at 6 months after vit D treatment no patients displayed severe cGVHD versus 3 at the beginning. Moreover, at that time 5 patients have complete remission and were not receiving immunosuppressive treatment. This is a very important information since previous studies have shown that 30% to 70% of patients surviving more than 100 days after transplantation require immunosuppressive treatment for more than 2 years[15,20]. Taking into account the adverse effects of these drugs the use of vit D would be most helpful in these patients. In fact, the reduction of physical activity during high-dose chemotherapy and bone marrow aplasia in these patients due to infections, general weakness, isolation together with nutritional restrictions, gastrointestinal disturbances and a reduced exposure to the sun could lead to the appearance of a prolonged vitamin D deficiency for more than 6 months after allo-HSCT[59].

Another interesting finding in our study is related to the considerable reduction of cGVHD relapses or progressions. Thus, at 6 months after the beginning of treatment with vit D, 9 of 12 patients had no relapse or progression. Accordingly, immunosuppresive treatment could be stopped in 5 patients. Comparing patients who did or did not receive vit D with respect to improvement of cGVHD and withdraw immunosupresion we use a cohort of 12 patients with similar characteristics in terms of cGVHD extension and NIH classification who did not receive vit D in order to compare their outcome to

those receiving vit D who did not relapse prior to treatment. At six months 3 patients who where receiving vit D were free from systemic immunosupression versus 4 patients who did not receive vit D and global response was better in those receiving vit D. Regarding those patients who had already relapsed at the time of vit D treatment, each patient can be considered as his own control prior to and after vit D and, in this regard, 3 out of 6 patients who had relapsed prior to vit D did not relapse after treatment. Furthermore, during the time of study we did not observe any adverse effects related to vitamin D.

In conclusion, treatment with vitamin D appears to be effective, safe and inexpensive for the management of patients with cGVHD. The current study establishes the basis for further studies which are required with a larger number of patients to better assess the potential immune-modulatory effect of vitamin D on the chronic GVHD setting.

CONCLUSIONS

1. The NIH scoring system has prognostic value in patients undergoing allo-PBSCT and, together with the type of onset, must to be considered to predict the outcome of patients who develop cGVHD and should be taken into account to adapt immunosuppressive strategies and decrease the risk to patients.

2. Liver function tests (an increased total bilirrubin, alkaline phosphatase and GGT > 2 times above the upper normal limit) together with absolute lymphocyte count (< 0.750 x 10^9 / L) at day +100 are non invasive, simple, fast and efficient predictors of extensive or severe cGVHD development after allo-PBSCT.

3. Beclomethasone dipropionate in the absence of systemic steroids, is a highly effective initial therapeutic approach for gastrointestinal cGVHD.

4. Treatment with vitamin D in association with immunosuppressive drugs appears to be effective, safe and inexpensive for the management of patients with refractory cGVHD.

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