Universidad de Granada

FACTORES PRONÓSTICOS CONSOLIDADOS Y EMERGENTES EN EL MIELOMA MÚLTIPLE

Tesis presentada por

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Para optar al grado de Doctor

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Granada, 1 de septiembre de 2016

Fdo.: Manuel Jurado Chacón

"Humildad, Señor, humildad"

AGRADECIMIENTOS

-A mi mujer.
-A mis hijas.
-A mi familia.
-A los buenos maestros.
-A los buenos compañeros.
-A los pacientes.

GLOSARIO DE ABREVIATURAS

ACAR	Anomalías citogenéticas de alto riesgo
AL	Amiloidosis primaria
CFM	Citometría de flujo multiparamétrica
CLL	Cadenas ligeras libres
CPc	Células plasmáticas clonales
CRAB	*Calcium elevation, Renal failure, Anemia, lytic Bone lesions*
CrCl	Aclaramiento de creatinina
CVRS	Calidad de vida relacionada con la salud
DM2	Diabetes mellitus tipo 2
EE	Enfermedad estable
EMR	Enfermedad mínima residual
FISH	Hibridación fluorescente in situ
GEM	Grupo Español de Mieloma
GMSI	Gammapatía Monoclonal de Significado Incierto
GWAS	*Genome-wide association Studies*
Hb	Hemoglobina
HLC	*Heavy/Light Chain*
Ig	Inmunoglobulina
IMMEnSE	*International Multiple Myeloma Research Consortium*
IMWG	*International Myeloma Working Group*
IPI	Índice pronóstico internacional
ISS	*International Staging System*
LDH	Lactato deshidrogenasa
MMS	Mieloma múltiple smoldering
MMRR	Mieloma múltiple en recaída o refractario
PEG	Perfil de expresión génica
PET/TC	Tomografía de emisión de positrones/ Tomografía computerizada
RC	Remisión completa
R-ISS	*Revised- International Staging System*
RNM	Resonancia nuclear magnética
sCLLc	Cociente de CLL en suero
sCr	Creatinina sérica
SEER	Registro Surveillance, Epidemiology, and End Results
SLP	Supervivencia libre de progresión
SNP	*Single nucleotide polymorphism*
TAPH	Trasplante autólogo de progenitores hematopoyéticos
TFGe	Tasa de filtración glomerular estimada
TPH	Trasplante de progenitores hematopoyéticos

ÍNDICE

I. INTRODUCCIÓN

1. Definición del mieloma múltiple

El mieloma múltiple (MM) sintomático es una neoplasia hematológica muy heterogénea desde el punto de vista clínico y molecular, caracterizada por la proliferación de células plasmáticas clonales (CPc) en la médula ósea, acompañada por la presencia de un componente monoclonal en suero y/o en orina en la mayoría de los casos, y asociada a disfunción orgánica, que se evidencia mediante las características CRAB (hipercalcemia, insuficiencia renal, anemia, y lesiones óseas). Se enmarca dentro de la clasificación de neoplasias linfoides de célula B madura (Swerdlow SH *et al*, 2016).

El diagnóstico del MM se realiza en base a criterios diagnósticos consensuados por el *International Myeloma Working Group* (IMWG) en determinados momentos (IMWG, 2003) (Rajkumar SV *et al*, 2014). La definición del MM ha ido modificándose con el tiempo, como consecuencia de la mejora las tecnologías disponibles y su capacidad para establecer el diagnóstico y pronóstico. Incluso en la actualidad, en base a los últimos criterios diagnósticos de 2014, la definición sigue siendo imperfecta y en ocasiones, pueden existir dudas diagnósticas que, sin embargo, se suelen disipar con un estrecho seguimiento del paciente en cuestión.

De acuerdo con los actuales criterios (Rajkumar SV *et al*, 2014), se requiere la presencia de una plasmocitosis clonal en médula ósea del 10% o superior, o bien un plasmocitoma demostrado por biopsia, junto con alguno de los siguientes eventos definitorios de MM (Tabla 1):

1. Evidencia de daño orgánico atribuible al MM (CRAB). En particular:
    - Hipercalcemia >2.75 mmol/L (>11 mg/dL).
    - Insuficiencia renal: creatinina sérica (sCr) >177 μmol/L (>2 mg/dL) o aclaramiento de Cr (CrCl) <40 ml/min.

- Anemia: hemoglobina (Hb) <100 g/L o >20 g/L por debajo del límite inferior de la normalidad.
- Lesiones óseas: una o más lesiones osteolíticas, en la radiología convencional, tomografía computerizada o tomografía de emisión de positrones con tomografía computerizada (PET/CT).

2. Uno o más de los siguientes biomarcadores de malignidad:
   - Plasmocitosis clonal medular >60%.
   - Cociente de cadenas ligeras libres en suero (implicada/no implicada) (sCLLc i/ni) ≥100.
   - Más de una lesión focal (de al menos 5 mm de tamaño) en la resonancia nuclear magnética (RNM).

El componente monoclonal puede estar constituido por una inmunoglobulina (Ig) completa o por una cadena ligera libre (CLL). La presencia de una proteína monoclonal en suero u orina no es obligatoria para el diagnóstico, sólo divide el MM en secretor y no secretor. Menos de un 2% de todos los MM se presentan como verdadero MM no secretor (Chawla SS *et al*, 2015).

El porcentaje de CPc en médula ósea debe ser evaluado mediante estudio morfológico (no por citometría de flujo) en una muestra de aspirado medular o biopsia ósea.

La clonalidad será establecida por una restricción de cadena ligera mediante citometría de flujo, inmunohistoquímica o inmunofluorescencia. Si no se alcanza el porcentaje del 10%, se requiere la presencia de más de una lesión ósea (así se diferencia el MM del plasmocitoma solitario con mínima infiltración medular).

Solo la insuficiencia renal secundaria a nefropatía por cilindros de cadenas ligeras, en base a la histopatología o la presencia de un nivel de CLL implicada elevado,

habitualmente > 1500 mg/L, se considera un evento definitorio de MM. Se recomienda biopsia renal en casos dudosos, en particular si la CLL implicada es <500 mg/L.

Tabla 1. Criterios diagnósticos del mieloma múltiple

1. PLASMOCITOSIS CLONAL EN MÉDULA ÓSEA ≥ 10% ó PLASMOCITOMA DEMOSTRADO POR BIOPSIA, Y	
2. EVENTO DEFINITORIO DE MIELOMA	
2.1. DAÑO ORGÁNICO (CRAB)	**2.2 BIOMARCADOR DE MALIGNIDAD**
o Hipercalcemia >2.75 mmol/L (>11 mg/dL).	**o Plasmocitosis clonal medular >60%.**
o IR: sCr >177 μmol/L (>2 mg/dL) ó CrCl < 40 ml/min.	**o sCLLc (i/ni) ≥100.**
o Anemia: Hb <100 g/L ó >20 g/L por debajo del límite inferior de la normalidad.	**o > 1 lesión focal (≥5 mm) en RNM.**
o Lesiones óseas: 1 ó > lesiones osteolíticas en RC, CT ó PET/CT.	

CRAB: *Calcium- Renal Insufficiency- Anemia- Bone lesions*. IR: Insuficiencia Renal. sCr: Creatinina sérica. CrCl: Aclaramiento de creatinina. RC: Radiología Convencional. CT: Tomografía Computerizada. PET: Tomografía de Emisión de Positrones. sCLLc(i/u): Cociente de Cadenas Ligeras libres en suero (implicada/no implicada). RNM: Resonancia Nuclear Magnética.

2. Patogénesis del mieloma múltiple

Los linfocitos B se originan a partir de células madre hematopoyéticas pluripotenciales y son los responsables de la inmunidad humoral. El receptor para el antígeno de las células B les permite reconocer y responder a los antígenos, desencadenando respuestas celulares de distintos tipos. La primera fase de diferenciación de los linfocitos B ocurre en la médula ósea. Sus precursores (células proB, preB y células B inmaduras) reordenan los genes que codifican para las cadenas pesada y ligera del receptor para el antígeno de las céluas B, y posteriormente maduran en los órganos linfoides secundarios, donde reconocen antígenos, proliferan y se diferencian a CP secretoras de anticuerpos.

El MM representa, desde el punto de vista ontogénico, la expresión final de un espectro de neoplasias de células B. Puede considerarse una enfermedad multipaso, que tiene su origen en células B maduras, tras pasar el centro germinal (Figura 1). El clon tumoral estaría representado por la contrapartida neoplásica de las células plasmáticas diferenciadas de larga duración, productoras de inmunoglobulinas, consideradas clave para la memoria inmunológica. En base a los estudios de secuenciación de la región variable de la cadena pesada del gen de las inmunoglobulinas, el evento genético inicial se considera que tiene lugar en el propio centro germinal, facilitado por los procesos de hipermutación somática y cambio del gen de la cadena pesada de las inmunoglobulinas, que condiciona el cambio de isotipo. Diferentes cambios genéticos y en el microambiente medular dan lugar a una transformación maligna de estas células (Palumbo A & Anderson K, 2011).

Los cambios genéticos primarios iniciales más comunes ocurren a nivel de la región del gen de la cadena pesada, en el cromosoma 14 (q32.33), que puede sufrir traslocaciones,

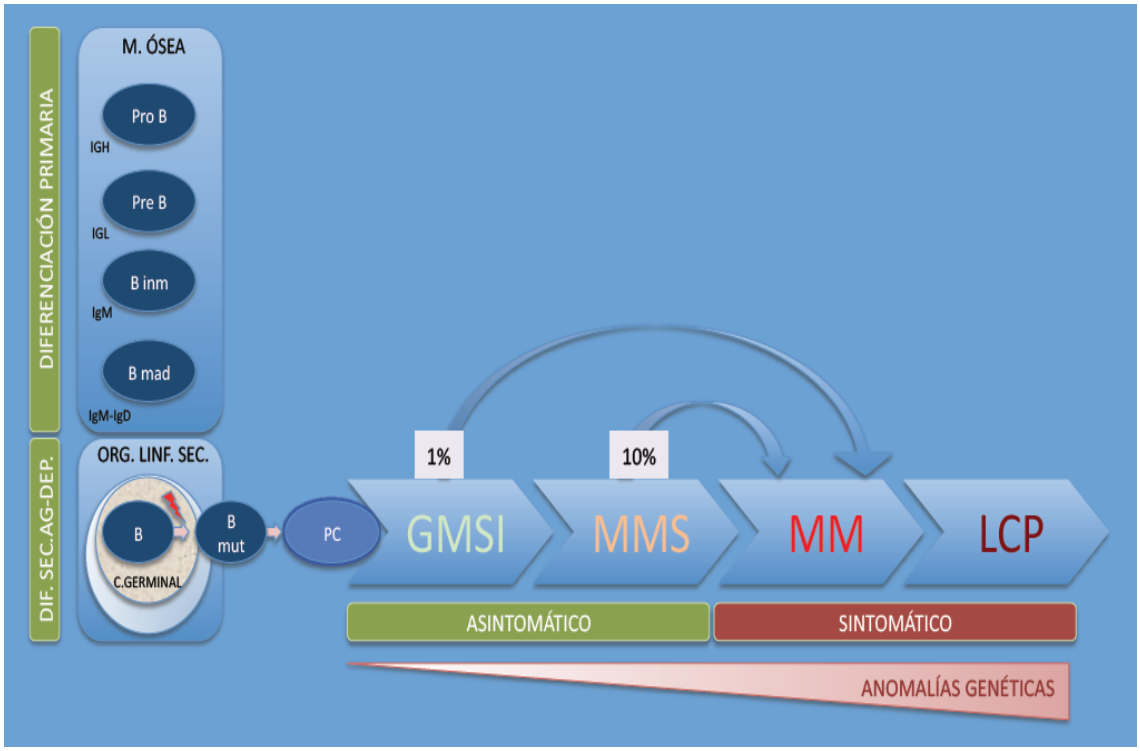
en particular t(14;16) y t(4;14), con *MAF* (Fonseca R *et al*, 2003; Avet-Loiseau H *et al,* 2011) y *MMSET* (Zhan F *et al*, 2006), respectivamente.

Los cambios genéticos secundarios están implicados en la progresión de la enfermedad y afectan a anomalías en *MYC* (8q24), activación de *NRAS* y *KRAS*, mutaciones en *FGFR3* y *TP53*, así como la inactivación de inhibidores de quinasas dependientes de ciclinas *CDKN2A* y *CDKN2C* (Manier S *et al*, 2016). Al mismo tiempo, pueden existir anomalías epigenéticas tales como alteraciones en la expresión de micro RNA y modificaciones en la metilación de los genes (Walker BA *et al*, 2014; Corre J *et al*, 2015).

Las anomalías genéticas alteran la expresión de moléculas de adhesión en las células tumorales, así como las respuestas a estímulos de crecimiento en el microambiente. La interacción entre las CPc y otras células del microambiente o proteínas de la matriz extracelular (colágeno, fibronectina, laminina, vitronectina) está mediada por receptores de superficie celular (integrinas, selectinas, etc), que inducen proteínas reguladoras del ciclo celular y proteinas que inhiben la apoptosis, provocando en última instancia el crecimiento tumoral, migración tumoral y resistencia al tratamiento. La inducción de moléculas que estimulan la angiogénesis, como el factor de crecimiento vascular endotelial contribuyen al crecimiento tumoral.

El microambiente medular juega un papel muy destacado en la progresión de la enfermedad (Bianchi *et al*, 2014). A pesar de los recientes avances, la patogénesis del MM se conoce sólo parcialmente (Bianchi *et al*, 2015a). Sin embargo, la mayor comprensión de las vías metabólicas implicadas ha permitido establecer nuevas dianas terapéuticas, haciendo posibles nuevos abordajes terapéuticos.

Figura 1. Origen del MM. MM como modelo de enfermedad multipaso



ProB: célula progenitora B (reordenamiento del gen de la cadena pesada). PreB: célula pre-B (reordenamiento del gen de la cadena ligera). B inm: célula B inmadura (expresa IgM de membrana). B mad: célula B transicional en proceso de maduración, que expresa IgM e IgD y migra a los órganos linfoides secundarios. B: Las células B maduras en el centro germinal sufren un proceso de hipermutación somática y cambio de isotipo. Se establece un proceso de selección de clones en un tipo de células denominadas centrocitos, que pueden especializarse hacia célula B memoria o hacia CP. GMSI: Gammapatía monoclonal de significado incierto. MMS: MM Smoldering. LCP: Leucemia de células plasmáticas.

3. Epidemiología del mieloma múltiple

El MM representa aproximadamente un 1-2% del total de neoplasias malignas y alrededor de un 10-15% de las neoplasias hematológicas (Alexander DD *et al*, 2007; Smith A *et al*, 2009; Siegel RL *et al*, 2016). A pesar de los recientes avances, concretados en una mejor definición del pronóstico y de los criterios de respuesta, una optimización del uso del trasplante de progenitores hematopoyéticos (TPH), un mejor tratamiento de soporte y el uso de combinaciones de nuevos fármacos, el MM sigue siendo incurable en la mayoría de los casos, aunque existe evidencia creciente sobre la posibilidad de cronificar la enfermedad y conseguir una cura funcional en un grupo seleccionado de pacientes (Barlogie B *et al*, 2014). Estos pacientes pertenecerían en su mayoría a una categoría de bajo riesgo de acuerdo con subgrupos moleculares basados en perfiles de expresión génica (PEG) (Chng WJ *et al*, 2016). Para valorar la potencial curación se requiere un seguimiento mínimo de 10 años.

3.1 Incidencia a nivel mundial

El número de casos nuevos de MM en un periodo de tiempo es muy variable en función del tipo de población y país. En general, suele ser mayor en paises nórdicos y menor en paises asiáticos. Por ejemplo, la tasa estandarizada de incidencia de MM en Suecia es de 4.8 casos por 100,000 personas/año (Turesson I *et al*, 2010) y 5.4/100.000 en Reino Unido (Vélez R et al, 2016), mientras que en Korea es de 1.4/100,000 (Lee JH *et al*, 2010) y en Taiwan de tan sólo 0.75/100.000 (Huang SY *et al*, 2007). Estas diferencias geográficas y raciales (Waxman AJ *et al*, 2010) se pueden explicar en parte por factores genéticos, así como por la diferente exposición a factores ambientales. Los datos más relevantes provienen de Estados Unidos, del programa *Surveillance, Epidemiology, and*

End Results del *National Cancer Institute*, en los que se aprecia una discreta tendencia progresiva al aumento en la incidencia, así como al descenso en la mortalidad en los últimos años. En Estados Unidos se estima que habrá 30,330 nuevos casos en 2016, 17,900 en varones y 12,430 en mujeres (Siegel RL *et al*, 2016).

3.2 **Incidencia en España. Registros de base poblacional**

Los registros de cáncer de base poblacional incluyen todos los casos nuevos de MM en un área geográfica determinada y una de sus principales misiones es monitorizar la incidencia y supervivencia de los pacientes, sirviendo de base para la investigación epidemiológica del cáncer. Por otra parte, los registros hospitalarios recogen todos los casos diagnosticados en un hospital concreto, y están implicados fundamentalmente en el manejo clínico de los pacientes. Ningún registro es perfecto. Los primeros están basados en unos estándares de calidad internacionalmente validados e incluyen todos los datos sociodemográficos, así como la existencia de otras neoplasias asociadas, pero sólo incorporan datos clínicos muy básicos. Los segundos incluyen más datos clínicos como el tipo de tratamiento y la respuesta al mismo, así como los antecedentes familiares y personales, y cualquier otra información obtenida de la historia clínica.

En Granada existe un registro de cáncer de base poblacional que comenzó su actividad en enero de 1985, siendo el tercero más antiguo de España. Cubre toda la población de la provincia de Granada, 919,455 habitantes en 2014. Es miembro de la *European Network of Cancer Registries* y de la *International Association of Cancer Registries*, y sus actividades están coordinadas por la *International Agency for Research on Cancer*.

La incidencia de MM en Granada es 2.8 para hombres y 2.4 para mujeres en 2007 (Forman D *et al*, 2013). Un reciente estudio de la Red Española de Registros de Cáncer (Galceran J *et al*, 2014) estima para el año 2014 en España una tasa de incidencia

ajustada a la población estándar mundial de 3 para hombres y 2 para mujeres, por cada 100.000 habitantes.

3.3 Factores de riesgo

El MM es una enfermedad multifactorial. La edad, la historia familiar, el sexo varón, la raza negra, factores genéticos y la gammapatía monoclonal de significado incierto (GMSI), han sido descritos como factores de riesgo.

Edad y sexo. La incidencia del MM, como la de otras neoplasias hematológicas, aumenta con la edad. La mediana de edad al diagnóstico se sitúa en torno a los 70 años. El número de casos en pacientes mayores de 65 años diagnosticados anualmente se estima que aumentará un 77% en 2030, debido al envejecimiento progresivo de la población (Wildes TM *et al*, 2014). Este hecho, junto con el coste asociado a su manejo y la creciente mejoría en la supervivencia, hace preveer que el impacto sanitario del MM aumentará significativamente en los próximos años. La incidencia es algo mayor en varones en la mayoría de registros. La proporción varón/mujer es 1.5 en España (Galceran J *et al*, 2014), 1.8 en Taiwan (Huang SY *et al*, 2007), 1.06 en Inglaterra (Renshaw C *et al*, 2010), 1.33 en Estados Unidos (Rifkin RM *et al*, 2015).

Antecedentes familiares. El riesgo de padecer MM está elevado cuando hay antecedentes familiares de otra neoplasia hematológica (Wang *et al*, 2012; van Valkenburg *et al*, 2016) y es aún mayor cuando existen antecedentes de MM lo que sugiere una predisposición genética compartida y/o exposición a factores medioambientales. El hecho de presentar un familiar de primer grado con una neoplasia hematológica aumenta el riesgo de padecer MM (OR=1.29) y si se trata de un familiar con MM, el riesgo aumenta aún más (OR=1.9), especialmente en hombres (OR=4.13) (Schinasi LH *et al*, 2016). El MM muestra una asociación con antecedente familiar de

cáncer de colon (riesgo relativo 1.91) lo que sugiere que el MM puede compartir susceptibilidad genética con otros tipos de cáncer (Frank C *et al*, 2016).

Raza. El MM es la neoplasia hematológica más frecuente en Estados Unidos entre las personas de raza negra, con una tasa estandarizada de incidencia 2-3 veces superior a la población de raza blanca (Dores GM *et al*, 2008). Las razones no se conocen hasta la fecha. No se han podido demostrar diferencias a nivel genómico que expliquen esta mayor incidencia en afroamericanos, ni diferencias en la prevalencia de firmas de alto riesgo en los perfiles de expresión génica (PEG) (Baker A *et al*, 2013).

Factores genéticos. Los factores genéticos asociados al riesgo de padecer MM se conocen cada vez con mayor precisión, gracias fundamentalmente al desarrollo de los estudios de genes candidato y de los estudios de asociación de genoma completo o *genome-wide association studies* (GWAS) (Manolio TA, 2013). A pesar de un efecto modesto como marcadores de riesgo y de las inciertas consecuencias funcionales implicadas, el conocimiento de determinadas variantes genéticas situadas en unas regiones de riesgo concretas, está contribuyendo a la comprensión de la patogénesis, así como a la valoración del pronóstico individual (Hunter DJ, 2012).

La genómica ha contribuido considerablemente al conocimiento del MM. Para llevar a cabo muchos de estos estudios, se precisa generalmente un gran tamaño muestral, tanto en casos como en controles, por lo que en los últimos años ha existido una tendencia creciente a crear consorcios, como el *International Multiple Myeloma Research consortium* (IMMEnSE) (Martino A *et al*, 2012; Martino A *et al*, 2014; Campa D *et al*, 2015), que ha permitido avanzar en el conocimiento de la epidemiología molecular y factores de riesgo del MM (Morgan GJ *et al*, 2013).

GMSI. El MM está virtualmente precedido en la mayoría de los casos por una GMSI (Weiss BM *et al*, 2009; Landgren O *et al*, 2009), siendo éste el principal factor de riesgo

para desarrollar un MM (Sergentanis TN *et al*, 2015). No obstante, en el momento del diagnóstico del MM sólo se tienen la certeza de un diagnóstico previo de GMSI en un porcentaje muy pequeño de casos (Sigurdardottir EE *et al*, 2015), presentando este grupo una mejor supervivencia. El riesgo de progresión de la GMSI no-IgM es de aproximadamente un 1% al año (Turesson I *et al*, 2014). Entre la GMSI y el MM, existe una entidad intermedia, el MM smoldering (MMS) (Rajkumar SV *et al*, 2015a), en la cual aún no existe CRAB. El riesgo de progresión a MM de estos pacientes es de un 10% al año en los primeros 5 años (Kyle RA *et al*, 2007). Se ha definido una categoría de alto riesgo en el MMS con mayor riesgo de progresión, en base a datos bioquímicos, inmunofenotípicos, citogenéticos y de pruebas de imagen (Rajkumar SV *et al*, 2015a) y otra de ultra-alto riesgo (Waxman *et al*, 2014).

Otros. Diversos factores de riesgo han sido implicados en el riesgo de padecer MM con diferentes niveles de evidencia. Entre ellos, cabe destacar:

a. La obesidad (Larsson SC & Wolk A, 2007; Wallin A & Larsson SC, 2011), que puede considerarse el único factor de riesgo conocido potencialmente reversible. También se ha asociado al riesgo de GMSI (Landgren O *et al*, 2010).
b. La diabetes mellitus tipo 2 (DM2) (Castillo JJ *et al*, 2012).
c. Exposición ocupacional, en particular a pesticidas (Perrotta C *et al*, 2012; Chang ET & Delzell E, 2016) y otros agentes químicos (Liu T *et al*, 2013).
d. Dieta, en particular en relación con el bajo consumo de pescado, vegetales, ajo y bebidas como el te (Lwin ST *et al*, 2015; Wang Q *et al*, 2012).
e. La actividad física reducida (Patel AV *et al*, 2015).
f. El uso de determinados fármacos como la eritromicina ha sido asociado a un incremento del riesgo de MM en hombres (Nuyujukian DS *et al*, 2014).

g. Determinadas infecciones, como la infección por el virus de la inmunodeficiencia humana (Cheung MC *et a*l, 2005) o el virus de la hepatitis B (Franceschi S *et al*, 2011).

h. Enfermedades autoinmunes (Hemminki K *et al*, 2012; Hemminki K *et al*, 2016).

4. Clínica del mieloma múltiple

El MM presenta una enorme heterogeneidad clínica y molecular (Greenberg AJ *et al*, 2014), existiendo cierta relación entre la presentación clínica y las alteraciones citogenéticas. Es precisamente la heterogeneidad intraclonal o intratumoral (Brioli A *et al*, 2014) un importante obstáculo que impide la curación del MM, ya que aumenta paulatinamente desde la GMSI al MM.

Las manifestaciones clínicas más características del MM son el dolor óseo, el síndrome anémico, las relacionadas con la insuficiencia renal, la hipercalcemia, y las infecciones.

La enfermedad ósea se observa aproximadamente en el 80% de los pacientes al diagnóstico, siendo la columna vertebral la localización anatómica más frecuentemente afectada (Tosi P, 2013). De ahí que el dolor óseo sea una de las manifestaciones clínicas más frecuentes. Las modernas técnicas de imagen, en particular el PET/TC y la RNM han ayudado enormemente a detectar enfermedad ósea con mucha mayor sensibilidad que la radiología convencional.

El síndrome anémico está presente aproximadamente en el 73% de los casos, y está relacionado con la infiltración medular y con la IR (Palumbo A *et al*, 2011).

La incidencia de insuficiencia renal al diagnóstico oscila entre el 20% y el 50%, según sea la definición empleada, sCr > 2 mg/dL o una tasa de filtración glomerular estimada (TFGe) < 60 ml/min/m^2. La insuficiencia renal debería ser considerada una emergencia médica (Wirk B, 2011) debido a su impacto pronóstico.

Las infecciones son una causa mayor de morbilidad y mortalidad. El riesgo de desarrollar una infección está incrementado por 7 en comparación con población sana. La edad avanzada y el sexo varón aumentan aún más el riesgo de infecciones (Blimark

C *et al*, 2015). Antes de comenzar el tratamiento, la neumonía es una de las manifestaciones infecciosas más frecuentes (Teh BW *et al*, 2014). Determinadas comorbilidades como la DM2, la obesidad o las enfermedades respiratorias aumentan a su vez la probabilidad de presentar infecciones (Jung SH *et al*, 2014; Harpsøe MC *et al*, 2016).

Aunque los síntomas de neuropatía periférica (parestesias, entumecimiento, sensación de quemazón, debilidad) suelen estar asociados con el tratamiento, aproximadamente un 20% de los pacientes pueden presentar manifestaciones de neuropatía basal (Terpos E *et al*, 2015) y probablemente este porcentaje aumentaría si se efectuase un estudio electrofisiológico basal.

En ocasiones el primer síntoma puede consistir en un cuadro constitucional, una fractura patológica, manifestaciones cutáneas o la presencia de una tumoración. Más raramente, puede debutar como insuficiencia hepática (Coffey D *et al*, 2012), derrame pleural (Zhang LL *et al*, 2014), alteraciones orales (Cardoso RC *et al*, 2014), ascitis (Mitra S *et al*, 2015) y hemorragia retroperitoneal (Alwadhi A *et al*, 2016), entre otros.

5. Datos de laboratorio

El laboratorio juega un papel fundamental en el diagnóstico, pronóstico y manejo clínico del MM.

Hemograma. El hemograma puede evidenciar alguna citopenia, en particular anemia. Como ocurre en otros trastornos linfoproliferativos de célula B, los pacientes con MM también pueden desarrollar anemia hemolítica autoinmune (Kashyap R *et al*, 2014).

Presencia de CPc circulantes. Se debe realizar un análisis basal de la presencia de CPc circulantes en sangre periférica, mediante estudio morfológico e inmunofenotípico, para documentar en su caso la presencia de leucemia de células plasmáticas primaria, una variante rara y agresiva de MM definida por la presencia de más de un 20% de CPc o un valor absoluto de CPc $\geq 2x\ 10^9$ /L, aunque se ha propuesto disminuir estos valores a 5% y $\geq 0.5x\ 10^9$ /L, respectivamente (Fernández de Larrea C *et al*, 2013; Musto P *et al*, 2016).

Bioquímica general. El perfil de bioquímica general debe incluir la sCr, nivel del calcio sérico (corregido en función de la albúmina según la fórmula: calcio corregido = calcio + (4-albúmina) • 0.8 mg/dL), albúmina sérica, β2-microglobulina, proteínas totales y lactato deshidrogenasa (LDH).

La medición de la función renal puede realizarse mediante sCr o CrCl, pero es más adecuado utilizar la tasa TFGe, mediante la fórmula *Modification of Diet in Renal Disease* (MDRD) o la propuesta por la *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) (Dimopoulos MA *et al*, 2010; Terpos E *et al*, 2013):

-Fórmula MDRD: TFG (ml/min/1.73 m^2) = 186 • $(sCr/88.4)^{-1.154}$ • $edad^{-0.203}$ • 0.742 (si mujer) • 1.212 (si origen afro-americano).

-Fórmula CKD-EPI : TFG (ml/min/1.73 m^2) = 177.6 • $sCr^{-0.65}$ • CysC (cistatina C)$^{-0.57}$ • $edad^{-0.20}$ • 0.82 (si mujer) • 1.11 (si raza negra).

Así, mediante el TFGe se diferencian 5 estadíos (Tabla 2):

Tabla 2. Medición de la función renal mediante TFGe

ESTADÍO DISFUNCIÓN RENAL	TFGe
NORMAL	≥ 90
DESCENSO LIGERO FG	60 - 89
DESCENSO MODERADO FG	30 - 59
DESCENSO SEVERO FG	15 - 29
IR TERMINAL	< 15 ó Diálisis

Estudio inmunológico. El estudio inmunológico abarca electroforesis de proteínas en sangre y orina de 24 horas, inmunofijación en sangre / orina, cuantificación de inmunoglobulinas, cuantificación del componente monoclonal en sangre / orina, y CLL. Se sugiere el uso de un algoritmo para optimizar la aproximación diagnóstica (Willrich MAV *et al*, 2016). El análisis de las inmunoglobulinas intactas (*Heavy/Light Chain*; HLC), así como su ratio, en pacientes con MM IgA o Ig G cuantifica la cantidad de Ig implicada de forma más precisa que otros métodos (Koulieris E *et al*, 2012) y puede ayudar en el manejo clínico (Bradwell A *et al*, 2013), estando asociado a la supervivencia como un factor de riesgo independiente (Ludwig H *et al*, 2013; Ludwig H *et al,* 2016).

Estudio microbiológico. Desde el punto de vista microbiológico, es importante conocer el perfil serológico vírico basal (hepatitis B y C, virus de la inmunodeficiencia humana, citomegalovirus) así como documentar mediante cultivo cualquier proceso infeccioso.

Aspirado medular y biopsia de médula ósea. El estudio del aspirado medular debe incluir un análisis morfológico e inmunofenotípico, así como el estudio citogenético mediante hibridación fluorescente in situ (FISH) realizado en la población de CPc mediante selección positiva.

La biopsia ósea a nivel de cresta iliaca es recomendable ya que complementa la información derivada del aspirado, aportando una valoración más precisa de la celularidad y del grado de infiltración medular por CPc. Además, permite realizar inmunohistoquímica y otros estudios, por lo que añade información pronóstica de interés (Stifter S *et al*, 2010; Gabriel J *et al*, 2016). En ocasiones, es más recomendable realizar una biopsia ósea dirigida a lugares accesibles con afectación ósea demostrada previamente por pruebas de imagen.

Estudios de genómica. Los estudios de genómica en general aún no se usan en la práctica clínica diaria, pero existe evidencia creciente sobre su interés en el manejo de los pacientes con MM. El desarrollo de modernas técnicas moleculares (microarrays, secuenciación masiva) han confirmado el alto nivel de heterogeneidad a nivel molecular del MM (Corre J *et al*, 2015; Manier S *et al*, 2016). La utilidad clínica práctica de estos nuevos biomarcadores moleculares está aún por definir.

6. Diagnóstico y diagnóstico diferencial del mieloma múltiple

El diagnóstico del MM se ha establecido históricamente en base a criterios consensuados. Los últimos criterios establecidos por el IMWG (Rajkumar SV *et al*, 2014) han supuesto un avance en la definición del MM, al incorporar información derivada de las modernas técnicas de imagen, así como otros biomarcadores de malignidad como las CLL. Sin embargo, existen situaciones en las que alcanzar un diagnóstico de certeza puede ser difícil, por lo que es necesario realizar una adecuada valoración de otras entidades limítrofes. En la Tabla 3 se refleja el diagnóstico diferencial del MM (Rajkumar SV, 2016).

En el diagnóstico diferencial, la valoración del daño tisular a nivel óseo ocupa un lugar muy destacado. Las modernas técnicas de imagen, en particular el PET/CT aportan ventajas respecto de la radiología convencional, por su mayor sensibilidad y su capacidad para detectar enfermedad extramedular y progresión precoz (Zamagni E *et al*, 2015; Nanni C *et al*, 2016). La RNM es la mejor técnica para valorar afectación de columna vertebral (Dimopoulos MA *et al*, 2015).

Tabla 3. Diagnóstico diferencial del mieloma múltiple

ENTIDAD	CM	CPcMO	CRAB	OBSERVACIONES
GMSI no IgM	<3 g/dL (s)	<10%	No CRAB	Necesarios los 3
GMSI IgM	IgM <3 g/dL (s)	Infiltr. lp <10%	No datos de SLP	Necesarios los 3
GMSI CL	No expr. CPI (IF) <500 mg/24h (o)	<10%	No CRAB	Todos necesarios
MMS	IgG óIgA ≥3 g/dL(s) ó ≥500 mg/24h (o)	y/o 10-60%	y No CRAB	Ambos
MW	IgM	Infiltr. lp ≥10%	Datos de SLP	Todos necesarios
MWS	IgM ≥3 g/dL	y/o Infiltr. lp ≥10%	y No datos de SLP	Ambos
PS	-	Ausente	No CRAB	Lesión única B* TI: resto neg
PSMIM	-	<10%	No CRAB	Lesión única B* TI: resto neg
POEMS	Casi siempre λ	<60%	Polineuropatía	1cM+1cm
AM AL	s y/o o (la mayoría)	La mayoría	SSRA	Rojo Congo+ Amiloide rel.CL

CM: Proteína Monoclonal. CPc MO: Plasmocitosis clonal en médula ósea. CLLc: Cadenas Ligeras Libres, cociente (valor normal 0.26-1.65). GMSI: Gammapatía Monoclonal de Significado Incierto. s.suero. o:orina. CRAB: *Calcium-Renal Insufficiency-Anemia-Bone lesions*. Infiltr.lpl.: infiltración linfoplasmocítica. SLP: Síndrome Linfoproliferativo (datos atribuibles: anemia, síndrome constitucional, hiperviscosidad, adenopatías, hepatoesplenomegalia). CL: Cadena Ligera. CPI: Cadena Pesada Inmunoglobulina. IF: Inmunofijación. CLi: Cadena Ligera implicada. MMS: Mieloma Múltiple Smoldering. MW: Macroglobulinemia de Waldenström. MWS: MW Smoldering. PS: Plasmocitoma Solitario. B*: Lesión solitaria (hueso o tejido blando) demostrada por biopsia con plasmocitosis clonal,

TI: Técnicas de imagen (RNM;TC,PET/CT).PSMIM: PS con Mínima Infiltración Medular. cM: criterios Mayores:lesiones óseas escleróticas, enfermedad de Castleman, aumento VEGFA >3. Cm: Criterios menores. AM AL: Amiloidosis. SSRA: Síndrome sistémico relacionado con amiloide (riñón, hígado, corazón, gastrointestinal, polineuropatía).

7. Pronóstico del mieloma múltiple

El MM es una neoplasia tremendamente heterogénea desde el punto de vista clínico y pronóstico. El pronóstico en el MM se suele medir en términos de supervivencia global, supervivencia libre de progresión, calidad de vida relacionada con la salud, y mortalidad precoz. En la medida en que se mejore la valoración pronóstica, el manejo del paciente podrá ser más individualizado y adaptado a su propio riesgo. En general, el pronóstico en onco-hematología gira en torno a 4 grupos de factores, dos relacionados con características del paciente y dos relacionados con las del propio tumor. Todos los factores pronósticos demostrados para el MM se pueden agrupar en 4 categorías (Figura 2):

-Dependientes de la biología del tumor (Agresividad).

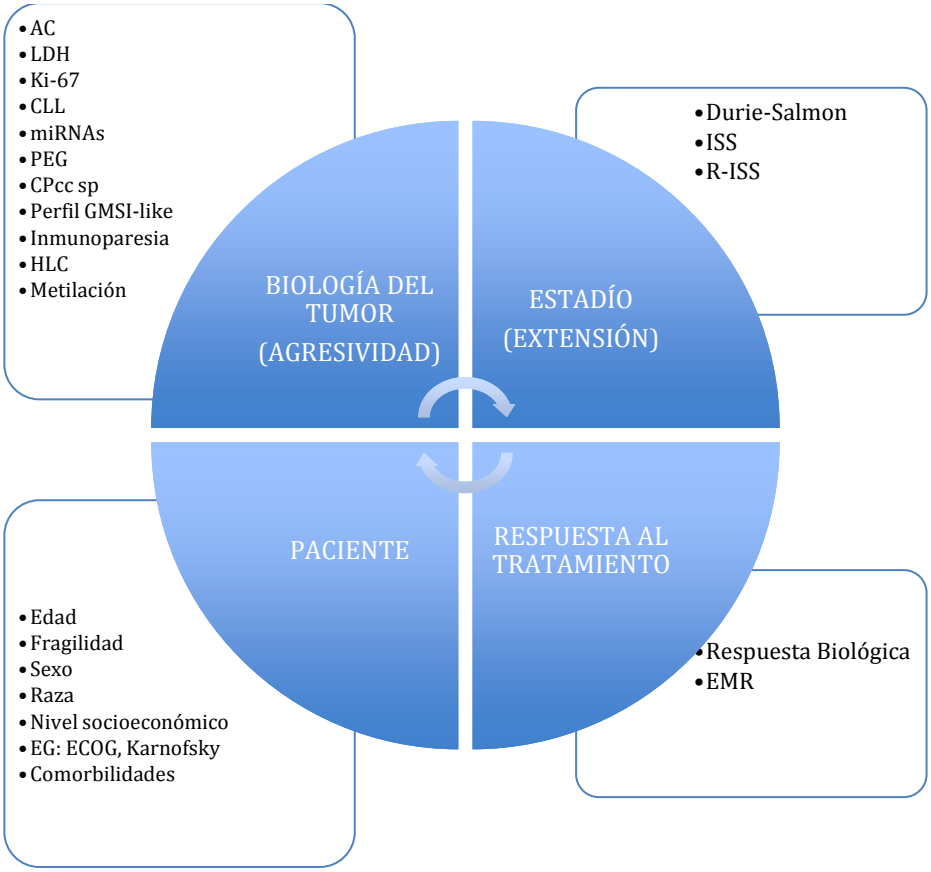
-Dependientes de la extensión (Estadío)

-Dependientes del paciente

-Derivados de la respuesta al tratamiento

El pronóstico en el MM es un área dinámica en contínuo desarrollo. Son muchos los marcadores con interés pronóstico potencial, en particular en la esfera biológica. Sin embargo, para que un factor pronóstico sea utilizado en la práctica clínica debe de cumplir una serie de requisitos. En primer lugar, debe existir evidencia sólida sobre su papel como factor pronóstico independiente. Por otra parte, el sistema de medición debe estar estandarizado para garantizar la comparabilidad. Además, los resultados deben estar disponibles en un plazo razonable de tiempo. Por último, no debe resultar muy caro. En todo este proceso, el papel del IMWG es crucial. Sin embargo, en la actualidad no existe una sistemática global y estandarizada para la valoración del pronóstico en los pacientes con MM.

Figura 2. Clasificación de los factores pronósticos en MM



AC: Anomalías citogenéticas. LDH: Lactatodeshidrogenasa. CLL: Cadenas Ligeras Libres. miRNAs: microRNAs. PEG: Perfil de Expresión Génica. CPcc sp: Células Plasmáticas clonales circulantes en sangre periférica. GMSI: Gammapatía Monoclonal de Significado Incierto. HLC: *Heavy Light Chain*. EG: Estado General. ECOG: *Eastern Cooperative Oncology Group*. ISS: *International Staging System*. EMR: Enfermedad Mínima Residual.

7.1 Factores pronósticos asociados al paciente

Edad. La edad es un factor pronóstico de primer orden (Bringhen S *et al*, 2013; Chretien ML *et al*, 2014). La mediana de edad al diagnóstico en general supera los 66 años, por lo que es recomendable usar una escala de valoración geriátrica (Palumbo A *et al*, 2015a).

Fragilidad. Una escala de fragilidad (basada en la edad, comorbilidades y la condición física y cognitiva) puede predecir mortalidad y el riesgo de toxicidad en pacientes mayores con MM. Por ejemplo, la supervivencia a 3 años en los pacientes fit es del 84% mientras que en los pacientes frágiles es del 57% (Palumbo A *et al*, 2015a).

Sexo. El sexo también juega un papel pronóstico en el MM, siendo la supervivencia global en hombres, en la mayoría de los estudios, menor que en mujeres (Ludwig H *et al*, 2010; Pozzi S *et al*, 2013). Esta diferencia alcanza significación estadística en algunos estudios (Kristinsson SY *et al*, 2007; Kumar SK *et al*, 2008; Turesson I *et al*, 2009).

Raza. La raza es objeto de disparidad en la incidencia y resultados clínicos del MM. En el registro SEER se observa una evolución de la supervivencia global peor en la raza negra (Waxman AJ *et al*, 2010). Estas diferencias pueden deberse en parte a factores socioeconómicos que condicionan menor acceso a determinados tratamientos incluido el TPH.

Nivel socioeconómico. El nivel socioeconómico bajo se ha asociado con una supervivencia global menor en el MM (Kristinsson SY *et al*, 2009; Renshaw C *et al*, 2010). Tras ajustar por otras variables sociodemográficas, un bajo nivel socioeconómico se relaciona de forma independiente con baja supervivencia (Fiala MA *et al*, 2015). Además, el nivel socioeconómico ha sido relacionado específicamente con los resultados del TPH autólogo (TAPH) (Hong S *et al*, 2016).

Otros factores sociodemográficos. Además de los ingresos económicos, otros factores como el estado marital (distinto de casado) y el estado de aseguramiento sanitario (no asegurado) se asocian con pobre supervivencia global (Costa LJ *et al*, 2016). En este estudio, al ajustar por factores sociodemográficos, no se encuentra asociación entre raza y supervivencia.

Estado funcional. El estado funcional general, tanto si se mide con la escala de Karnofsky (Karnofsky DA & Burchenal JH, 1949) como con la escala del Grupo Eastern Cooperative Oncology Group (ECOG) (Oken MM *et al*, 1982), se asocia con la supervivencia global. Su valor pronóstico está tan asumido que aún en nuestros días se sigue utilizando como un criterio de exclusión para la participación en ensayos clínicos.

Comorbilidad. La coexistencia de otros trastornos junto con la enfermedad primaria de interés (comorbilidad) es un área de enorme importancia en Oncología (Sarfati D *et al*, 2016). A pesar de su posible asociación con el estado general y con la fragilidad, son conceptos independientes con impacto independiente en los resultados. Tras décadas de estudios, aún no existe una aproximación estándar para medir la comorbilidad en cáncer, y lo mismo se puede decir para el MM. Por ejemplo, la asociación de la obesidad con una mayor tasa de mortalidad por cualquier causa ha sido demostrada de forma consistente en numerosos estudios en las últimas décadas (The Global BMI Mortality Collaboration, 2016). Sin embargo, la evidencia del impacto pronóstico de determinadas comorbilidades como la obesidad (Teras LR *et al,* 2014) o la DM2 (Chou YS *et al*, 2012) en el MM es menor y ha sido demostrada o sugerida más recientemente. El MM puede asociarse a otros tipos de cáncer e incluso con otras neoplasias hematológicas linfoides (Pantic M *et al*, 2010; Huang C *et al*, 2016) o mieloides (Ríos-Tamayo R *et al*, 2000; Malhotra J *et al*, 2014).

7.2 Factores pronósticos relacionados con la biología del tumor

Anomalías citogenéticas. Las anomalías citogenéticas tienen un profundo impacto pronóstico en el MM y son la base, junto con la edad y el estadío *International Staging System* (ISS), para la estratificación del riesgo (Chng WJ *et al*, 2014), reflejada en la Tabla 4.

Aunque la citogenética convencional ha sido usada durante años, sus limitaciones han condicionado prácticamente su abandono, en beneficio del FISH como técnica de elección para la detección, en muestras con selección positiva utilizando habitualmente CD138, de una selección de sondas para anomalíaas citogenéticas de alto riesgo (ACAR): del(17p), t(4;14) y t(14;16). Otras alteraciones citogenéticas como las ganancias o pérdidas en el cromosoma 1 también implican alto riesgo (Nahi *et al*, 2015; Hebraud *et al*, 2014).

Tabla 4. Consenso del IMWG para la estratificación del riesgo

	ALTO RIESGO	RIESGO ESTÁNDAR	BAJO RIESGO
Parámetros	**ISS II/III y t(4;14) ó 17p13del**	**Otros**	**ISS I/II y Ausencia de t(4;14), 17p13del y +1q21 y Edad <55 años**
SG	**2**	**7**	**>10**
% pacientes	**20**	**60**	**20**
Tratamiento	**Trasplante alogénico, inmunoterapia, etc**	**Tratamiento convencional**	**Tratamiento convencional**

SG: Supervivencia global mediana (años). ISS: *International Staging System.*

Perfiles de expresión génica. Los perfiles de expresión génica (PEG) han mostrado que el análisis de una selección de genes mediante arrays o por PCR cuantitativa en tiempo real, añaden información significativa para la estratificación del riesgo (Sarasquete *et al*, 2013; Kuiper *et al*, 2015; Weinhold N *et al*, 2016; Chng WJ *et al*, 2016). Sin embargo, pocos centros aplican esta tecnología en la clínica diaria.

Elevación de LDH. La elevación de la LDH en el momento del diagnóstico se observa aproximadamente en un 15% de los casos y se asocia con enfermedad avanzada y supervivencia corta. Es un factor pronóstico independiente del ISS (Terpos E *et al*, 2010), por lo que ha sido incluido en el ISS revisado (R-ISS).

Expresión de Ki-67. Ki-67 es una proteína nuclear asociada a la proliferación celular, que puede estar relacionada con la agresividad intrínseca del clon tumoral (Thomas G *et al*, 2007; Kuranda *et al*, 2010), aunque su reproducibilidad intercentro ha sido cuestionada (Polley MYC *et al*, 2013).

CLL. Una ratio de CLL implicada / no implicada en suero (sCLLr i/ni) ≥100 es un biomarcador de malignidad que forma parte de los actuales criterios diagnósticos de MM. Este cociente fue usado para definir el MMS de alto riesgo (Larsen JT *et al*, 2012). La normalización del cociente sCLLr i/ni se asocia con un aumento significativo de la supervivencia libre de progresión y de la supervivencia global, incluso en pacientes con MM que no alcanzan respuesta completa (Moustafa MA *et al,* 2015).

MicroRNAs. MicroRNAs (miRNAs) son pequeñas moléculas de RNAs no codificante implicadas en la regulación de la expresión génica. Algunos miRNAs, por ejemplo miRNA-17–92, miRNA-15a y 16–1, son expresados de forma aberrante y se asocian a MM de alto riesgo (Gao X *et al*,2012; Ahmad N *et al*, 2014; Campo S *et al*, 2014).

CPc circulantes en sangre periférica. La presencia de CPc en sangre periférica (al diagnóstico y en recaída) es un marcador de MM de alto riesgo asociado a peor supervivencia global (Paiva B *et al*, 2013; Gonsalves WI *et al*, 2014).

CPc en médula ósea. El grado de diferenciación de las CPc en la médula ósea medido por CFM ha sido relacionado con la supervivencia libre de progresión y la supervivencia global (Paiva B *et al*, 2016a). Este estudio demuestra que la expresión de CD81 está regulada epigenéticamente. Las CPc menos diferenciadas presentan una alta expresión de genes relacionados con estadíos B inmaduros como PAX5 y muestran un perfil mutacional diferente a las CPc diferenciadas.

Perfil GMSI-like. La citometría de flujo multiparamétrica (CFM) juega un papel pronóstico muy relevante en el MM. Aproximadamente un 8% de pacientes con MM presentan un perfil GMSI-like por CFM, asociado a buen pronóstico (Paiva *et al*, 2013).

Inmunoparesia. La presencia de niveles normales de las inmunoglobulinas no implicadas se asocia a un pronóstico favorable (Kastritis E *et al*, 2014).

***Heavy Light chain*.** Este ensayo permite una medición precisa del componente monoclonal y de la inmunoglobulina policlonal no implicada del mismo isotipo que la proteina monoclonal. La inmunoglobulina no implicada se denomina par emparejado. La supresión (disminución > 50%) de los niveles del par emparejado se asocia a supervivencia global pobre (Ludwig H *et al,* 2016).

Alteración en los patrones de metilación Los fenómenos epigenéticos están mediados por la metilación del ADN. El potencial impacto de factores ambientales en la regulación epigenética ha despertado gran interés (Feil R & Fraga MF, 2012). Un patrón aberrante de mutilación tiene implicaciones en la patogénesis y en el pronóstico del MM (Walker BA *et al*, 2011). La combinación de un genoma altamente metilado con un

estado de baja metilación en *NFKB1* define un grupo de pacientes con mejor pronóstico (Fernández de Larrea *et al*, 2013a).

7.3 Extensión tumoral

La masa tumoral se ha intentado correlacionar con diversas clasificaciones por estadíos. Durante el final del siglo pasado se usó la clasificación de Durie y Salmon, pero a principios del actual siglo se describió el ISS, que sigue siendo utilizado hasta nuestros días, sirviendo de plataforma para construir nuevas clasificaciones pronósticas, como el ISS-revisado (R-ISS) (Palumbo A *et al*, 2015b) (Tabla 5)

Tabla 5: Clasificaciones pronósticas por estadíos

ISS		
ESTADÍO	CRITERIOS	SG
I	β2m <3.5 mg/L y Alb ≥3.5 g/dL	62
II	No cumple I ni III	44
III	β2m >5.5 mg/L	29

R-ISS		
ESTADÍO	CRITERIOS	SG
I	ISS I, no AC y LDHn	NA
II	No cumple I ni III	83
III	ISS III y AC ó LDHe	43

ISS: *International Staging System*. SG: Supervivencia Global mediana en meses. B2m: Beta-2-microglobulina. Alb: Albúmina. R-ISS: International Staging System Revisado.. AC: Anomalías cromosómicas de Alto Riesgo detectadas por FISH: del17p, t(4;14), t(14;16). LDH: lactatodeshidrogenasa (n:normal; e:elevada)

7.4 Respuesta al tratamiento

La valoración de la respuesta al tratamiento es la única variable pronóstica cuya información no está disponible en la valoración inicial. Los criterios de respuesta actualmente utilizados son definidos por el IMWG (Durie BGM *et al*, 2006) (Tabla 6).

Tabla 6. Criterios de respuesta

Categoría	Criterios
Remisión Completa	• Inmunofijación negativa en sangre y orina • Desaparición de los plasmocitomas de partes blandas • ≤ 5% CPc en médula ósea
Remisión Completa estricta	• Igual que la anterior, más: • sCLLr normal. • Ausencia de CPc con fenotipo patológico (por CFM de 4 colores)
Muy Buena Respuesta Parcial	• Componente monoclonal en suero y orina detectable por inmunofijación (no por electroforesis), o • Reducción del componente monoclonal en suero ≥ 90%, más componente en orina < 100 gr/24h
Respuesta Parcial	• Reducción del componente monoclonal en suero ≥ 50%, y > 90% en orina de 24h (o > 200 mg/orina 24h) • Descenso ≥ 50% en la diferencia de CLLi-ni (si no hay componente medible) • Descenso ≥ 50% en CPc en médula ósea (si no hay componente medible y la infiltración basal es > 30%) • Descenso ≥ 50% en el tamaño de los plasmocitomas, si los hubiera
Enfermedad Estable	• No cumple ninguna de las otras categorías
Enfermedad progresiva	• Aumento ≥ 25% con respecto al nivel más bajo alcanzado para uno o más: • Componente monoclonal en suero (el incremento absoluto debe ser ≥ 0,5 g/dL) • Componente monoclonal en orina (el incremento absoluto debe ser ≥ 200 mg/orina 24h) • Incremento > 25% en la diferencia de CLLi-ni (el incremento absoluto debe ser > 10 mg/L) • Incremento > 25% en CPc en médula (el incremento absoluto debe ser > 10 %) • Nuevas lesiones óseas o plasmocitomas de partes blandas, o aumento de tamaño de las previas. • Hipercalcemia atribuida al MM

Aunque estos criterios han demostrado su utilidad pronóstica, en los últimos años se ha observado que el grupo de pacientes con respuesta completa es heterogéneo. La utilidad de la respuesta completa estricta está siendo cuestionada (Martínez-López J *et al*, 2015). Un método que añade información sobre el grado de profundidad de la respuesta es el análisis de la enfermedad mínima residual (EMR), ya sea valorada mediante CFM (Paiva B *et al*, 2015a; Paiva B *et al*, 2016b) o mediante técnicas moleculares (Martínez-López J *et al*, 2014). El estudio de la EMR aporta precisión ya que es capaz de discriminar grupos de pacientes con distinto resultado y está siendo cada vez más usado en la práctica clínica (Paiva B *et al*, 2015b; Kumar S *et al*, 2016; Paiva B *et al*, 2016c). Este método debe ser complementados por técnicas de imagen, siendo en la actualidad el PET/TC (Nanni C *et al*, 2016) la técnica más apropiada (Tabla 7)

Tabla 7. Métodos de monitorización de la enfermedad mínima residual

	CFM	ASO-PCR	NGS	PET/CT
Aplicabilidad	≈100%	≈70%	≈90%	≈100%
Reproducibilidad	A	A	NR	M-A
Disponibilidad	A	M	B	M
Sensibilidad	10^{-5}-10^{-6}	10^{-5}-10^{-6}	10^{-6}	A (4 mm)
Tiempo	2-3 h	3-4 w[a]/≥5d[b]	≥7d	2h
Coste[c]	≈350	1500[a]/500[b]	≈700	≈2000
Cuantitativo	SI	SI	SI	SI
Muestra diagnóstico	No obligatoria	Obligatoria	Obligatoria	No obligatoria
Muestra fresca	SI (<36h)	NO	NO	NA
Muestra irregular	Influye	Influye	Influye	NA
Caracterización celular global	SI	NO	NO	NA
Estandarización	En proceso	SI	NR	NO

CFM: Citometría de Flujo Multiparamétrica (≥8 colores). ASO-PCR: *Allele-Specific Oligonucleotide Polimerase Chain Reaction.* NGS: *Next Generation Sequencing.* PET/CT: Tomografía de Emisión de Positrones con Tomografía Computerizada. A: Aumentada. NR: No reportada. M: Media. B:Baja. H: hora. W:semana. D:día. [a]: Identificación. [b]:Seguimiento. [C]:coste en USD ($).

8. Tratamiento

Los resultados clínicos se han asociado históricamente con la eficacia de los tratamientos empleados, en términos de valoración de la respuesta, supervivencia libre de progresión y supervivencia global, incorporándose en los últimos años la calidad de vida relacionada con la salud. Durante décadas, desde los años sesenta a los noventa, sólo se disponía de los corticoides y la quimioterapia (básicamente, agentes alquilantes como melfalán o ciclofosfamida, y otros como adriamicina y vincristina) como arsenal terapéutico. Durante esta época los avances en supervivencia libre de progresión y supervivencia global fueron muy limitados.

El tratamiento del MM ha ido cambiando radicalmente en los últimos años, en base a la aparición de nuevos agentes, que frecuentemente en combinaciones de dos o más fármacos, han demostrado su eficacia en términos de calidad de vida relacionada con la salud (Sonneveld P *et al*, 2013), supervivencia libre de progresión y supervivencia global (Kumar SK *et al*, 2014).

En la actualidad, el esfuerzo de la comunidad internacional centrado en la investigación del MM es enorme. La mayoría de países europeos disponen de grupos cooperativos que gestionan el desarrollo de cientos de ensayos clínicos que contribuyen a profundizar en el conocimiento de la enfermedad y mejorar los resultados. En España, el Grupo Español de Mieloma (GEM) es un grupo destacado a nivel internacional por sus excelentes contribuciones (San Miguel JF *et al* 2008; Lahuerta JJ *et al*, 2008; Rosiñol L *et al*, 2012; Mateos MV *et al*, 2013; Martínez-López J *et al*, 2014; Bladé J *et al*, 2015; Mateos MV *et al*, 2016a; Mateos MV *et al*, 2016b; Paiva B *et al*, 2016a; Paiva B *et al*, 2016b; Paiva B *et al*, 2016c). El IMWG cuenta con un nutrido grupo de investigadores españoles y entre sus principales cometidos, está actualizar y estandarizar en lo posible

los aspectos relacionados con el diagnóstico, pronóstico y tratamiento de esta enfermedad (Chng WJ *et al*, 2014; Rajkumar SV *et al*, 2014; Palumbo A *et al*, 2015b; Sonneveld P *et al*, 2016; Laubach J *et al*, 2016; Kumar S *et al*, 2016).

Los dos grupos de fármacos más importantes en la actualidad son los inhibidores del proteosoma y los inmunomoduladores. Los corticoides y alquilantes siguen usándose, aunque existe un cierto debate sobre la utilidad de estos últimos en la actualidad. Otros grupos de fármacos como los anticuerpos monoclonales, inhibidores del punto de control, etc, están siendo investigados intensivamente (Bianchi G *et al*, 2015b).

Así, nuevos fármacos están incorporándose paulatinamente a nuestro arsenal terapéutico y nuevas combinaciones de fármacos están siendo utilizadas para incrementar los beneficios terapéuticos. El MM es probablemente la neoplasia hematológica en la que más fármacos se han aprobado en los últimos años. La *Food and Drug Administration* (FDA) ha aprobado cuatro nuevos fármacos sólo en 2015: panobinostat, daratumumab, ixazomib y elotuzumab

En un contexto socio-sanitario de crisis económica en el que se gestionan recursos limitados, incluso en países desarrollados, se plantea la necesidad de establecer criterios de coste-efectividad para garantizar un acceso equilibrado así como un uso racional y optimizado de dichos medicamentos, minimizando riesgos y coste, y maximizando beneficio clínico.

Clásicamente, los pacientes se dividen de acuerdo al tratamiento, aquellos candidatos a recibir TAPH y los no candidatos. El MM sigue siendo en la mayoría de centros la indicación más frecuente de TAPH y su frecuencia va aumentando, lo que demuestra que su utilidad permanece a pesar de la utilización de los nuevos agentes (Auner HW *et al*, 2014).

Por otra parte, también la aproximación terapéutica se clasifica en tratamiento de primera línea, para pacientes con MM de nuevo diagnóstico (Mateos MV *et al*, 2015; Moreau P *et al*, 2015) y tratamiento de la recaída, en pacientes con MM en recaída o refractarios (MMRR) (Nooka AK *et al*, 2015; Laubach J *et al*, 2016).

Las etapas del tratamiento del MM se pueden dividir en: inducción, intensificación (trasplante, si procede), consolidación y mantenimiento.

Los regímenes más usados en la actualidad, desde una perspectiva de tratamiento adaptado al riesgo, han sido recientemente revisados (Rajkumar SV, 2016). El tratamiento continuo parece ofrecer mejores resultados que un tratamiento con una duración limitada (Palumbo A *et al*, 2015c). El tratamiento para los pacientes de alto riesgo citogenético ha sido recientemente revisado (Sonneveld P *et al*, 2016) por el IMWG.

El concepto de línea terapéutica ha sido recientemente enfatizado (Rajkumar SV *et al*, 2015b). Tanto en los ensayos clínicos como en pacientes de la vida real es importante estandarizar en cada caso el numero de línea para permitir la comparación de resultados, ya que es conocido que a medida que el MM progresa, con cada recaída, la efectividad de un determinado tratamiento es habitualmente menor que si el mismo tratamiento se usa en etapas más tempranas.

A pesar de la indudable relevancia de los ensayos clínicos, estos se centran generalmente en un grupo seleccionado de pacientes. Es por ello que, además de los ensayos clínicos, son necesarios estudios de base poblacional para conocer los resultados en pacientes no seleccionados de la vida real.

8.1. Tratamiento en pacientes candidatos

El tratamiento con altas dosis de quimioterapia (generalmente, melfalán 200 mg/m^2) y TAPH como intensificación en pacientes candidatos con MM de nuevo diagnóstico sigue estando indicado (Shah N *et al*, 2015) a pesar de la incorporación de los nuevos agentes. Los factores a tener en cuenta son la edad y las comorbilidades. La edad superior a los 65 años ha dejado de ser un factor limitante en años recientes. Existe un índice de comorbilidad específico para el TPH (Sorror ML *et al*, 2005; Sorror ML *et al*, 2014) que ha sido validado (Raimondi R *et al*, 2012) y es predictivo de la supervivencia global (Saad A *et al*, 2014).

El doble TAPH en tándem puede ser considerado una opción en pacientes que no alcanzan muy buena respuesta parcial tras el primer TAPH o en pacientes seleccionados con alto riesgo. El trasplante alogénico como primera línea para pacientes con MM de nuevo diagnóstico debería seguir siendo considerado un procedimiento experimental (Rajkumar SV, 2016). Las indicaciones para el TPH en el MMRR han sido recientemente revisadas (Giralt S *et al*, 2015).

8.2. Tratamiento en pacientes no candidatos

En este contexto, el objetivo del tratamiento también debe ser alcanzar la mejor respuesta posible y mantenerla, pero siempre hay que buscar el equilibrio con la tolerancia. Las escalas de valoración geriátrica (Palumbo A *et al*, 2015a) pueden ser útiles para decidir el tratamiento óptimo, que debería ser individualizado y adaptado al riesgo.

El IMWG ha consensuado unas recomendaciones para el manejo de estos pacientes (Palumbo A *et al*, 2014).

9. Evaluación de la respuesta al tratamiento

La respuesta al tratamiento debe ser monitorizada mediante los criterios de respuesta consensuados por el IMWG (Durie BGM *et al*, 2006). Durante la inducción se debe monitorizar la respuesta biológica en cada ciclo. Tras cada fase del tratamiento, en particular tras la inducción, se realizará una evaluación global que incluye estudio medular y pruebas de imagen.

La tendencia actual es incorporar a la actividad clínica diaria los estudios de EMR. El IMWG acaba de establecer unos criterios de consenso para la valoración de la EMR en el MM (Kumar S *et al*, 2016). Si determinadas decisiones clínicas como suspender el tratamiento de mantenimiento pueden basarse en los resultados de la EMR es aún objeto de debate y probablemente se precisen ensayos clínicos para contestar a dichas preguntas. Existe un gran cuerpo de evidencia que relaciona la profundidad de la respuesta con los resultados clínicos (Martínez-López J *et al*, 2014; Paiva B *et al*, 2015a; Rawstron AC *et al,* 2015). Para conseguir una curación del MM, primero es preciso conseguir una respuesta profunda y mantenerla en el tiempo (Barlogie B *et al*, 2014).

La recaída es el gran obstáculo para conseguir la curación en el MM. Los pacientes deben ser vigilados estrechamente para detectar la posible recaída en fases tempranas (recaída biológica) en vez de identificar recaídas clínicas, ya que potencialmente un tratamiento más precoz, en un escenario clínico más favorable, puede obtener mejores resultados, en particular tras el TPH (Zamarin D *et al*, 2013; Fernández de Larrea C *et al*, 2014).

II. JUSTIFICACIÓN

El MM es una neoplasia muy heterogénea desde el punto de vista clínico y molecular. Esta marcada heterogeneidad justifica una aproximación adaptada al riesgo, que debe estar basada en la evidencia, en particular en un momento en que las posibilidades de elección dentro del arsenal terapéutico son cada vez mayores.

A pesar de los esfuerzos por estratificar el riesgo, las clasificaciones actuales no pueden justificar por sí solas la enorme diferencia en resultados, en términos fundamentalmente de supervivencia global y mortalidad precoz.

Aunque pueden existir excelentes factores pronósticos que se usan sólo en el laboratorio y en la investigación, un factor pronóstico aplicable a la clínica debería ser una variable fácil de medir, relativamente barata, validada, estandarizada y consensuada, para ayudar en la toma de decisiones clínicas.

Las variables más usadas para estratificar el riesgo en la práctica clínica diaria actualmente son la edad, el ISS y las ACAR detectadas mediante FISH. Sin embargo, el número de factores pronósticos ha crecido en los últimos años. El nivel de evidencia de dichos factores pronósticos es variable, así como su potencial aplicabilidad clínica.

En la investigación sobre el MM, como ocurre en otras entidades, existe una cierta dicotomía entre la investigación clínico-epidemiológica (representada por los ensayos clínicos y los estudios de base poblacional, liderada por clínicos y epidemiólogos) y la investigación básica (representada por la aplicación de técnicas moleculares, liderada por genetistas y biólogos moleculares). Tanto a nivel clínico como a nivel molecular se han ido incorporando nuevos factores pronósticos. Sin embargo, el perfil de la mayoría de estudios sólo tiene en cuenta un grupo de ellos, lo que puede dar lugar a una visión parcial de la realidad. Los modernos métodos de secuenciación masiva son cada vez más rápidos y baratos, y por tanto, aplicables a la clínica. Los nuevos factores pronósticos deberán integrar información clínica y molecular para ser robustos y de

utilidad en la práctica clínica. Por tanto, es necesario contar con datos clínico-epidemiológicos de calidad, que incluyan todas las variables de interés en el momento del diagnóstico, así como variables dinámicas que es preciso monitorizar en el seguimiento de los pacientes. Al mismo tiempo, es necesario incluir datos moleculares. Con la integración de todos estos factores pronósticos, se podrán construir mejores herramientas de estratificación del riesgo que sirvan para adaptar el tratamiento de forma individualizada y hacer realidad la medicina de precisión aplicada al MM.

En la esfera clínica, el interés por el impacto clínico de la comorbilidad en onco-hematología es creciente y el MM no es una excepción. Sin embargo, en la actualidad carecemos de una aproximación integral y consensuada para medir la comorbilidad en el mieloma en pacientes de la vida real. Por otra parte, los ensayos clínicos no son una herramienta adecuada para valorar comorbilidad, ya que en muchas ocasiones la presencia de una determinada comorbilidad se usa como criterio de exclusión.

En la esfera genómica, los métodos de secuenciación masiva están aportando nueva información sobre epidemiología molecular, epigenética, farmacogenómica, EMR, etc. De igual forma, el impacto pronóstico de determinadas mutaciones, variantes genéticas, anomalías en el número de copias, etc, está siendo resaltado en el contexto del MM.

Por todo ello, es preciso entatizar la importancia del momento del diagnóstico de los pacientes con MM para realizar un estudio basal integral de todos los factores pronósticos consolidados con validez clínica demostrada, para conseguir una estratificación individualizada del riesgo lo más precisa posible. La elección de factores pronósticos no sólo debería basarse en los resultados de los ensayos clínicos, ya que éstos utilizan pacientes seleccionados y su validez externa debe ser cuestionada. Los estudios de base poblacional de amplias series con largo seguimiento en el MM son escasos, pero aportan información complementaria a la de los ensayos, minimizando los

sesgos de selección, clasificación y confusión. Estos estudios de base poblacional deberían de realizarse de forma ideal en el marco de registros de cáncer con una metodología estandarizada y unos estándares de calidad internacional bien definidos.

La integración de datos clínicos, epidemiológicos y biológicos es necesaria para una valoración pronóstica precisa. Sin embargo, la incorporación de determinados factores pronósticos emergentes potenciales a la práctica habitual es un proceso lento y selectivo, que exige una cuidadosa valoración de la evidencia y aplicabilidad por parte de las organizaciones. En este sentido, es muy imporante el papel del IMWG.

III. OBJETIVOS

Objetivo general

Analizar la tendencia de la supervivencia global en una cohorte de pacientes con MM diagnosticados en Granada a lo largo de las últimas tres décadas, así como el impacto pronóstico de la comorbilidad en términos de supervivencia global y mortalidad precoz, en el contexto de los factores pronósticos consolidados más usados en la práctica clínica habitual y otros potenciales factores pronósticos.

Objetivos específicos

1. Conocer la tendencia en términos de supervivencia global, de una cohorte de pacientes diagnosticados de MM sintomático, en el contexto de un estudio de base poblacional de un solo centro, durante un período de estudio de tres décadas, incluyendo entre los potenciales factores pronósticos una aproximación integral de las comorbilidades basales.
2. Estudiar la tendencia en términos de mortalidad precoz, durante un período de estudio de treinta y un años, de una cohorte de pacientes diagnosticados de MM síntomático, en el contexto de un estudio de base poblacional de un solo centro, incluyendo entre los potenciales factores pronósticos una aproximación integral de las comorbilidades basales, para analizar su impacto pronóstico selectivo y dependiente del tiempo.
3. Analizar el impacto del tipo de hospital (hospital comarcal *versus* hospital de referencia) en la supervivencia global de los pacientes diagnosticados de MM en nuestro entorno sanitario.
4. Evaluar el impacto de la presencia de determinadas variantes genéticas relacionadas con la DM2, como factor de riesgo de MM, y determinar si un modelo predictivo que incluya esta información ayuda a predecir el riesgo de desarrollar MM.
5. Evaluar el impacto en términos de supervivencia global de la presencia de determinadas variantes genéticas relacionadas con la DM2, en pacientes con MM.

IV. METODOLOGÍA

1. Metodología para los objetivos 1-3

1.1 Diseño de los estudios

Estudio de cohortes.

1.2. Población y periodo de estudio

Pacientes diagnosticados de MM de nuevo diagnóstico sintomático incluidos en el RCBP de Granada. El periodo de estudio fue variable en función de los objetivos definidos y la secuencia de los estudios: desde 1985 a 2014 para el abordaje del objetivo 1; desde 1985 a 2015 para alcanzar el objetivo 2; y desde 1993 a 2006 para el objetivo 3.

Los pacientes debían cumplir los criterios diagnósticos establecidos por el IMWG.

La población de estudio para el objetivo 1 incluyó 582 pacientes (302 mujeres y 280 hombres). En el objetivo 2 se incluyeron 621 pacientes (322 mujeres y 299 hombres).

La población de estudio para el objetivo 3 incluyó a todos los pacientes diagnosticados de MM durante el mismo período de estudio y con los mismos criterios diagnósticos en 5 hospitales públicos andaluces, uno de ellos de referencia (Hospital Universitario Virgen de las Nieves) y 4 comarcales con similares características: Hospital Valle de los Pedroches (Córdoba), Hospital de La Línea de la Concepción (Cádiz), Hospital Santa Ana de Motril y Hospital de Baza (ambos en Granada). En total se incluyeron 431 pacientes (222 mujeres y 209 hombres).

1.3. Variables de estudio y fuentes de información

Las variables analizadas para los objetivos 1-2 fueron:

1.3.1. Sociodemográficas

-Edad al diagnóstico, en años.

-Sexo.

1.3.2. Antropométricas

-Índice de masa corporal.

-Pérdida de peso anterior al diagnóstico.

1.3.3. Antecedentes

-Fecha del primer síntoma relacionado con el MM.

-Evento definitorio de MM.

-Comorbilidad basal: se han recogido todas las comorbilidades con potencial impacto pronóstico, diferenciando dos grupos:

a. Entidades específicas: hipertensión arterial, obesidad, DM2, dislipemia, úlcera péptica, neoplasia maligna previa o concomitante, infección por virus de hepatitis B, enfermedad tromboembólica, amiloidosis, infección por virus de hepatitis C, esplenectomía, trasplante renal, e infección por virus de la inmunodeficiencia humana.

b. Agrupaciones de patologías por órganos o aparatos: cardiopatía, enfermedad pulmonar, trastorno neurológico, enfermedad psiquiátrica, hepatopatía, enfermedad reumática, enfermedad cutánea. Los grupos de patologías han sido analizados como grupo y de forma individualizada. Por ejemplo, dentro del grupo cardiopatía existen distintas entidades: enfermedad valvular, insuficiencia cardiaca congestiva, arritmia, enfermedad coronaria, grupo mixto, y otras. Para el grupo de hepatopatía: cirrosis, esteatosis, otras, etc.

-Existencia de enfermedad precursora previa (GMSI o MMS).

1.3.4. Relacionadas con el diagnóstico

-Fecha de diagnóstico.

-Retraso diagnóstico. Es el tiempo transcurrido (en meses) desde la aparición del primer síntoma o evento definitorio de MM hasta que se confirma el diagnóstico (aspirado medular o biopsia).

-Estado funcional (ECOG).

-Subtipo de MM.

-Porcentaje de infiltración medular por células plasmáticas (morfología e inmunofenotipo).

-ACAR (FISH).

-Estadío ISS.

-Enfermedad ósea (PET/TC), enfermedad extramedular.

-sCLLc.

-LDH.

-sCr.

-TFGe (MDRD).

1.3.5. Evolución y desenlace

-Fecha de inicio del tratamiento.

-Retraso terapéutico, en días.

-Tratamiento de inducción.

-TPH, número y tipo.

-Fecha de defunción.

-Causa principal de la muerte.

-Supervivencia global, en meses.

Las variables utilizadas para el objetivo 3 fueron:

-Edad al diagnóstico.

-Sexo.

-Fecha de diagnóstico.

-Fecha de muerte.

-Estadío Durie-Salmon.

-Tipo de MM.

-Tipo de hospital.

-Existencia previa de GMSI.

-sCr.

-TPH.

-Supervivencia global, en meses.

La fuente de información básica para los objetivos 1-3 ha sido el Registro Clínico de MM de base poblacional creado en 2011 (Ríos-Tamayo R et al, 2011) en el que, desde ese momento, se incluyen de forma prospectiva todos los pacientes con MM de nuevo diagnóstico, con toda la información clínica disponible a lo largo de todo el curso clínico del paciente, hasta el fallecimiento. Igualmente, se revisó retrospectivamente toda la información clínica disponible de los pacientes hasta 1993. En el período 1985-1992 no se encuentra disponible información clínica.

1.4. Análisis de datos

Para el análisis de los datos se utilizó el paquete estadístico SPSS v.20. Se realizó una estadística descriptiva de las variables independientes. Las variables cuantitativas se expresan como media, mediana y rango. Las variables cualitativas se expresan como frecuencia y porcentaje. Para la comparación entre variables categóricas, se usó la prueba de la ji al cuadrado (χ^2). Para la comparación de medias se usó la prueba de la t de Student. La supervivencia global y sus intervalos de confianza al 95% (IC 95%) fueron estimados en meses por el método de Kaplan-Meier, desde la fecha del diagnóstico (primer aspirado medular o biopsia) hasta la fecha de fallecimiento, pérdida de seguimiento o fin de estudio. La fuente de información para el estado vital fue el Índice Nacional de Defunciones. Las diferencias en las curvas de supervivencia fueron valoradas mediante la prueba del log-rank. Para cada variable se estimó su hazard ratio

(HR) mediante el modelo de regresión de Cox. Para valorar el impacto simultáneo de diversas variables predictoras en la supervivencia se llevó a cabo un análisis multivariante, introduciendo en el modelo de riesgos proporcionales de Cox aquellas variables con un valor de $p \leq 0.05$ y efectuando un análisis hacia atrás (backward analysis). No se usó imputación para los datos faltantes.

2. Metodología para los objetivos 4-5

2.1 Diseño de los estudios

Estudio de casos y controles (objetivo 4). Estudio de cohortes (objetivo 5).

2.2. Población y periodo de estudio

Para el objetivo 4, la población de casos y controles consistió en 1420 pacientes con MM (705 mujeres y 715 hombres) y 1858 controles (916 mujeres y 942 hombres) incluidos en el consorcio internacional IMMEnSE. Los controles fueron donantes de sangre o sujetos hospitalizados con un diagnóstico no relacionado con cáncer, pertenecientes a la misma área geográfica que los casos.

Para el objetivo 5, la población de estudio consistió en 936 pacientes (454 mujeres y 482 hombres) del consorcio IMMEnSE. Estos pacientes son aquellos para los que se disponía de datos de supervivencia en el momento de llevar a cabo el estudio. La población para la replicación consistió en 700 pacientes (296 mujeres y 404 hombres) del grupo alemán de Heidelberg. Las características de ambas poblaciones en lo referente a la edad mediana al diagnóstico y sexo fueron similares.

2.3. Variables de estudio y fuentes de información

Las variables utilizadas para el objetivo 4 fueron:

-Edad al diagnóstico, en años.

-Sexo.

-País de origen.

-58 variantes genéticas asociadas con DM2 e identificadas en estudios GWAS.

Las variables utilizadas para el objetivo 5 fueron:

-Edad al diagnóstico, en años.

-Sexo.

-Fecha de diagnóstico.

-País de origen.

-Fecha de muerte.

-Estadío Durie-Salmon.

-58 variantes genéticas asociadas con DM2 e identificadas en estudios GWAS.

2.4. <u>Análisis de datos</u>

Para los objetivos 4-5, se ha utilizado la base de datos del consorcio IMMEnSE. Para el análisis de los datos se utilizó el paquete estadístico SPSS v.20.

En el objetivo 4 se usó un modelo de regresión logística para valorar los efectos de las variantes genéticas en el riesgo de MM usando modelos de herencia co-dominante, dominante, recesiva y log-aditiva, analizando sus respectivas OR así como los IC 95%. El análisis global fue ajustado por la edad al diagnóstico, el sexo y el país de origen. Los tests *Hardy-Weinberg Equilibrium* (HWE) fueron realizados en el grupo control mediante una prueba estándard (observado-esperado) de χ^2. El cálculo del poder estadístico se realizó con Quanto vs 12.4. Para el análisis *multiple testing* se usó el método M_{eff} (Nyholt DR, 2004), que considera el número de loci y el número de modelos de herencia estudiados. Se construyó un modelo predictivo incluyendo la edad, el sexo y las variantes que mostraron asociación con MM en el análisis individual ($p < 0.05$). Se estimó el área bajo la curva de una curva ROC (*Receiver Operating Characteristic*) para valorar el poder discriminativo comparativo de este modelo con

respecto al modelo de referencia que incluyó sólo edad y sexo. Se usó el *log-likelyhood ratio test* (LR test) para comparar las diferencias entre dichos modelos. La confirmación de las diferencias entre los modelos se realizó por análisis de permutaciones.

Para el objetivo 5 se analiza la supervivencia global por el método de Kaplan-Meier, midiendo las diferencias entre grupos mediante el test log-rank. Se estimó un HR para cada variante genética usando el modelo de regresión de Cox, ajustando por edad, sexo, país y estadío Durie-Salmon (cohorte IMMEnSE) o edad, sexo y ensayo clínico (cohorte Heidelberg). Las estimaciones de asociación fueron calculadas de acuerdo con los modelos de herencia dominante, recesiva y log-aditiva, con el alelo mayor como referencia para el análisis de regresión. Igualmente se realizó un análisis de interacción gen-sexo para determinar si la asociación entre las variantes genéticas y la supervivencia global fueron de similar magnitud entre ambos sexos. Para confirmar las asociaciones significativas, se realizó un meta-análisis combinado los datos de la población IMMEnSE con la cohorte Heidelberg. El estadístico I^2 fue usado para valorar la heterogeneidad entre ambos estudios y el HR combinado fue estimado usando el modelo de efecto aleatorio, que asegura la fiabilidad de los resultados teniendo en cuenta que los datos provienen de estudios con un diseño diferente. Para los cálculos del meta-análisis se usó Stata v.12. Para el análisis funcional *In silico* se usaron las bases de datos de Haploreg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) y ENCODE (https://genome.ucsc.edu/ENCODE/). Los datos de *expression quantitative trait loci* (eQTL) del grupo de Heidelberg han sido previamente señalados (Weinhold *et al*,2015).

V. RESULTADOS

Resumen artículo 1

Ríos-Tamayo R, Sánchez MJ, Puerta JM, Sainz J, Chang DY-L, Rodríguez T, et al. Trends in survival of multiple myeloma: a thirty-year population-based study in a single institution. Cancer Epidemiol 2015;39:693-699.

Este trabajo responde al objetivo 1.

Introducción. A pesar de los indudables progresos realizados en la supervivencia global de los pacientes con MM de nuevo diagnóstico en los años recientes, esta enfermedad sigue siendo considerada incurable. La mayoría de los datos de supervivencia provienen de ensayos clínicos que lógicamente incluyen pacientes altamente seleccionados. Los datos sobre supervivencia global en pacientes no seleccionados, de la vida real, son poco conocidos. El interés por conocer estos datos es aún mayor cuando el estudio es de base poblacional, ya que se incluyen todos los pacientes diagnosticados de MM, y cuando el período de estudio es muy amplio, lo que permite establecer con claridad una tendencia temporal en los resultados.

Métodos. Se estimó la supervivencia global en una cohorte de pacientes con MM de nuevo diagnóstico diagnosticados a lo largo de tres décadas, en un estudio de base poblacional realizado en el Servicio de Hematología del Hospital Universitario Virgen de las Nieves. Para valorar la supervivencia se consideraron períodos de 5 años. Además de todas las variables de interés pronóstico habituales, se incluyeron en el estudio nuevas variables con potencial interés, en particular, una aproximación integral a la comorbilidad, incluyendo además de la función renal, un panel de veinte comorbilidades.

Resultados. 582 pacientes con MM de nuevo diagnóstico fueron diagnosticados y seguidos a lo largo del proceso desde 1985 a 2014, observando una mejoría progresiva

de la supervivencia global con el tiempo, mayor para los pacientes diagnosticados entre 2010 y 2014. Estas diferencias en las curvas de supervivencia global alcanzan significación estadística en pacientes menores de 65 años, sin embargo esto no se evidencia para pacientes con 65 años o mayores.

Los factores pronósticos asociados con la supervivencia global son el ISS, el nivel sérico de LDH, la presencia de insuficiencia renal, la realización de TAPH y la presencia basal de amiloidosis asociada al MM.

Interpretación. La supervivencia global está mejorando progresivamente en pacientes no seleccionados con MM de nuevo diagnóstico, en particular tras el uso generalizado de los nuevos agentes.

Una valoración integral de la comorbilidad puede ayudar a explicar parte de la enorme heterogeneidad clínica en los resultados clínicos que caracteriza a esta enfermedad.

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Trends in survival of multiple myeloma: A thirty-year population-based study in a single institution

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ABSTRACT

Background: Despite the progress made in recent years, multiple myeloma is still considered an incurable disease. Most survival data come from clinical trials. Little is known about the outcome in unselected real-life patients.
Methods: Overall survival was analyzed in a cohort of newly diagnosed symptomatic multiple myeloma patients, over the last three decades, in a single institution population-based study.
Results: 582 consecutive myeloma patients were included in the study. Survival increased over time in patients younger than 65 years but did not reach statistical significance in patients with 65 years or older. The prognostic factors associated with overall survival were the International Staging System, the serum lactate dehydrogenase level, the renal impairment, the realization of autologous stem cell transplantation, and the presence of concomitant amyloidosis. Overall survival shows a steady improvement over time.
Interpretation: The survival of myeloma is improving progressively in real-life patients, particularly after the widespread use of the novel agents. A comprehensive assessment of comorbidity can help to explain the huge heterogeneity of myeloma outcome. The optimization of current therapeutic resources as well as the incorporation of new drugs will allow further improvement of survival in the coming years.

1. Introduction

Multiple myeloma (MM) is a biologically complex and clinically heterogeneous disease [1,2] whose definition has been recently updated [3]. Symptomatic or active MM is characterized by a clonal expansion of plasma cells (PC) in the bone marrow (BM), the detection in most cases of a monoclonal immunoglobulin in serum and/or urine and the presence of end-organ damage. It accounts for approximately 1% of neoplastic diseases and 13% of hematologic cancers [4–6]. MM is always virtually preceded by a premalignant stage, termed monoclonal gammopathy of undetermined significance. Sometimes, a more advanced but still asymptomatic disease known as smoldering MM (SMM) may appear behaving as an

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intermediate entity between monoclonal gammopathy of undetermined significance and MM.

It has been shown that the course of MM is highly variable with both long-term and short-term surviving patients. At present, the prognosis of newly diagnosed MM (NDMM) is based on the International Staging System (ISS) as well as on the interphase fluorescence in situ hybridization (FISH) results [7]. These tools have represented a major advance in the prognostic evaluation of MM. However, many studies have identified other prognostic factors with additional capability to predict part of this heterogeneity in survival. Therefore, comorbidity should be taken into account to improve the prognostic assessment in MM [8].

There is no doubt that treatment has a major impact on the outcome. Overall survival (OS) has improved slightly over time with the use of conventional therapy (corticosteroids, anthracyclines and alkylating agents) whereas during the past decade, the widespread use of the novel agents has led to a marked improvement in OS. Fortunately, we can now face the future of MM as a chronic disease. In addition, the operational cure could be an achievable objective in a selected group of patients. Notwithstanding, the benefit in terms of survival varies between different studies and comorbidity could be responsible for part of this heterogeneity. The available information on OS studies is derived primarily from clinical trials, in which patients with common comorbidities are excluded. Little is known about OS in population-based series with real-life patients. The aim of this single institution population-based study is to report trends on OS over 30 years in a large population of unselected NDMM patients.

2. Materials and methods

2.1. Patients

The Granada Cancer Registry is a population-based cancer registry that works since 1985 and covers a population of 905.285 inhabitants, of which 442.523 belong to the reference area of our hospital. This Registry is integrated into the European Network of Cancer Registries, using internationally standardized work rules and procedures, ensuring the quality of the information. The main source of information is the electronic clinical record, but all other public and private available sources are continuously analyzed. In 2011, a comparison was made between the two available registries (hospital-based and population-based) [9] and a specific population-based monographic MM clinical registry (MMCR) was created. According to the International Classification of Diseases, only patients with C90.0 code were included, excluding patients with primary plasma cell leukemia (C90.1), extramedullary plasmacytoma (C90.2) or solitary plasmacytoma (C90.3). From January 1985 to January 2015, all NDMM patients who had their current residence at the time of diagnosis in Granada and met the diagnostic criteria of the International Myeloma Working Group (IMWG) [10], were included in the MMCR and are the basis of this study, which was performed according to the Declaration of Helsinki (Ethics Committee approval number C-14, CEI-Gr, 2014). Although our policy is to include those patients diagnosed post-mortem and by death certificate, provided they met the mentioned criteria, no case was incorporated by this method. Demographic and survival data are available for the whole cohort, but clinical data were incorporated in 1993.

2.2. Variables

All common baseline prognostic factors were recorded, such as age, subtype of myeloma, renal function, Eastern Cooperative Oncology Group (ECOG) performance status score, the presence of cytogenetic abnormalities by FISH and ISS. Renal function was assessed by serum creatinine (sCr, mg/dL) and the estimated glomerular filtration rate (eGFR). Other variables of increasing interest were also included in the study, such as the Body Mass Index (BMI), the occurrence of weight loss before diagnosis, the delay in diagnosis, the serum free light chain ratio (FLCr), lactate dehydrogenase (LDH), BMPC as measured by morphology and flow cytometry, the documentation of lytic lesions, the use of bortezomib in induction, the realization of autologous SCT (ASCT) and the calendar period. Comorbidity was divided in twenty

Table 1
Baseline patient characteristics according to calendar periods.

Characteristic	1985–1989	1990–1994	1995–1999	2000–2004	2005–2009	2010–2014	Total
No.(%)	63(10.8)	66(11.3)	107(18.4)	108(18.6)	109(18.7)	129(22.2)	582
Age, years							
Median	64	67	70	67	68	65	66
Range	28–83	35–85	31–91	39–87	21–88	12–91	12–91
Sex, *n*(%)							
Male sex	29(46)	32(48.5)	51(47.7)	53(49.1)	41(37.6)	74(57.4)	280(48.1)
Subtype, *n*(%)							
IgG	–	2(33.3)	28(58.3)	54(55.7)	61(57)	68(52.7)	213(55)
IgA	–	1(16.7)	11(22.9)	26(26.8)	26(24.3)	35(27.1)	99(25.6)
LC	–	3(50)	8(16.7)	9(9.3)	17(15.9)	22(17.1)	59(15.2)
NS	–	0	1(2.1)	7(7.2)	2(1.9)	3(2.3)	13(3.4)
IgD	–	0	0	1(1)	0	1(0.8)	2(0.5)
IgM	–	0	0	0	1(0.9)	0	1(0.3)
ISS, *n*(%)							
I	–	1(50)	1(8.3)	10(27.8)	20(22.2)	38(30.9)	70(26.6)
II	–	1(50)	4(33.3)	9(25)	23(25.6)	32(26)	69(26.2)
III	–	0	7(58.3)	17(42.2)	47(52.2)	53(43.1)	124(47.1)
eGFR < 60, %	–	50	72.8	53.2	54.9	45.4	52
%BMPC, mean	–	37	27.7	27.9	25	23.4	25.5
BMI ≥30, no.(%)	–	2(50)	4(26.7)	18(40)	26(32.9)	36(29.3)	86(32.3)

Abbreviations: Ig = immunoglobulin, LC = light chain only, NS = non-secretory, ISS = International Staging System, eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2), BMPC = bone marrow plasma cells, BMI = Body Mass index (kg/m^2).

components besides renal impairment (RI), as previously reported [8]. Obesity was defined as a BMI $\geq$30.0 kg/m^2.

2.3. Therapy

Patients were treated with conventional chemotherapy until 2006, when we started to use a bortezomib-based induction approach. ASCT has been used since 1995. Treatment at the time of relapse is not standardized, but lenalidomide is available for this indication since 2008.

2.4. Survival

Median OS was calculated in months (m) from the date of diagnosis (first bone marrow aspirate or biopsy) until the date of death, loss to follow-up, or end of study (January 1, 2015), whichever occurred first. Patients were divided for analysis in six calendar periods (1985 to 1989, 1990 to 1994, 1995 to1999, 2000 to 2004, 2005 to 2009, and 2010 to 2014). The delay to diagnosis was calculated as time between the first symptomatic presentation and the date of definitive diagnosis. The source of information for vital status of patients was the National Index of Deaths as well as the Andalusian Registry of Mortality.

2.5. Statistical analysis

Comparisons for categorical variables among different groups were made with the χ^2-test. Comparisons of means of quantitative continuous variables between two groups were made with the *t*-test. Median follow-up time was calculated among individuals with censored data. OS curves were estimated using the Kaplan–Meier method, and comparisons among groups were carried out with the log-rank test. Cox proportional hazards were used for the calculation of hazard ratios (HR) for each variable. For multivariate analysis, factors with prognostic significance at 0.05 level were introduced into a Cox proportional hazards model (backward analysis). All *P*-values were two-sided. No imputation for missing data has been used. Data were analyzed with SPSS v20 software.

3. Results

A total of 594 patients were recruited in MMCR until January 1, 2015. Patients with smoldering myeloma (n=8) and primary plasma cell leukemia (n=4) were excluded. The remaining 582 patients were the basis of the study, 280 men (48.1%) and 302 women. In only 6 patients (1.03%) survival data were not available for analysis (all of them were diagnosed before 1992). 31 patients (5.3%) were considered only for palliative care. Median age was 66 years (range, 12–91). Women had a significantly higher mean age than men (67.4 versus 64.2 years; P=0.001); despite being older, women showed a slight trend to better median OS [28.1; 95% confidence interval (95% CI), 21–35.1 versus 24.2 m; 95% CI, 18–30.4; P=0.108]. The main clinical and laboratory baseline characteristics of patients are shown in Table 1. A complete assessment of comorbidity was available in 381 patients (65,5%). Over half of these patients in which comorbidity data were known, had moderate or severe RI, as measured by eGFR < 60%. On the other hand, 28% of the same group of patients had sCr of 2 mg/dL or higher. The distribution of comorbidities in decreasing order of frequency is shown in Table 2. The subtype of heart disease was arrhytmia 6.8%, congestive heart failure 6%, coronary artery disease 4.7%, isolated heart valve disease 0.3%, and other 2.1%; among patients with lung disease, we found chronic obstructive pulmonary disease 8.1%, asthma 5.2%, and other 2.2%; regarding liver disease, steatosis 2.1%, cirrhosis 1.6%, and other 1.3%; in respect to amyloidosis, 0.3% were immunoglobulin light chain amyloidosis, 1% reactive amyloidosis, and 0.8% undefined. Table 3 shows the induction therapy used and the distribution of SCT performed according with age category and calendar period. Hundred and twelve patients (19.2%) underwent stem cell transplant (109 ASCT and 3 allogeneic), in most cases as part of first-line therapy; 20 of them received a second transplant (17 allogeneic and 3 ASCT). The median follow-up was 28 m. The median diagnostic delay was 4 m and the median time from diagnosis to treatment was 13 days. At

Table 2
Comorbidity frequencies (%).

Hypertension	44.9
Obesity	32.3
Heart disease	19.9
Type 2 diabetes	18.9
Lung disease	15.5
Hyperlipaemia	12.3
Neurological disorder	9.7
Peptic ulcer	8.4
Prior malignancy	8.1
Psychiatric disorder	7.9
Hepatitis B virus	5.2
Liver disease	5
Rheumatologic disorder	3.7
Thromboembolism	3.7
Amyloidosis	2.1
Cutaneous disease	1.8
Hepatitis C virus	1.6
Splenectomy	0.8
Renal transplant	0.5
Human immunodeficiency virus	0.3

Table 3
Induction therapy and SCT according to age and calendar period.

	Age <65 years				Age ≥65 years			
	1995–1999	2000–2004	2005–2009	2010–2014	1995–1999	2000–2004	2005–2009	2010–2014
MP, no.(%)	10(47.6)	1(2.2)	0	1(1.6)	18(75)	33(64.7)	27(46.6)	4(6.2)
VAD	11(52.4)	40(88.9)	33(78.6)	3(4.8)	3(12.5)	13(25.5)	18(31)	0
VCMP/VBAP	0	3(6.7)	2(4.8)	0	0	2(3.9)	0	0
VD	0	0	4(9.5)	19(30.6)	0	0	1(1.7)	9(13.8)
VMP	0	0	2(4.8)	1(1.6)	0	0	8(13.8)	33(50.8)
VCD	0	0	0	20(32.3)	0	0	0	7(10.8)
VRD	0	0	0	13(21)	0	0	0	0
OTHER	0	1(2.2)	1(2.4)	5(8.1)	3(12.5)	3(5.9)	4(6.9)	12(18.5)
SCT1, *n*	11	30	28	36	1	4	1	0
SCT2, *n*	2	10	2	6	0	0	0	0

Abbreviations: SCT = stem cell transplant, MP = melphalan–prednisone, VAD = vincristine–doxorubicin–dexamethasone, VCMP/VBAD = vincristine–cyclophosphamide–melphalan–prednisone/vincristine–carmustine (BCNU)–doxorubicin–prednisone, VD = bortezomib–dexamethasone, VMP = bortezomib–melphalan–prednisone, VCD = bortezomib–cyclophosphamide–dexamethasone, VRD = bortezomib–lenalidomide–dexamethasone, Other: other Bortezomib-based combinations.

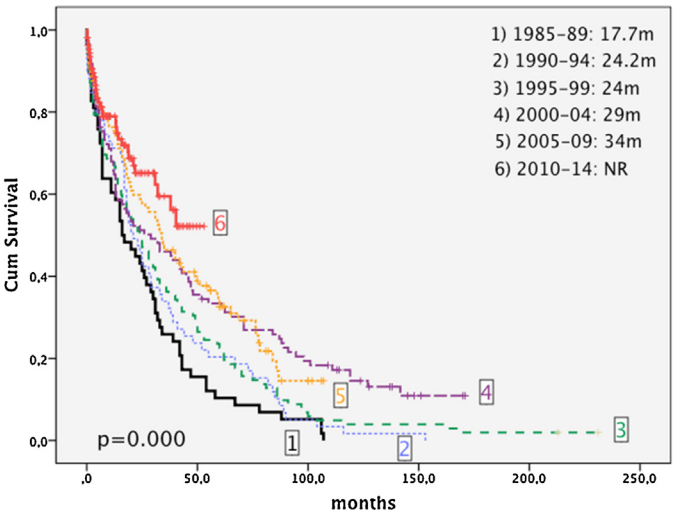

Fig. 1. Overall survival according with the six calendar periods.

the end of the study, 129 patients (22.6%) remain alive. The median OS for the whole cohort was 26 m (95% CI, 21.2–30.8).

Fig. 1 shows OS curves for the whole cohort ($n = 576$). Median OS over the six calendar periods has been increasing steadily and is not reached for the last period. Fig. 2 shows OS according with the age category. The improvement in survival reached statistical significance only in patients under 65 years ($n = 242$)($P < 0.001$). In those with 65 years or older ($n = 334$), median OS has doubled in the last calendar period (30.9 m, 95% CI, 14.6–47.2) with respect to the first one (14.9 m, IC 95%, 0.5–29.3), but these differences did not reach statistical significance ($P = 0.226$). However, all palliative patients ($n = 31$) belonged to this category of age. When palliative patients were excluded ($n = 303$), the improvement in OS over time reached a marginal statistical significance ($P = 0.094$). Data of Cox univariate and multivariate OS analyses are shown in Tables 4 and 5, respectively. Interestingly, in the final multivariate Cox model, ISS, LDH, the presence of concurrent amyloidosis, RI, and the realization of a front-line ASCT, were independent predictive factors for OS. This model is equally valid if patients considered only for palliative care are excluded. Among the whole panel of comorbidities, only RI and the amyloidosis show a role as independent prognostic factors.

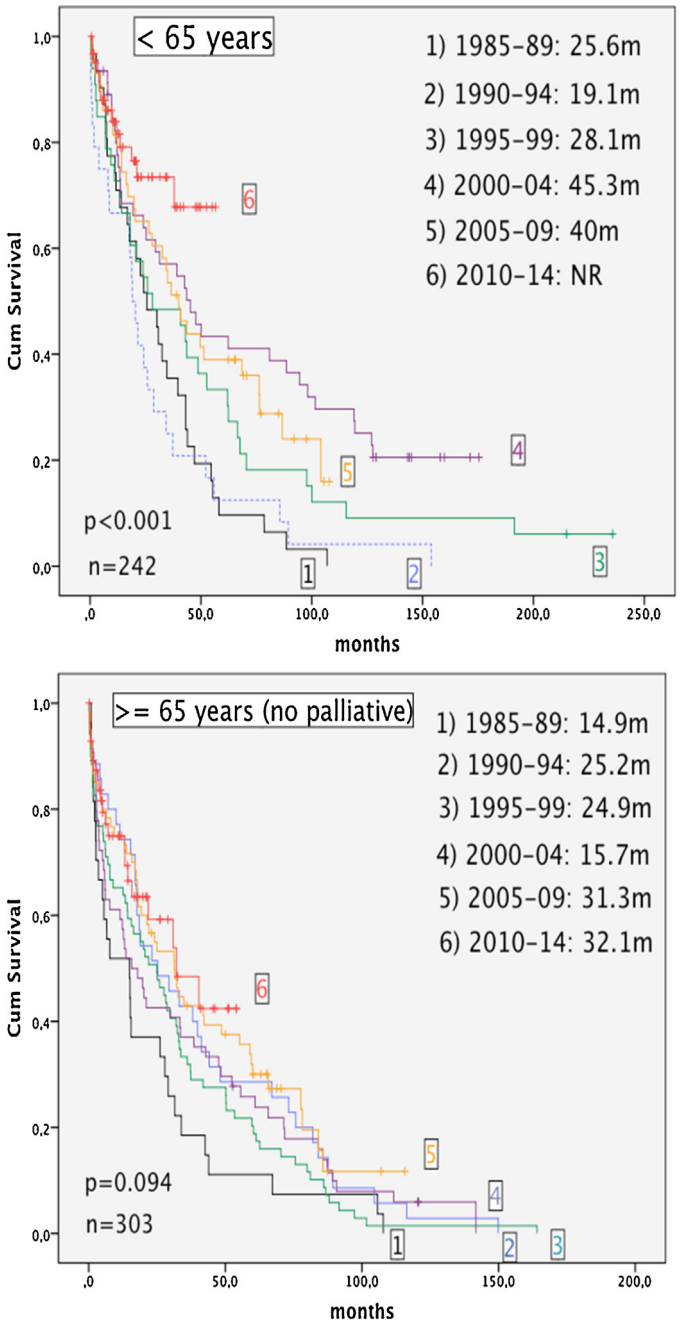

Fig. 2. Overall survival according to age category and calendar periods.

4. Discussion

The progress in epidemiology and genomics has helped to unravel the complex pathogenesis and the clinical and molecular heterogeneity of MM. Despite recent advances, MM remains one hematological malignancy with a poor outcome [11]. However, long-term survival can be achievable in a selected group of patients. The widespread use of new and more effective treatment probably explains much of the increased survival, but improvements in survival are not uniform across Europe [12]. OS remains the most important primary end-point in both clinical trials (designed with enough follow-up) and population-based MM studies. On the other hand, improving OS is the main clinical objective in MM therapy. Cancer registries have an essential role in monitoring improvements in cancer survival.

The prognosis of NDMM patients should be determined accurately according to standardized and uniformly used criteria. Outside clinical trials, an evidence-based and risk-adapted therapy should be used whenever possible. However, current prognostic tools cannot elucidate the wide heterogeneity in results. A comprehensive assessment of comorbidity might help to explain part of this heterogeneity, contributing to provide a more customized therapy.

This is the first single institution population-based study aiming to focus on the role of comorbidity in the survival of NDMM patients. Our results emphasize the great improvement obtained in recent years in OS, especially in patients under 65 years. Several circumstances have contributed to improve OS in MM, such as a better supportive care, a more intensive use of transplantation, a proper patient selection and a risk-adapted therapy approach. Nevertheless, it has been the use of novel agents and new drugs combinations what has dramatically changed the outcome of patients.

Several groups have reported on progressive OS improvement in MM [13–24]. Table 6 summarizes the results of the most relevant studies about survival in MM. According to the type of

Table 4
Univariate regression analyses.

Variables	HR	95% CI	*P*-value
Age (years)	1.03	1.02–1.04	<0.001
ISS 2 (vs 1)	1.99	1.21–3.29	0.007
ISS 3 (vs 1)	3.26	2.08–5.12	<0.001
PS, ECOG 2 (vs 0)	3.86	1.19–12.58	0.025
PS, ECOG 3 (vs 0)	5.54	1.68–18.21	0.005
PS, ECOG 4 (vs 0)	11.69	3.05–44.81	<0.001
MM LC	1.56	1.12–2.19	0.009
Palliative care	4.88	3.26–7.29	<0.001
Lytic bone lesions	1.16	1.06–1.27	0.001
Amyloidosis	2.24	1.05–4.76	0.036
Psychiatric disorder	1.76	1.11–2.79	0.017
Neurological disorder	1.55	1.02–2.35	0.039
LDH	1.00	1.00–1.002	<0.001
FLCr(i/u)	1	1–1.001	0.023
sCr	1.17	1.11–1.23	<0.001
eGFR (MDRD)	0.98	0.97–0.99	<0.001
BMPC (Mor)	1.01	1.00–1.02	0.003
BMPC (Im)	1.01	1.00–1.02	0.050
ASCT	0.34	0.25–0.45	<0.001
Period 4 (vs 1)	0.61	0.44–0.85	0.003
Period 5 (vs 1)	0.60	0.43–0.84	0.003
Period 6 (vs 1)	0.47	0.31–0.70	<0.001
B-based induction	0.63	0.44–0.88	0.008

[a]Only variables with significant *P*-value are shown.
Abbreviations: HR = hazard ratio, CI = confidence interval, ISS = International Staging System, PS(ECOG) = performance status (Eastern Cooperative Oncology Group), ELC = light chain, LDH = lactate dehydrogenase (U/L), sCr = serum creatinine (mg/dL), eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2), FLCr(i/u) = free light chain ratio (involved/uninvolved), BMPC = bone marrow plasma cells, Mor = morphology, Im = immunophenotype, ASCT = autologous stem cell transplant, B = Bortezomib.

Table 5
Cox regression model according to the inclusion or exclusion of palliative patients.

	Whole cohort			No palliative patients		
Variables	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ISS 2 (vs 1)	1.97	1.09–3.54	0.024	2.02	1.10–3.70	0.024
ISS 3 (vs 1)	2.37	1.32–4.20	0.004	2.18	1.18–4.02	0.012
LDH	1.002	1–1.002	0.001	1.001	1–1.002	0.001
Amyloidosis	11.32	2.35–54.55	0.002	16.01	3.36–76.84	0.001
sCr	1.11	1.02–1.21	0.013	1.15	1.06–1.24	<0.001
ASCT	0.26	0.14–0.49	<0.001	0.26	0.14–0.48	<0.001

[a]Only variables with significant *P*-value are shown.
Abbreviations: HR = hazard ratio, CI = confidence interval, ISS = International Staging System, LDH = lactate dehydrogenase (U/L), sCr = serum creatinine (mg/dL), BMPC = bone marrow plasma cells, Mor = morphology, Im = immunophenotype, ASCT = autologous stem cell transplant, B = bortezomib.

study, most of them are multicenter and non population-based studies. Kumar et al. [14], at Mayo Clinic, has recently reported excellent OS, even in patients older than 65 years, but this is not a population-based study. Furthermore, they used a lenalidomide-based regimen as frontline induction in 66% of patients diagnosed between 2006 and 2010. This strategy has not been available in Europe, outside of a clinical trial, until 2015. The first single institution population-based study to report on trends in OS in an unselected MM population over a long time period was by Turesson et al. [16], showing a median OS of 22.2 m. This study was conducted in Mälmo (Sweden) and remains the largest single institution population-based study reported to date. Our study has several similarities with the previously mentioned study. First, contrary to the usual, the percentage of men (48%) is lower than that of women. Second, the outcome is better in women than in men, albeit in our study this difference did not reach statistical significance. Third, the pattern of improved OS was more evident for patients under 65 years, whereas in patients older than 65 years a more modest progress has been demonstrated. Unfortunately, the study by Turesson et al. did not include information about therapy for individual patients nor data on clinical variables of prognostic interest. In addition, they included patients with asymptomatic disease and more than 10% of their cases did not receive any treatment. No other single-institution population-based study has been reported until now. Our study is the first population-based study from a single institution in which baseline clinical data and a comprehensive comorbidity assessment were included. We have shown a steady pattern of OS improvement over time for the whole cohort, more accused in the last five years, when OS has tripled over that achieved in the nineties. The strengths of our study are the inclusion of clinical information in a large population of well-characterized symptomatic MM patients at the MMCR. Patients have been treated uniformly in a single institution, with a specific unit of monoclonal gammopathies, where all the clinical information is recorded prospectively in a continuously updated database, including emerging variables of interest that have not been analyzed in previous studies. Patients with SMM were excluded, provided that the current standard of care is waiting until symptomatic disease develops. However, a recent Spanish study [25] showed that early treatment in a subgroup of high-risk SMM could delay progression and increase OS. Therefore, monitoring of these asymptomatic patients should follow a risk-based approach to ensure early treatment when needed. A strict control of data quality was performed before the survival analyses were carried out, according with current recommendation. The limitations of our study include the lack of clinical information from 1985 to 1992, limited data of cytogenetic abnormalities, and some missing data for certain variables. Demographic and survival data are presented for the whole cohort, but clinical data were incorporated in 1993.

There is increasing evidence indicating that comorbidity plays an emerging role in the outcome of MM. A great effort has been

Table 6
Results of recent studies on NDMM OS.

Authors [Ref.]	Type of study	Study period	Year of publication	No. of patients	Median OS (months)
Kumar et al. [13]	Single institution	1971–2006	2008	2981	44.8 (after 1996)
Kumar et al. [14]	Single institution	2001–2010	2014	1038	62.4
Kristinsson et al. [15]	Nationwide PB (Sweden)	1973–2003	2007	14381	Best 5-year RSR 0.36
Turesson et al. [16]	Single institution PB	1950–2005	2010	773	22.2
Kastritis et al. [17]	Multicenter (Greece)	1985–NI	2009	1376	48 (after 2000)
Dimopoulos et al. [18]	Multicenter (Greece)	1990–2011	2014	1773	54 (after 2005)
Liwing et al. [19]	Multicenter PB (Sweden)	2000–2011	2014	1638	33.6 vs 82.8 (HD)
Pozzi et al. [20]	Multicenter PB (Italy)	1988–2009	2013	1206	50.9% (OS at 5 year)
Ludwig et al. [21]	Multicenter	1982–2002	2010	10549	44.4
Geng et al. [22]	Single institution	2006–2011	2013	264	61
Ozaki et al. [23]	Multicenter (Japan)	2004–2009	2014	318	55.2 (ISS III)
Oortgiesen et al. [24]	Multicenter PB (Netherlands)	2005–2013	2014	270	49.5

Abbreviations: NDMM = newly diagnosed multiple myeloma, OS = overall survival, PB = population-based, RSR = relative survival ratio, HD = high-dose therapy.

made in SCT-related setting [26–29]. However, most patients with MM are ineligible for SCT. There is not a standardized approach to measure comorbidity in older MM patients, in which comorbidity is more prevalent. Aging of the population and the progressive increase in OS of MM patients will highlight even more the role of comorbidity in next years. Comorbidity should be faced with a simple, practical and useful method to become a common tool in daily clinical practice. The clinical history of every patient should include all past or concomitant diseases. Hence, the relevant list of comorbidities should be easily found in the current electronic medical records. The Charlson Comorbidity Index [30] was developed in a cohort of 559 medical patients and tested in a cohort of 685 patients, combining a scoring for 16 disease categories and a scoring for age. It was designed for general use in longitudinal studies and is not specific for MM. The Freiburg Comorbidity Index was developed in 127 MM patients [31] and validated in 466 MM patients [32]. In the initial study, Kleber et al. included only 10 prognostic factors in their univariate analysis, of which only 7 were comorbidities (lung disease, RI, malignancy, hepatic impairment, cardiac impairment, hypertension and diabetes). This score considered only three factors, RI (eGFR), lung impairment and Karnofsky performance status (KPS). We agree with Kleber et al. that KPS or PS as measured by ECOG score have an impact on MM outcome, but strictly, PS cannot be considered a specific comorbidity but rather the global impact on health of several factors. This scoring system did not took into account other potentially relevant comorbidities, such as obesity [33], hyperlipaemia, thromboembolism, hepatitis C virus infection, human immunodeficiency virus, renal transplant, splenectomy, neurological disorders, psychiatric disorders, rheumatologic disorders, and amyloidosis. Our approach is broader to explore the full impact of any potentially relevant comorbidity. Contrary to what happened in the study of Kleber et al., our multivariate analysis shows that ISS remains an independent factor associated with OS, along with LDH and ASCT. The two only comorbidities that show an independent prognostic impact are RI and amyloidosis. This model is valid for the whole cohort and also when palliative patients are excluded.

The population-based studies are a necessary tool to complement the information derived from clinical trials, helping to achieve a more objective landscape of the outcome in real-life unselected MM patients. More and larger population-based studies are warranted to confirm our results and better highlight the contribution of new prognostic variables on the outcome of NDMM patients.

Authorship contribution statement

All authors provided substantial contributions in the acquisition, analysis or interpretation of data. R.R.-T. is the head of the Monoclonal Gammopathies Unit and responsible for the clinical care of outpatients. M.J. A.R., L.M. P.A.G. and E.L.-F. are responsible of the admitted patients and Stem Cell Transplantation Unit. R.R.-T. created the MM clinical registry, in collaboration with M.J.S.-P. and D.Y.L.C. R.R.-T. analyzed all the data, performed the statistical analyses and wrote the first draft of the manuscript. R.R.-T., T.R., J.L. G., P.N., and P.G. performed the laboratory analyses. R.R-T, J.J.J.-M, J. S. and M.J. supervised the project. J.M.P., J.M.D.-P. and P.L. were involved in the enrolment of patients, clinical data collection and analysis. All authors revised the article at any stage and finally approved the version to be published.

Conflict of interest

R.R.-T has received research support and honoraria as speaking fee from Celgene and Johnson & Johnson. The rest of the authors declare no conflict of interest.

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Resumen artículo 2

Ríos-Tamayo R, Sainz J, Martínez-López J, Puerta JM, Chang DYL, Rodríguez T, et al. Early mortality in multiple myeloma: the time-dependent impact of comorbidity. A population-based study in 621 real-life patients. Am J Hematol 2016;91(7):700-705.

Este trabajo responde al objetivo 2.

Introducción. El MM es una neoplasia muy heterogénea desde diversos puntos de vista, incluido el clínico, con resultados en términos de supervivencia muy variables. Esta diversidad de resultados no puede ser completamente justificada mediante los actuales sistemas de clasificación pronóstica, lo que induce a pensar que otras variables con impacto pronóstico no están siendo medidas adecuadamente.

A pesar de la tendencia favorable en el incremento de la supervivencia global en los años recientes, se ha prestado poca atención a la primera parte de la curva, que refleja la mortalidad precoz. La mortalidad precoz es un serio problema para seguir mejorando la supervivencia global.

Métodos. Se analizó el papel de una valoración integral de la comorbilidad basal en una cohorte de pacientes no seleccionados con MM de nuevo diagnóstico incluidos en el registro clínico de MM de Granada, durante un período de 31 años, para estudiar su potencial impacto en la mortalidad precoz, ajustando por los factores pronósticos establecidos. La mortalidad precoz se midió a los 2, 6 y 12 meses tras el diagnóstico. Para valorar la tendencia temporal, se dividió el período de estudio en quinquenios.

Se realizó un análisis de regresión logística multivariante secuencial a los 2, 6 y 12 meses, para ver el impacto selectivo y dinámico, dependiente del tiempo, de cada comorbilidad.

Resultados. 621 pacientes con MM de nuevo diagnóstico fueron incluidos en el estudio, de los cuales en 426 (68,6%) se disponía de una valoración de comorbilidad basal completa. El análisis demostró que la comorbilidad es un factor pronóstico independiente para mortalidad precoz, de forma diferencial y dependiente del tiempo. Ajustando por el resto de factores pronósticos, la enfermedad respiratoria a los 2 meses, la enfermedad hepática a los 6 meses y la infección por el virus de la hepatitis C a los 12 meses, fueron respectivamente asociados con mortalidad precoz, junto con la insuficiencia renal, que actúa como un denominador común en todos los puntos de corte analizados.

La tendencia de la mortalidad precoz muestra un descenso lento pero progresivo a lo largo del estudio en los 3 puntos de corte analizados.

La causa fundamental del fallecimiento se conoce en 222 pacientes. La insuficiencia renal es la causa fundamental (36.2%) a los 2 meses, mientras que la infección es la causa fundamental a los 6 y 12 meses (38% y 42.9%), respectivamente. En conjunto, la infección es la causa fundamental de mortalidad antes de los 12 meses.

Interpretación. Este es el primer estudio de un solo centro, de base poblacional, que valora el impacto de la comorbilidad en la mortalidad precoz en el MM de nuevo diagnóstico. Junto con la edad y la insuficiencia renal, el análisis integral de la comorbilidad basal tiene un papel crítico en los resultados en salud de los pacientes con MM en términos de mortalidad precoz, con un impacto selectivo y dependiente del tiempo. El impacto de la comorbilidad en la mortalidad precoz es específico y las variables predictoras no tienen por qué coincidir con las que inciden en la supervivencia global.

RESEARCH ARTICLE

Early mortality in multiple myeloma: the time-dependent impact of comorbidity:

A population-based study in 621 real-life patients

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Multiple myeloma is a heterogeneous disease with variable survival; this variability cannot be fully explained by the current systems of risk stratification. Early mortality remains a serious obstacle to further improve the trend toward increased survival demonstrated in recent years. However, the definition of early mortality is not standardized yet. Importantly, no study has focused on the impact of comorbidity on early mortality in multiple myeloma to date. Therefore, we analyzed the role of baseline comorbidity in a large population-based cohort of 621 real-life myeloma patients over a 31-year period. To evaluate early mortality, a sequential multivariate regression model at 2, 6, and 12 months from diagnosis was performed. It was demonstrated that comorbidity had an independent impact on early mortality, which is differential and time-dependent. Besides renal failure, respiratory disease at 2 months, liver disease at 6 months, and hepatitis virus C infection at 12 months, were, respectively, associated with early mortality, adjusting for other well-established prognostic factors. On the other hand, the long-term monitoring in our study points out a modest downward trend in early mortality over time. This is the first single institution population-based study aiming to assess the impact of comorbidity on early mortality in multiple myeloma. It is suggested that early mortality should be analyzed at three key time points (2, 6, and 12 months), in order to allow comparisons between studies. Comorbidity plays a critical role in the outcome of myeloma patients in terms of early mortality.
Am. J. Hematol. 91:700–704, 2016.

Introduction

Multiple myeloma (MM) is a complex and heterogeneous disease [1–3], with variable survival for newly diagnosed patients, ranging from a few months to more than 10 years [4,5]. Despite the recent progress in the prognosis evaluation and therapy of myeloma patients [6–14], the current risk stratification systems cannot predict the outcome in a particular patient accurately.

There has been a major improvement in the long-term survival of MM patients in the last decade. However, little attention is usually paid to the first part of the survival curve. Early mortality (EM) draws the shape of the survival curve in the beginning of the follow-up. The concept of EM is not standardized. A cutoff at 2 (EM2) [15–17], 6 (EM6) [18–20], or even 12 months (EM12) [21,22] is commonly used. Kastritis et al. [23] first reported EM results along the three cutoff points previously referred. Later cutoff have been eventually analyzed [24]. The clinical impact of EM has been mostly evaluated in clinical trials, in the context of multicenter studies. However, little is known about EM in unselected real-life MM patients. Data about EM at the population-level are scarce. Costa et al. [22] have recently reported extracted information from the National Cancer Institute Surveillance Epidemiology and End Result Registry, showing a decline in EM over time. However, to date no single institution population-based study has systematically evaluated changes in EM for over three decades. Only a few studies have been reported from a single center but none of them is a population-based study. In addition, specific risk factors for EM are largely unknown outside clinical trials. Data about the role of diagnostic and treatment delays in EM of MM are limited. Although a few studies have analyzed the role of a number of risk factors and certain comorbidities in the outcome of MM [17,21,25], no study has specifically focused on the impact of comorbidity on EM in MM. We therefore analyzed our population-based MM clinical registry focusing on the impact of potential risk factors for EM, including a

Additional Supporting Information may be found in the online version of this article.

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Conflict of interest: Nothing to report.
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comprehensive comorbidity approach. We hypothesized that EM should be higher in a cohort of unselected patients with respect to data of highly selected patients enrolled onto clinical trials. On the other hand, we supposed that the impact of comorbidity could be different according to the selected cutoff point analyzed.

Methods

Patients. From January 1985 to December 2015, 631 newly diagnosed myeloma patients who had their current residence at the time of diagnosis in Granada and met the diagnostic criteria of the International Myeloma Working Group (in accordance with the existing criteria in each period) [1,26], were included in our population-based MM clinical registry and are the basis of this study, which was performed according to the Declaration of Helsinki (Ethics Committee approval number C-14, CEI-Gr, 2014). Patients not fit for MM-directed therapy were analyzed separately.

Variables. At the moment of diagnosis, the following baseline prognostic factors were recorded: age, sex, Eastern Cooperative Oncology Group performance status score, the occurrence of weight loss, body mass index, number of comorbidities, diagnostic delay, treatment delay, presence of previously documented precursor disease [27], International Staging System (ISS), light chain MM [28], percentage of bone marrow plasma cells by morphology, serum creatinine (mg/dL), estimated glomerular filtration rate (mL/min/1.73 m^2), free light chain ratio (involved/uninvolved), lactate dehydrogenase (U/L), high-risk [t(4;14), t(14;16), +1q, and del(17p)] cytogenetic abnormalities by fluorescent in situ hybridization, and extramedullary disease by positron emission tomography with ^{18}fluorine-fluoro-deoxyglucose integrated with computed tomography.

Demographic variables and survival data are available for the whole cohort since 1985, but clinical data were incorporated in 1993. Clinical and laboratory variables have been incorporated prospectively since 2011 [29] and retrospectively for the period 1993–2010. Moreover, due to the duration of the study, these variables have been incorporated to the registry progressively, once it has been demonstrated its usefulness. For instance, despite that the revised ISS is better than the classical ISS for risk stratification in real-life patients [30], we decided not to use this new staging criteria due to the relatively low number of patients with available cytogenetic data in our cohort.

The delay to diagnosis was calculated as time (months) between the first symptomatic presentation and the date of definitive diagnosis. The treatment delay was measured in days from date of diagnosis to date of initiation of treatment.

Data about comorbidities and therapy of our patient cohort were gathered from medical records and have been reported elsewhere [31]. In short, 20 baseline comorbidities were analyzed: hypertension, obesity, heart disease, type 2 diabetes, respiratory disease, hyperlipaemia, neurological disorder, peptic ulcer, prior malignancy, psychiatric disorder, hepatitis B virus infection, liver disease, rheumatologic disorder, thromboembolism, amyloidosis, cutaneous disease, hepatitis C virus infection, splenectomy, renal transplant, and human immunodeficiency virus infection. Data for major specific entities are available within each category of disease. For instance, within the category liver disease, the specific entities are cirrhosis, steatosis, or other.

Survival. Survival was calculated in months from the date of diagnosis (first bone marrow aspirate or biopsy) until the date of death, loss to follow-up, or end of study (December 31, 2015), whichever occurred first.

Mortality has been analyzed at three key cut-off points: EM2, EM6, and EM12. In order to evaluate possible temporary changes in EM, the study was divided into six periods of 5 years (the last one, 6 years). The source of information for vital status of patients was the National Index of Deaths.

Statistical analysis. Comparisons for categorical variables among different groups were made with the χ^2-test, using Fisher's exact test when appropriate. Comparisons of means of quantitative continuous variables between two groups were made with the *t*-test. Survival curves were estimated using the Kaplan–Meier method, and comparisons among groups were carried out with the log-rank test. Odds ratios (OR) and 95% confidence interval (CI) were estimated using univariate and multivariate binary logistic regression models to test independent variables as risk factors for EM. Predictive variables with $P < 0.1$ in univariate analysis were entered in the multivariate logistic regression model for each cutoff analyzed. A backward selection method was used to reach the estimated model for each cutoff. All *P*-values were two-sided. No imputation for missing data has been used. Data were analyzed with SPSS v20 software.

Results

Six hundred and thirty-one newly diagnosed myeloma patients were recruited in our MM clinical registry during the period of study. Patients with smoldering MM ($n = 10$) were excluded. The remaining 621 were evaluable for survival analysis and are the basis of the study, 299 men (48.1%) and 322 women. Six patients (1%) lack survival data (all of them were diagnosed before 1992). Median age was 67 years (range, 12–91). There were four patients with primary plasma cell leukemia. Women had a significantly higher mean age than men (67.5 vs. 64.5 years; $P = 0.001$). A complete assessment of comorbidity was available in 426 patients (68.6%) at the moment of diagnosis.

TABLE I. Baseline Patient Characteristics

Characteristics	Unfit	Fit	*P*-value
n (%)	40 (6.4)	581 (93.6)	
Age (y), Mean ± SD	82.37 ± 4.95	64.96 ± 10.84	**<0.001**
Sex, *n* (male/female) (%)	19/21 (6.4/6.5)	280/301 (93.6/93.5)	1
PS, ECOG 3–4, *n* (%)	15 (55.5)	59 (24.2)	**0.004**
WL, *n* (%)	12 (44.4)	85 (29.1)	0.131
BMI, Mean ± SD	27.59 ± 4.73	28.24 ± 4.63	0.549
NC, Mean ± SD	2.94 ± 1.88	1.85 ± 1.50	**<0.001**
DD (m), Mean ± SD	4.55 ± 5.77	6.10 ± 7.14	0.313
TD (d), Mean ± SD	19.90 ± 32.31	41.03 ± 113.05	0.406
PPD, *n* (%)	4 (12.1)	31 (7.8)	0.330
ISS III, *n* (%)	24 (85.7)	129 (46.4)	**<0.001**
LC MM, *n* (%)	6 (18.2)	67 (17)	0.812
BMPC, Mean ± SD	26.00 ± 22.27	25.61 ± 21.79	0.927
sCr, Mean ± SD	2.84 ± 2.66	1.98 ± 2.14	**0.033**
eGFR, Mean ± SD	38.77 ± 30.82	59.21 ± 35.71	**0.002**
FLCr(i/u), Mean ± SD	1,008.68 ± 1756	715.77 ± 2008	0.606
LDH, Mean ± SD	295.12 ± 158.92	305.98 ± 256.84	0.833
HR FISH, *n* (%)	3 (37.5)	22 (26.8)	0.680
EMD, *n* (%)	2 (22.2)	22 (18.5)	0.676

Abbreviations: BMI, body mass index (Kg/m^2); BMPC, bone marrow plasma cells (morphology); d, days; DD, diagnostic delay; eGFR, estimated glomerular filtration rate (mL/min/1.73 m^2); EMD, extramedullary disease by PET/CT (Positron Emission Tomography/Computed Tomography); FLCr(i/u), free light chain ratio (involved/uninvolved); HR FISH, high risk fluorescent *in situ* hybridization; ISS, international staging system; LC, light chain; LDH, lactate dehydrogenase (U/L); m, months; n, number of patients; NC, number of comorbidities; PPD, prior precursor disease; PS (ECOG), performance status (Eastern Cooperative Oncology Group); sCr, serum creatinine (mg/dL); SD, standard deviation; TD, treatment delay; WL, weight loss; y, years.

Forty patients (6.4%) were considered not fit for MM-directed therapy. As expected, median overall survival for this group was extremely short compared with the whole cohort, 3.7 (95% CI, 0–9.45) versus 31.2 months (95% CI, 26.6–35.7), respectively, $P < 0.001$ (Supporting Information Fig. 1A). When only patients with comorbidities known were analyzed, similar data were found (Supporting Information Fig. 1B). Therefore, the analysis was performed including or excluding this special group of patients.

Table I shows baseline characteristics of patients. As expected, patients unfit were significantly older, having more comorbidities, ISS III, grade 3–4 performance status, and severe renal failure.

Data of EM in accordance with the period of time and the type of patient are shown in Supporting Information Table I. For the whole cohort, EM2, EM6, and EM12 were 12.7%, 22.2%, and 31.9%, respectively. Excluding patients not fit for MM-directed therapy, a significant decrease in EM was observed, 10.6%, 20%, and 28.6%, respectively. The evolution of the percentage of EM2, EM6, and EM12 throughout the study period is highlighted in Fig. 1.

The univariate analysis in the cohort of patients with comorbidities known is shown in Table II. Age, performance status, treatment delay, ISS, the percentage of bone marrow plasma cell, and renal function (as measured by serum creatininine or by estimated glomerular filtration rate) were significant predictors of EM, in the three cutoff points used. Interestingly, the number of comorbidities is a significant predictor for EM2, but it gradually loses its significance thereafter. On the other hand, the presence of hypertension, heart disease, hepatitis virus C infection, respiratory disease, and liver disease were differential

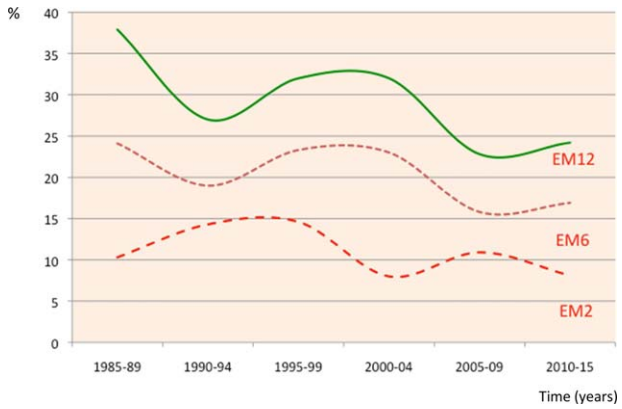

Figure 1. Evolution of early mortality (EM) at 2, 6, and 12 months over time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

predictors for EM. Hypertension and respiratory disease are mainly associated with EM2, whereas hepatitis virus C infection predicts for EM6 and EM12. Heart disease and liver disease were significant predictors of EM, in the three cutoff points used.

The multivariate analysis (Table III) was performed separately in both the whole cohort of patients with comorbidities known ($n = 426$) and the cohort of fit patients ($n = 393$), after excluding patients unfit for MM-therapy. In this multivariate binary regression model, only age and serum creatinine were independent risk factors for EM in all the cutoff points analyzed. Focusing on the cohort of fit patients, the presence of respiratory disease was associated with EM2, whereas liver disease was an independent predictor for EM6, and finally, the hepatitis virus C infection besides ISS were significantly associated to EM12. This model fits well when unfit patients were included, with only two remarks: in relation to EM2, the association of respiratory disease is only marginal, but liver disease reach significance; with respect to EM6, the hepatitis virus C infection also behaves as an independent predictor.

The survival for specific entities in relation to liver disease, heart disease, and respiratory disease are highlighted in Supporting Information Figs. 2, 3 and 4, respectively.

The analysis of the main cause of death is available in 222 cases, and it is summarized in Supporting Information Table II. In accordance with other studies, overall, infection remains the main cause of death for EM in our study. Interestingly, a gradual increase in the frequency of infection, bleeding and progressive disease is shown over the three cutoff points to analyze EM, whereas a progressive decrease is demonstrated for renal failure and cardiovascular disease as the main cause of death.

Discussion

Here, we evaluate systematically the impact of prognostic factors and comorbidities on EM in MM patients. Given that the concept of EM has not been standardized and has been shown to be highly variable among previous studies (ranging from 2 months till 2 years), we decided to analyze our data considering the most frequently used periods, that is, EM2 (very early mortality), EM6 (early-early mortality), and EM12 (early-late mortality). Taking into consideration the growing relevance of EM in both clinical trials and population-based studies, common efforts should be made to achieve a standardized concept, in order to allow comparisons, since the current variability in the definition of EM preclude reaching firm conclusions. On the other hand, given the strong impact of patients not fit for MM-directed therapy on both long-term survival and EM, we also decided to perform a sub-analysis excluding this specific subgroup of patients, which are commonly excluded from clinical trials and probably are also excluded in non population-based studies.

Until now, only a few studies have focused on EM in MM [15–24]. Supporting Information Table III highlights the results of these studies, emphasizing the differences in the type of study and the cutoff used to assess EM. Augustson et al. [15] attracted the attention about EM in 2005 analyzing the results of five trials; they found an EM2 of 10%, being bacterial infection the cause of early death in 45%. Since then, no work has specifically focused on EM in MM, until 2013, when Kastritis et al. [23] analyzed 509 patients from Athens, showing 6% EM2, 13% EM6, and 18% EM12. This was the first study in which the three cutoff points (EM2, EM6, EM12) were used, and we agree with these authors in the convenience of using this approach. In 2014, Kumar et al. [21] and Dimopoulos et al. [16] showed some new data on EM. In the Mayo Clinic study, they found only a 13% of EM12. This is a tertiary referral center in which patients were seen within 30 days of diagnosis and probably did not include many critically sick patients, as the authors stated. The Greek Myeloma Study Group showed that renal failure is associated with EM2, ranging from 12% in patients with severe renal failure to 3% in patients with mild or no renal failure. More recently, Holmström et al. [19] presented a nationwide Danish MM database of patients ineligible for high-dose therapy, showing 22% EM6 associated with performance status, ISS, and lactate dehydrogenase. The most common causes of death were infections, cardiovascular failure and renal failure. O'Donnell et al. [24] pointed out in a single center study from Boston, a 32% EM within 2 years, showing association with ISS, bone disease and renal failure. Finally, Hsu et al. [17] in a single institution study from Taipei, have shown 12.6% EM2, being infections, renal failure and cardiac failure the main causes of death.

To the best of our knowledge, this is the first single institution population-based study focused on EM in newly diagnosed MM patients. Our registry-based study ensures the inclusion of all incident MM cases and avoids a potential referral bias. In addition, this study has the largest timeframe (31 years) reported to date, allowing a better evaluation of the evolution of EM. Remarkably, this study shows for the first time a differential and time-dependent impact of specific comorbidities on well-defined cutoff points to assess EM dynamically. Moreover, this study is the first one to investigate the potential role of diagnostic and treatment delays in EM. When considering the number of patients, this study is the third largest single institution study to date, being surpassed only by those studies by Kumar et al. [21] and O'Donnell et al. [24], which are non-population–based studies. The limitations of our study include the lack of clinical and laboratory information from 1985 to 1992, limited data of cytogenetic abnormalities, and some missing data for certain variables.

In this study, we demonstrate that the age, the presence of renal failure, and respiratory disease are independent risk factors for EM2. On the other hand, age, renal failure, and liver disease are the main predictors for EM6. Finally, age, renal failure, ISS, and hepatitis virus C infection, were significantly associated to EM12. It should be emphasized that renal failure is the only common denominator for the three cutoff points, besides age. The model works similarly when applied to the entire cohort or when unfit patients are excluded.

As expected, the EM in our unselected MM population is higher than the reported in recent clinical trials, in which a very selected group of patients is analyzed. Recent clinical trials reported an EM2 as low as 3.8% [32], an EM6 ranging from 6.9% [33] to 12.8% [34] and an EM12 ranging from 10.8% to 31.7%. Nonetheless, our EM2 (10.6%) is very similar to the one highlighted by Augustson et al. [15] (10%), and lower than the one recently reported by Hsu et al. [17] (12.6%). Our EM6 (20%) is higher than that reported by Kastritis et al. [23] (13%) but lower than that showed by Holmström et al. [25] (22%), although this study included only elderly patients. Finally,

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TABLE II. Univariate Analysis in the Cohort of Patients with Comorbidities Known ($n = 426$)

	EM2		EM6		EM12	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.08 (1.04–1.11)	**0.001**	1.07 (1.04–1.10	**<0.001**	1.05 (1.03–1.07)	**<0.001**
ECOG	2.22 (1.48–3.33)	**<0.001**	2.59 (1.81–3.72)	**<0.001**	2.46 (1.66–3.63)	**<0.001**
WL	1.47 (0.97–2.23)	**0.072**	1.48 (1.04–2.09)	**0.028**	1.43 (1.04–1.96)	**0.027**
NC	1.20 (1.01–1.43)	**0.043**	1.15 (0.99–1.33)	**0.069**	1.14 (0.99–1.30)	**0.071**
TD	0.94 (0.90–0.98)	**0.050**	0.97 (0.95–0.99)	**0.004**	0.97 (0.95–0.99)	**<0.001**
PPD	0.69 (0.20–2.34)	0.548	0.48 (0.16–1.39)	0.175	0.32 (0.11–0.94)	**0.038**
ISS	3.30 (1.64–6.66)	**0.001**	3.20 (1.91–5.37)	**<0.001**	2.46 (1.67–3.63)	**<0.001**
LCMM	2.16 (1.07–4.39)	**0.032**	2.16 (1.18–3.94)	**0.012**	1.58 (0.89–2.81)	0.116
BMPC	1.02 (1.00–1.03)	**0.024**	1.02 (1.01–1.03)	**0.007**	1.02 (1.01–1.04)	**<0.001**
sCr	1.24 (1.11–1.38)	**<0.001**	1.33 (1.18–1.49)	**<0.001**	1.27 (1.13–1.43)	**<0.001**
eGFR	0.98 (0.97–0.99)	**<0.001**	0.98 (0.97–0.99)	**<0.001**	0.98 (0.97–0.99)	**<0.001**
FLCr (i/u)	1.00 (1.00–1.00)	0.420	1.00 (1.00–1.00)	0.490	1.00 (1.00–1.00)	**0.049**
LDH	1.00 (1.00–1.00)	0.266	1.00 (1.00–1.00)	0.112	1.00 (1.00–1.00)	**0.012**
HY	2.02 (1.11–3.70)	**0.022**	1.61 (1.00–2.60)	**0.051**	1.47 (0.96–2.27)	**0.079**
HD	1.87 (0.98–3.57)	**0.056**	1.86 (1.09–3.19)	**0.024**	1.77 (1.08–2.92)	**0.025**
HCV	2.13 (0.43–10.54)	0.355	4.92 (1.29–18.75)	**0.019**	8.59 (1.76–42.01)	**0.008**
RD	2.18 (1.11–4.30)	**0.024**	1.71 (0.94–3.10)	**0.079**	1.54 (0.88–2.70)	0.131
LD	2.78 (1.04–7.43)	**0.041**	3.14 (1.33–7.43)	**0.009**	2.71 (1.16–6.34)	**0.021**

Abbreviations: BMPC, bone marrow plasma cells (morphology); CI, confidence interval; eGFR, estimated glomerular filtration rate (mL/min/1.73 m^2); EM2, early mortality at 2 months; EM6, early mortality at 6 months; EM12, early mortality at 12 months; FLCr(i/u), Free Light Chain ratio (involved/uninvolved); HCV, hepatitis C virus; HD, heart disease; HY, hypertension; ISS, international staging system; LC, light chain; LD, liver disease; LDH, lactate dehydrogenase (U/L); NC, number of comorbidities; OR, odds ratio; PPD, prior precursor disease; PS (ECOG), performance status (Eastern Cooperative Oncology Group); RD, respiratory disease; sCr, serum creatinine (mg/dL); TD, treatment delay; WL, weight loss.
Only variables with any *P* value <0.1 are shown.

TABLE III. Multivariate Binary Regression Model in the Cohort of Patients with Comorbidities Known ($n = 426$)

	Whole cohort ($n = 426$)			Fit patients ($n = 393$)		
Variables	OR	95% CI	*P*-value	OR	95% CI	*P*-value
EM2						
Age	1.08	1.04–1.12	**<0.001**	1.05	1.00–1.09	**0.039**
sCr	1.25	1.11–1.40	**<0.001**	1.26	1.10–1.43	**0.001**
LD	4.01	1.36–11.84	**0.012**	2.99	0.75–11.90	0.120
RD	2.00	0.90–4.43	0.087	2.72	1.10–6.74	**0.030**
EM6						
Age	1.07	1.04–1.10	**<0.001**	1.05	1.02–1.09	**0.003**
sCr	1.34	1.18–1.52	**<0.001**	1.35	1.18–1.54	**<0.001**
LD	3.24	1.15–9.10	**0.026**	3.45	1.06–11.19	**0.039**
HCV	5.34	1.03–27.80	**0.047**	4.63	0.84–25.63	0.079
EM12						
Age	1.06	1.03–1.09	**<0.001**	1.03	1.00–1.07	**0.033**
sCr	1.17	1.01–1.35	**0.033**	1.17	1.01–1.35	**0.040**
HCV	7.51	1.29–43.91	**0.025**	6.95	1.14–42.35	**0.035**
ISS (III vs. I)	3.09	1.27–7.53	**0.013**	3.46	1.28–9.33	**0.014**

Abbreviations: CI, confidence interval; EM2, early mortality at 2 months; EM6, early mortality at 6 months; EM12, early mortality at 12 months; HCV, hepatitis C virus; ISS, international staging system; LD, liver disease; OR, odd ratio; RD, respiratory disease; sCr, serum creatinine (mg/dL).
Only variables with significant *P*-value are shown.

our EM12 (28.6%) was higher than the one previously described by Kumar et al. [21] (13%) but identical to the one reported by Costa et al. (28.6%) [22]. None of the above-mentioned studies except ours is a single-institution population-based study.

The evolution of EM2, EM6, and EM12 over the six calendar periods shows only a slight steady decline, probably reflecting that renal failure continues to lead a poor outcome even after the widespread use of the novel agents.

There is increasing evidence indicating that comorbidity plays an emerging role in the outcome of MM. However, the predictors for overall survival are different from those associated with EM. Renal failure remains a strong and steady predictor of EM, but the relative impact of the other comorbidities associated with EM seems to be time-dependent in our study, in particular for respiratory disease, liver disease, and hepatitis C virus infection. Gonsalves et al. [35] have recently reported a series of recommendations aimed at reducing EM in MM. All highlighted items are important. Probably, there is room for improvement in the prevention and management of infection. Avoiding unnecessary diagnostic and treatment delay as well as implementation of strategies to restore renal function are also key points. Interestingly, they emphasized that comorbidities affect prognosis of MM but currently there are no prospective MM studies that assess outcomes in patients with specific pre-existing comorbidities. Although the role of comorbidities in EM of MM patients still remains to be determined, our study provides evidence in this regard.

There is no gold-standard approach to measuring comorbidity in the context of cancer [36]. Many approaches have been proposed; however, any approach to summarizing comorbidity into a single metric, by necessity, results in loss of information [37]. Common efforts should be made to include patients with comorbidities in clinical trials, which are the safest setting to capture data [38].

In summary, the management of MM patients with comorbidities is a challenge that should be addressed improving the measurement of comorbidity and the coordination of care. Population-based studies are necessary to identify prognostic factors for EM in real-life unselected MM patients, complementing the information derived from clinical trials. We suggest that, in the near future, EM in MM should be faced building a comprehensive and dynamic comorbidity approach helping to quantify and highlight the selective and time-dependent role of specific comorbidities in the short term outcome of newly diagnosed MM patients.

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Resumen artículo 3

Ríos-Tamayo R, González-Silva M, Molina E, García-Fernández JR, Clavero ME, Durán JM, et al. Impacto del tipo de hospital en la supervivencia de pacientes con mieloma múltiple: estudio MICORE. Rev Clin Esp. 2013, 213(7):330-5.

Este trabajo responde al objetivo 3.

Introducción. El MM es una neoplasia hematológica que sigue siendo considerada incurable en la mayoría de los casos y presenta una enorme heterogeneidad clínica en términos de supervivencia global. En el contexto de la red de hospitales del Sistema Sanitario Público Andaluz, los pacientes son diagnosticados y tratados en distintos tipos de hospitales, básicamente hospitales comarcales u hospitales de referencia (de carácter provincial o regional). Existen pocos estudios en nuestro entorno sanitario que valoren las posibles diferencias en la supervivencia global en función del tipo de centro donde los pacientes son tratados. El objetivo de este estudio es analizar si existen diferencias en nuestro entorno sanitario en la supervivencia global de los pacientes con MM en función del tipo de centro donde son tratados.

Métodos. Estudio de cohortes, retrospectivo, multicéntrico, de base poblacional, en el que se han registrado todos los casos de MM de nuevo diagnóstico desde enero de 1993 a diciembre de 2006 en 5 hospitales públicos andaluces, uno de ellos de referencia (Hospital Universitario Virgen de las Nieves) y 4 comarcales con similares características: Hospital Valle de los Pedroches (Córdoba), Hospital de La Línea de la Concepción (Cádiz), Hospital Santa Ana de Motril y Hospital de Baza (ambos en Granada).

La variable resultado fue la supervivencia global, medida en meses, hasta diciembre de 2011, fecha de finalización del seguimiento. Los datos de los tres hospitales de la

provincia de Granada fueron contrastados con el Registro de Cáncer de Granada; los otros dos disponían de registro propio.

Resultados. Durante los 14 años del período de inclusión del estudio, se incluyeron 431 pacientes, residentes en las diferentes áreas hospitalarias de los centros participantes, de los cuales 256 pertenecían al hospital de referencia (59,4%) y el resto a hospitales comarcales.

No se apreciaron diferencias en la supervivencia entre los cuatro hospitales comarcales (p = 0,490). La mediana de supervivencia de los pacientes del hospital de referencia fue de 20 meses (IC95% 14,9-25,1) *versus* 23 meses (IC95% 16-30) global para los hospitales comarcales (p = 0,473).

En el análisis univariante, las variables asociadas a la mortalidad fueron la edad, el estadio de Durie-Salmon (III), la insuficiencia renal y el MM de cadenas ligeras. En el análisis multivariante, solo se mantuvo la asociación con la edad, el estadio de Durie-Salmon, y la insuficiencia renal. El HR para la mortalidad entre los pacientes atendidos en hospitales comarcales *versus* hospital de referencia, ajustado por edad, sexo, estadio de Durie-Salmon, sCr y componente monoclonal fue de 0,72 (IC95% 0,48-1,07; p = 0,1).

Interpretación. En este estudio, el nivel de complejidad del hospital donde se atiende a los pacientes con MM de nuevo diagnóstico no se asocia con la mortalidad. El estudio confirma el valor pronóstico de la edad, el estadio de Durie-Salmon y la insuficiencia renal. Nuestros resultados avalan el modelo de atención sanitaria que actualmente funciona en nuestro entorno.

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ORIGINAL ARTICLE

The impact of the type of hospital on survival of multiple myeloma patients: The MICORE study☆

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KEYWORDS
Multiple myeloma;
Hospital;
Survival analysis

Abstract
Objective: To analyze the impact of the type of hospital in overall survival of multiple myeloma patients.
Patients and method: A survival analysis was performed of all patients ($n = 431$) diagnosed in 5 public hospitals (4 community hospitals and one university hospital) during the period 1993–2006.
Results: Patients attended to in community hospitals differ significantly from those seen in the university hospital in the following variables: mean age (70 years [31–92] versus 67.9 (35–91), $p = .038$); percentage of stage III patients (62.6% versus 69.1%, $p = .033$), and percentage of patients who had autologous stem cell transplant (8.2% versus 18.2%, $p = .026$). The variables associated with mortality in the multivariate analysis were age ($p < .001$), stage (III versus I; $p = .03$) and renal failure ($p = .04$). The type of hospital did not reach statistical significance (hazard ratio of 0.72 (95% confidence interval 0.48–1.07), $p = .1$].
Conclusions: The type of hospital is not significantly associated with mortality in multiple myeloma patients. These data support our current model of health care, in which the

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community hospitals are responsible for the primary care of these patients, in a coordinated work with the university hospital.

PALABRAS CLAVE
Mieloma múltiple;
Hospital;
Análisis de supervivencia

Impacto del tipo de hospital en la supervivencia de pacientes con mieloma múltiple: estudio MICORE

Resumen
Objetivo: Analizar el impacto del tipo de hospital en la supervivencia global de los pacientes con mieloma múltiple.
Pacientes y método: Análisis de supervivencia de todos los pacientes (n = 431) diagnosticados en 5 hospitales públicos (4 comarcales y uno universitario), durante el periodo 1993–2006.
Resultados: Los pacientes atendidos en los hospitales comarcales difieren significativamente de los atendidos en el hospital de referencia en las siguientes variables: edad media (70 años [rango 31–92] versus 67,9 [rango 35–91]; p = 0,038), porcentaje de pacientes en estadio III (62,6 versus 69,1%; p = 0,033), y porcentaje de pacientes sometidos a trasplante autólogo de médula ósea (8,2 versus 18,2%; p = 0,026). En el análisis multivariante, las variables asociadas de forma significativa con la mortalidad fueron la edad (p < 0,001), el estadio (III respecto a I; p = 0,03) y la insuficiencia renal (p = 0,04). El tipo de hospital no alcanzó significación estadística (*hazard ratio* de 0,72 [intervalo de confianza al 95% 0,48–1,07], p = 0,1).
Conclusiones: El tipo de hospital no se asocia de forma significativa con la mortalidad en pacientes con mieloma múltiple. Estos datos apoyan el actual modelo de atención a estos pacientes, en el que los hospitales comarcales son responsables de su manejo primario, de forma coordinada con el hospital universitario.

Background

Multiple myeloma (MM) is a cancer characterized by the proliferation of clonal plasma cells in the bone marrow microenvironment, accompanied in most cases by the presence of a monoclonal component in serum and/or urine and is associated with dysfunction of the target organs.[1] MM is preceded in most cases by the precursor disease known as monoclonal gammopathy of undetermined significance (MGUS).[2–4] MM represents approximately 1–2% of all malignant cancers and approximately 10–15% of hematologic malignancies.[5] Despite recent advances, specifically a better definition of the prognosis[6,7] and response criteria,[8,9] an optimized use of hematopoietic progenitor transplantation (HPT),[10] better support treatment[11] and the use of new drug combinations,[12] MM continues to be incurable in most cases. MM has enormous clinical heterogeneity, which newly discovered genetic and molecular mechanisms are helping to explain,[13–15] thereby contributing to a better pathogenic understanding of the disease.[16] Although advances in survival have been achieved,[17] especially in young patients, this progress is not considered satisfactory by any means. Moreover, MM is one of the hematologic diseases that most deteriorates quality of life related to health.[18]

MM clinically presents in highly diverse forms,[19] and patients with MM are often initially evaluated by various specialists and subsequently referred for hematologic assessment. Patients with MM are often treated in regional hospitals; however, there have been few studies that compare the results of patients treated in these centers with those of patients treated in large reference hospitals. This aim of this study is to analyze whether there are differences in our healthcare community in the overall survival of patients with MM based on the type of center where they are treated.

Patients and methods

We conducted a retrospective, multicenter, population-based cohort study that recorded all incident cases of symptomatic MM, diagnosed according to the criteria of the International Myeloma Workgroup,[20] from January 1993 to December 2006 in 5 hospitals belonging to the Public Healthcare System of Andalusia (University Hospital Virgen de las Nieves [Granada], Hospital Valle de los Pedroches [Pozoblanco, Cordoba], Hospital de La Línea [La Línea de la Concepción, Cadiz], Hospital de Baza [Baza, Granada] and Hospital Santa Ana [Motril, Granada]). The first hospital was a reference hospital and the other 4 were regional.

The recorded predictors were age at diagnosis, gender, Durie-Salmon stage, secreted monoclonal paraprotein, previously known presence of MGUS, performance or not of an HPT, type of hospital (reference or regional) and serum creatinine (Crs), classified as ''renal failure'' if it was $\geq$2 mg/dL. The resulting variable was the overall survival (measured in months) up to December 2011, the end date for the follow-up. Survival was assessed using each center's own registries and the National Death Index. For the 3 hospitals in Granada, the data were compared with those from the Cancer Registry of Granada of the Andalusian School of Public Health.

Statistical analyses were performed using IBM SPSS (Statistical Package for Social Sciences) version 20. A descriptive statistical study was conducted for the independent variables. The quantitative variables were expressed as mean,

What we know

Despite advances in the last decade, the morbidity and mortality of multiple myeloma remains considerable. Patients with multiple myeloma are often treated in regional or first level hospitals. There have been few studies that compare the results of patients treated in these centers with those of patients treated in large reference hospitals. The aim of this study is to analyze whether there are differences in our healthcare community in the overall survival of patients with multiple myeloma based on the level of complexity of the center where they are treated.

What this article provides

The level of complexity of the hospital is not significantly associated with the mortality of patients with multiple myeloma. We observed a trend toward lower mortality in first-level hospitals.

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median and range. The qualitative variables are shown as frequencies and percentages. The comparison of means was performed using the Student *t*-test, and the qualitative variables were compared using the chi-squared test. Survival was calculated using the Kaplan–Meier method, and the differences in the survival curves were assessed using the log-rank test. To assess the simultaneous impact of various predictors on survival and calculate the strength of each predictor's effect, we used the Cox proportional hazards test.

Results

During the 14 years of patient enrollment in the study, MM was diagnosed in a total of 431 patients who were residents in the various hospital areas of the participating centers; 209 of the patients were male (48.5%) and 222 were female (51.5%), with a median age of 70 years (range 31–92). Table 1 shows the baseline characteristics of the patients and their distribution by hospital type. Two hundred fifty-six cases (59.4%) corresponded to reference hospitals and the remaining 175 (40.6%) to regional hospitals. The Durie-Salmon stage was known in only 209 patients (stage I in 25 [12%], stage II in 46 [22%] and stage III in 138 [66%]). Crs levels were known in only 196 cases and were $\geq$2 mg/dl in 57 cases (29.1%). We were able to determine the type of secreted monoclonal paraprotein in only 224 occasions (IgG in 118 patients [52.7%], IgA in 63 [28.1%], light chains in 32 [14.3%], IgD in 2 [0.9%] and without paraprotein or nonsecretor in 11 [4.9%]). Of the 169 patients in whom the precedent of MGUS was recorded, there was a prior MGUS in only 16 (9.5%). In 338 patients, there was information on autologous HPT (AHPT), which was performed on 53 occasions (15.7%).

The distribution, according to hospital type (regional versus reference), of the variables of interest with significant differences were the following: the mean age was 70 years versus 67.9 years ($p = .038$), the percentage of cases in Durie-Salmon stage III was 62.6% versus 69.1% ($p = .033$) and the percentage of patients who underwent AHPT was 8.2% versus 18.2% ($p = .026$), respectively.

The median overall survival of patients with MM was 20 months (95% confidence interval [95% CI] 14.9–25.1) in the reference hospital versus 23 months (95% CI 16–30) in the regional hospitals ($p = .473$). There were also no significant differences in survival rates among the 4 regional hospitals in the study ($p = .490$).

The results of the survival analysis using the Cox test are shown in Table 2. In the univariate analysis, the variables associated with mortality were age, Durie-Salmon stage (III), renal failure and light chain MM. In the multivariate analysis, the only association was with age, Durie-Salmon stage and renal failure. The hazard ratio for mortality among the patients treated in regional versus reference hospitals, adjusted for age, gender, Durie-Salmon stage, Crs levels and monoclonal component was 0.72 (95% CI 0.48–1.07; $p = .1$).

Discussion

The available literature describes an association between the level of complexity of the hospital and better results in a wide variety of clinical situations,[21] including cancer,[22] especially in their surgical management.[23] However, many of these studies suffer from methodological problems. There are few results concerning the medical handling of various types of cancer based on the level of complexity of the hospital. Oivanen et al[24] found no significant differences in the response rate, in the progression-free survival or in the survival of patients with MM based on the size of the hospital.

The network of public hospitals of the Public Healthcare System of Andalusia has various types of hospitals, which from lesser to greater complexity are high-resolution hospitals, regional hospitals, specialties hospitals and reference hospitals. Reference hospitals have a broader portfolio of services, better facilities and, in theory, more experienced staff because they treat a larger number of cases and have a certain monographic dedication. Therefore, it is plausible to assume that the results of treatment of a number of diseases, among them MM, may be better in reference hospitals than in regional hospitals of lesser complexity, where the staff have a more generic and comprehensive dedication and the clinical–biological support is less. However, in our community, MM is considered a chronic disease that can be managed in regional hospitals, with the patient referred to reference hospitals for certain additional tests or to undergo specific procedures, such as AHPT and radiation therapy. Nevertheless, there are few studies that compare the results of managing specific hematologic malignancies based on the level of complexity of the hospital. To the best of our knowledge, this is the first study on MM in our setting that addresses this subject.

The type of treatment was not included as an independent variable due to the fact that, during the study period, the therapeutic approach to symptomatic MM was based on a strict protocol. The protocol was based on whether the patient is a candidate for AHPT, and was limited to regimens based mainly on corticosteroids, alkylating agents,

Table 1 General epidemiological characteristics of patients with multiple myeloma enrolled in the MICORE study.

	Hospital				Type of hospital		p^a
	HVP	HLL	HM	HB	HCO	HRE	
Patients, n	46	39	46	44	175	256	
Age							
Median	73	73	64	72	71	69	**.038**
Range	41–87	32–92	44–89	31–89	31–92	35–91	
Gender							
Female, *n* (%)	19	22	24	20	85 (48.6)	137 (53.5)	.328
Durie-Salmon stage							
I, *n*	4	7	0	2	13	12	
II, *n*	12	7	4	1	24	22	
III, *n* (%)	25	25	4	8	62 (62.6)	76 (69.1)	**.033**
Serum creatinine							
≥2 mg/dl, *n* (%)	11	12	3	1	27 (28.4)	30 (29.7)	.876
Paraprotein							
IgG, *n* (%)	20	–	4	6	30 (45.5)	88 (55.7)	.427
IgA, *n* (%)	16	–	4	4	24 (36.4)	39 (24.7)	
Light chains, *n* (%)	6	–	3	0	9 (13.6)	23 (14.6)	
No secretor, *n* (%)	1	–	0	1	2 (3.0)	7 (4.4)	
IgD [*n* (%)]	1	–	0	0	1 (1.5)	1 (0.6)	
pMGUS							
Yes, *n* (%)	7	–	1	0	8 (15.4)	8 (6.8)	.080
AHPT							
Yes, *n* (%)	2	5	–	–	7 (8.2)	46 (18.2)	**.026**

Abbreviations: pMGUS, prior monoclonal gammopathy undetermined significance; HB, Hospital de Baza; HCO, regional hospitals (grouped data from HVP + HLL + HM + HB); HLL, Hospital de La Línea; HM, Hospital de Motril; HRE, Reference hospital (Hospital Virgen de las Nieves); HVP, Hospital Valle de los Pedroches; IgA, immunoglobulin A; IgD, immunoglobulin D; IgG, immunoglobulin G; *n*, number of patients; *p*, *p*-value (in bold if <.05); AHPT, autologous hematopoietic progenitor transplantation.
[a] Chi-squared.

doxorubicin and vincristine. Access to new agents (initially bortezomib) did not occur at the same time in all centers. We therefore decided to end the study in 2006 to avoid the potential bias of greater access to the new agents in the reference hospitals as compared with the regional hospitals. With regard to AHPT, each eligible case was referred to the corresponding reference hospital for their acceptance. The greater median age of patients treated in the regional hospitals may be one of the causes for which AHPT was performed less often in patients treated in these centers. Nevertheless, we would need to study the comorbidities profile, which generally also tends to increase with age. It is not unusual to find a second primary malignancy when diagnosing MM.[25]

This study has several limitations: (1) the number of participating centers was limited; however, 3 of the 8 Andalusian provinces are represented and include 20% of the reference hospitals (1/5) and 22.2% of the regional hospitals (4/18) of Andalusia; (2) we did not take into account the new international staging, given that its publication coincided with the study's end date; (3) conventional and interphase cytogenetics were not assessed due to lack of patients with such information; (4) we did not analyze the quality and duration of the response, because this was not recorded with uniform criteria; (5) the number of cases with information on a number of variables was low; and (6) we did not assess comorbidity, which is a known cause of the decreased use of chemotherapy.[26]

In the general hemato-oncologic context and in the specific case of MM, overall survival is the most objective measure for comparing the progress in this disease, even though there are other data of interest.[27] At present, especially in patients younger than 65 years, we have witnessed the positive impact of new therapeutic agents and the optimization of AHPT.

In our study, the level of complexity of the hospital that treats patients with MM is not significantly associated with mortality; however, we do observe a trend toward presenting less risk in regional hospitals. Moreover, the study confirmed the prognostic value of age, Durie-Salmon stage and renal failure. Our results confirm that the model for health care that is currently in operation in our community, according to which the regional hospitals play an important role in the diagnosis and treatment of MM, is appropriate. Nevertheless, due to the growing complexity in the management of this disease, the treatment needs to be coordinated from reference centers.

Table 2 Results of the survival analysis conducted on the patients with multiple myeloma enrolled in the MICORE Study (Cox test).

Variable	Univariate analysis				Multivariate analysis			
	Coeff.	HR	95% CI	*p*	Coeff.	HR	95% CI	*p*
Age	0.03	1.03	1.02–1.04	<.001	0.06	1.06	1.03–1.08	**<.001**
Gender								
Women versus men	−0.10	0.89	0.73–1.10	.29	−0.03	0.96	0.65–1.42	.86
Durie-Salmon stage								
I (reference category)								
II	−0.18	0.83	0.48–1.44	.52	0.14	1.15	0.54–2.44	.70
III	0.53	1.71	1.07–2.72	.02	0.76	2.14	1.08–4.26	**.03**
Renal failure	0.72	2.05	1.47–2.87	<.001	0.45	1.58	1.02–2.43	**.04**
Type of myeloma								
IgG (reference category)								
IgA	0.01	1.01	0.72–1.41	0.96	−0.16	0.84	0.53–1.36	.49
Light chains	0.65	1.91	1.27–2.89	0.00	0.22	1.25	0.73–2.14	.41
No secretor	−0.15	0.85	0.39–1.84	0.68	−0.52	0.59	0.20–1.72	.33
Hospital								
HCO versus HRE	0.07	1.07	0.87–1.31	0.49	−0.33	0.72	0.48–1.07	.10

Abbreviations: Coeff., coefficient; HCO, grouped regional hospitals; HR, hazard ratio; HRE, regional reference hospital; 95% CI, 95% confidence interval; IgA, immunoglobulin A; IgG, immunoglobulin G; *p*, *p*-value (in bold if <.05).
Renal failure: serum creatinine $\geq$2 mg/dl.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Resumen artículo 4

Ríos-Tamayo R, Lupiañez CB, Campa D, Martino A, Martínez-López J, Martínez-Bueno M, et al. Type 2 diabetes-related variants influence the risk of developing multiple myeloma: results from the IMMEnSE consortium. Endocr-Relat Cancer 2015;22:545-559.

Este trabajo responde al objetivo 4.

Introducción. Algunos estudios sugieren que la DM2 puede ser considerada un factor de riesgo para el desarrollo del MM. La DM2 es una de las comorbilidades más frecuentes en pacientes con MM y podría contribuir al proceso de mielomagénesis a través de mecanismos dependientes y no dependientes de insulina. Dado que tanto la DM2 como el MM tienen una base genética demostrada y ambas entidades comparten determinadas vías metabólicas y marcadores, es plausible pensar que existan factores de riesgo genéticos para DM2 que podrían estar también asociados con el riesgo a presentar MM.

Métodos. Estudio de casos y controles multicéntrico (1420 casos y 1858 controles) para evaluar si 58 variantes genéticas asociadas a DM2 e identificadas mediante GWAS, contribuyen al riesgo de desarrollar MM. Se usó regresión logística para valorar los efectos de los polimorfismos genéticos en el riesgo de desarrollar MM usando modelos de herencia co-dominante, dominante, recesivo y log-aditivo. Por otra parte, se planteó valorar si un modelo predictivo incluyendo edad, sexo y las variantes genéticas que demostraron una asociación significativa con MM en el análisis univariante, podría mejorar la capacidad discriminatoria para predecir el riesgo de MM en comparación con un modelo que sólo incluye edad y sexo como covariables. El análisis de curvas ROC se utilizó para evaluar la capacidad predictiva de cada modelo y el test LR se empleó para

analizar si ambos modelos eran estadísticamente diferentes. Los resultados del test LR fueron confirmados por análisis de randomización (10.000 modelos randomizados; el efecto de los SNPs sobre el riesgo a desarrollar MM fue neutralizado a través de la randomización de los genotipos).

Resultados. Los portadores de los genotipos $KCNQ1_{rs22378927T}$, $CDKN2A\text{-}2B_{rs2383208G/G}$, $IGF1_{rs35767T/T}$ y $MADD_{rs7944584T/T}$ presentaban un mayor riesgo de desarrollar MM, [OR = 1.32, IC 95% 1.01–1.71, (p = 0.039); OR = 1.86, IC 95% 1.12–3.11, (p = 0.016); OR = 2.13, IC 95% 1.35–3.37, (p = 0.0012); y OR = 1.33, IC 95% 1.06–1.67, (p = 0.014)], respectivamente. Por el contrario, los portadores de los genotipos $KCNJ11_{rs5215C}$, $KCNJ11_{rs5219T}$, $THADA_{rs7578597C}$, $FTO_{rs8050136A/A}$ y $LTA_{rs1041981C/C}$ mostraron un menor riesgo de desarrollar MM, [OR = 0.85, IC 95% 0.73–0.99, (p = 0.038); OR = 0.84, IC 95% 0.72–0.99, (p = 0.034); OR = 0.81, IC 95% 0.68–0.98, (p = 0.032); OR = 0.78, IC 95% 0.64–0.95, (p = 0.013) y OR = 0.76, IC 95% 0.58–0.99, (p = 0.042)], respectivamente. Aunque al corregir por el test de múltiples comparaciones ninguna de estas asociaciones permanecía estadísticamente significativa, la asociación del polimorfismo $IGF1_{rs35767}$ se acercaba a la significación estadística y mostraba que los portadores de esta variante presentaban un mayor riesgo de desarrollar MM (OR = 2.13, 95% CI 1.35–3.37, p = 0.0012).

Por otra parte, la construcción de un modelo predictivo incluyendo edad, sexo y 6 variantes genéticas asociadas ($p < 0.05$) con el riesgo de MM mostraba que dicho modelo presentaba una capacidad predictiva significantivamente mayor que el modelo incluyendo sólo variables demográficas (área bajo la curva 0.645 vs. 0.629, $p = 4.05 \bullet 10^{-6}$). Además, un análisis estratificado por sexo mostró un efecto modificador del sexo para los polimorfismos $ADAM30_{rs2641348}$ y $NOTCH2_{rs10923931}$ ($p_{interacción}$ = 0.001 y 0.0004, respecitvamente). Los hombres portadores del los alelos $ADAM30_{rs2641348C}$ y

NOTCH2$_{rs10923931T}$ tenían un riesgo significativamente menor de desarrollar MM mientras que un efecto opuesto pero no significativo se observaba en las mujeres.

Interpretación. Este estudio sugiere por primera vez que variantes genéticas en los genes *IGF1, KCNJ11, CDKN2A-2B, MADD, THADA, LTA, FTO, ADAM30 y NOTCH2* pueden influir en el riesgo de MM a través de mecanismos insulina-independientes.

Además este estudio sugiere que el genotipado de estas variantes asociadas a DM2 podría ser útil para mejorar la predicción del riesgo de desarrollar MM.

Type 2 diabetes-related variants influence the risk of developing multiple myeloma: results from the IMMEnSE consortium

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Abstract

Type 2 diabetes (T2D) has been suggested to be a risk factor for multiple myeloma (MM), but the relationship between the two traits is still not well understood. The aims of this study were to evaluate whether 58 genome-wide-association-studies (GWAS)-identified common variants for T2D influence the risk of developing MM and to determine whether predictive models built with these variants might help to predict the disease risk. We conducted a case–control study including 1420 MM patients and 1858 controls ascertained through the International Multiple Myeloma (IMMEnSE) consortium. Subjects carrying the *KCNQ1*$_{rs2237892T}$ allele or the *CDKN2A-2B*$_{rs2383208G/G}$, *IGF1*$_{rs35767T/T}$ and *MADD*$_{rs7944584T/T}$ genotypes had a significantly increased risk of MM (odds ratio (OR) = 1.32–2.13) whereas those carrying the *KCNJ11*$_{rs5215C}$, *KCNJ11*$_{rs5219T}$ and *THADA*$_{rs7578597C}$ alleles or the *FTO*$_{rs8050136A/A}$ and *LTA*$_{rs1041981C/C}$ genotypes showed a significantly decreased risk of developing the disease (OR = 0.76–0.85). Interestingly, a prediction model including those T2D-related variants associated with the risk of MM showed a significantly improved discriminatory ability to predict the disease when compared to a model without genetic information (area under the curve (AUC) = 0.645 vs AUC = 0.629; $P = 4.05 \times 10^{-06}$). A gender-stratified analysis also revealed a significant gender effect modification for *ADAM30*$_{rs2641348}$ and *NOTCH2*$_{rs10923931}$ variants ($P_{interaction} = 0.001$ and 0.0004, respectively). Men carrying the *ADAM30*$_{rs2641348C}$ and *NOTCH2*$_{rs10923931T}$ alleles had a significantly decreased risk of MM whereas an opposite but not significant effect was observed in women ($OR_M = 0.71$ and $OR_M = 0.66$ vs $OR_W = 1.22$ and $OR_W = 1.15$, respectively). These results suggest that TD2-related variants may influence the risk of developing MM and their genotyping might help to improve MM risk prediction models.

Key Words

- multiple myeloma
- diabetes
- genetic variants
- susceptibility

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Introduction

Multiple myeloma (MM) is a plasma-cell neoplasm of complex aetiology that may arise as a result of the interaction between adverse environmental and inherited genetic risk factors (Morgan *et al.* 2012). Although survival rates for MM have improved dramatically during the last two decades, likely due to the introduction of novel targeted therapies (proteasome inhibitors, immunomodulators and others), the disease outcome still remains poor with a 5-year overall survival rate not higher than 55% (Kumar *et al.* 2014).

Age, male gender, African ancestry and monoclonal gammopathy of uncertain significance (MGUS) have been established as major risk factors for MM (Alexander *et al.* 2007). In addition, exposure to a wide range of toxins as well as type 2 diabetes (T2D) and obesity have been suggested as important mediators of the complex process of myelomagenesis (Alexander *et al.* 2007, Lope *et al.* 2008, Wallin & Larsson 2011). Among these latter preventable factors, T2D has attracted significant attention since it has been consistently identified as a medical condition frequently found in MM patients (Khan *et al.* 2008, Richardson *et al.* 2009, Castillo *et al.* 2012) and it is thought to influence the myelomagenesis through hyperglycaemia and insulin-dependent and -independent mechanisms (Xu *et al.* 2014). In a recent well-powered meta-analysis, Castillo *et al.* (2012) observed that T2D was significantly associated with an increased risk of developing the disease (Castillo *et al.* 2012). This finding concurs with those previously reported in several epidemiological studies that showed a high incidence of T2D among MM patients ranging between 11 and 22% (Richardson *et al.* 2006, Badros *et al.* 2007). In addition, it has been reported that T2D may have a negative impact on MM prognosis (Chiu *et al.* 2006, Wu *et al.* 2014) and that the treatment with anti-diabetic drugs may effectively kill MM cells (Wu *et al.* 2014).

Considering that T2D and MM have strong genetic components and share several biological pathways and markers (Xu *et al.* 2014) and that T2D-related polymorphisms may influence the risk of developing solid cancer (Folsom *et al.* 2008, Cheng *et al.* 2011, Sainz *et al.* 2012, Ma *et al.* 2014), we hypothesized that genetic risk factors for T2D may be associated with the risk of developing MM. So far there have not been studies evaluating the impact of diabetogenic variants on the risk of developing hematological cancers. Therefore, we decided to conduct a

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multi-centre case–control study including 1420 MM patients and 1858 controls to evaluate whether 58 variants convincingly shown to be associated with T2D contribute to the risk of developing MM. We also aimed at determining whether predictive models including T2D-related variants significantly improve the discriminatory ability to predict the risk of MM.

Material and methods

Study population

The study population consisted of 1420 MM patients (705 women and 715 men) and 1858 controls (916 women and 942 men) ascertained through the International Multiple Myeloma (IMMEnSE) consortium (Supplementary Table 1, see section on supplementary data given at the end of this article), which has been described in detail elsewhere (Martino *et al.* 2012). The diagnosis of MM was assigned by physician and fulfilled the International Myeloma Working Group (IMWG) criteria (International Myeloma Working Group 2003). Controls were blood donors or hospitalized subjects with a diagnosis not related to cancer who were recruited in the same geographical area of the cases (Supplementary Table 1). Additional information concerning to the recruitment strategy of controls is shown in the Supplementary Material. The investigation was approved by the ethical committee of each participant institution, functioning according to the third edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London (www.rcplondon.ac.uk) and all participants gave their written informed consent to participate in the study.

SNP selection and genotyping

Fifty-eight genome-wide-association-studies (GWAS)-identified variants for T2D were selected to be genotyped in the IMMEnSE consortium population (Table 1 and Supplementary Material). The genotyping of the selected polymorphisms was carried out at GENYO (Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain) using KASPar assays (LGC Genomics, Hoddesdon, UK) according to manufacturer's instructions. For internal quality control, 5% of samples were randomly selected and included as duplicates. Concordance between the original and the duplicate samples for the 58 SNPs was $\geq$99.0%. Call rates for all SNPs were $\geq$90.0% with the exception of the *WFS1*$_{rs734312}$ SNP that was excluded from further analyses.

Statistical analysis

The Hardy–Weinberg Equilibrium (HWE) tests were performed in the control group by a standard observed-expected χ^2 test. Logistic regression analyses were used to assess the effects of the genetic polymorphisms on MM risk using co-dominant, dominant, recessive and log-additive inheritance models. Overall analyses were adjusted for age at diagnosis, gender and country of origin. All analyses were conducted using the statistical software SSPS (version 20.0). Statistical power was calculated using the Quanto vs12.4 (http://biostats.usc.edu/software) assuming a log-additive model.

In order to account for multiple testing, we calculated an adjusted significance level using the M_{eff} method (Nyholt 2004), which considers the number of independent marker loci (M_{effLi}=55) but also the number of models of inheritance tested (co-dominant, dominant, recessive and log-additive). Thus, the resulting threshold for the main effect analysis was 0.00022 ((0.05/55)/4) (Supplementary Material). Since a study-wide significance threshold considering all these factors is generally perceived as a 'too conservative' test, we also assessed the magnitude of observed associations between selected SNPs and risk of MM through a quantile–quantile (QQ) plot generated from the results of the IMMEnSE population. The observed association *P* values were ranked in order from smallest to largest on the *y*-axis and plotted against the expected results from a theoretical $\sim\chi^2$-distribution under the null hypothesis of no association on the *x*-axis. A deviation from the identity line would confirm that the number of corresponding associations is more than expected under the null hypothesis and therefore that these associations are likely to be true associations.

Predictive models and discriminative accuracy

We also examined the value of T2D-related polymorphisms for prediction of MM using stepwise logistic and Cox regression analyses. We built a prediction model including age and sex and those genetic variants that showed significant associations with MM in the single-SNP analysis ($P<0.05$). Then, using *P* values as a selection criterion, we dropped variables that have the highest *P* value and we stopped when all variables were significant defined by $P<0.10$. The area under the curve (AUC) of a receiver operating characteristic (ROC) curve analysis was used to assess the discriminative accuracy of this particular model compared with a reference model including age and sex as covariates. A −2log likelihood ratio (LR) test

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Table 1 Selected type-2 diabetes-related SNPs

Gene name	dbSNP rs#	Nucleotide substitution	Reference allele IMMEnSE	GWAS-identified risk allele for T2D	Location/Aa substitution	References
ADAM30	rs2641348	T/**C**[a]	T	C	L359P	Zeggini *et al*. (2008) and Lyssenko *et al*. (2009)
ADAMTS9	rs4607103	T/C	C	C	Near gene	Mohlke *et al*. (2008), Zeggini *et al*. (2008) and Shu *et al*. (2010)
ADCY5	rs11708067	T/C	T	T	Intronic	Dupuis *et al*. (2010) and Saxena *et al*. (2010)
ADRA2A	rs10885122	G/T	G	G	Near ADRA2A	Dupuis *et al*. (2010)
ARAPI, *CENTD2*	rs1552224	G/T	T	A	Near gene	Voight *et al*. (2010) and Nielsen *et al*. (2011)
BCL11A	rs10490072	C/T	T	T	Near gene	Zeggini *et al*. (2008)
CDC123	rs12779790	A/G	A	G	Near gene	Mohlke *et al*. (2008), Zeggini *et al*. (2008) and Shu *et al*. (2010)
CDKAL1	rs7754840	C/G	G	C	Intronic	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Florez *et al*. (2007) and Scott *et al*. (2007)
CDKN2A-2B	rs564398	T/C	T	T	Near gene	Diabetes Genetics Initiative of Broad
CDKN2A-2B	rs10811661	T/C	T	T	Near gene	Institute of Harvard and MIT, Lund
CDKN2A-2B	rs2383208	A/**G**[b]	A	A	Near gene	University, and Novartis Institutes of BioMedical Research *et al*. (2007), Scott *et al*. (2007), Mohlke *et al*. (2008), Zeggini *et al*. (2008), Takeuchi *et al*. (2009), Shu *et al*. (2010) and Yamauchi *et al*. (2010)
COL5A1	rs4240702	C/T	C	NS	Intronic	Bouatia-Naji *et al*. (2009)
CRY2	rs11605924	A/C	C	A	Intronic	Dupuis *et al*. (2010)
DCD	rs1153188	A/T	A	A	Near gene	Zeggini *et al*. (2008)
EXT2	rs1113132	C/G	C	C	Intronic	Florez *et al*. (2007) and Sladek *et al*. (2007)
FADS1	rs174550	C/T	A	T	Intronic	Dupuis *et al*. (2010)
FAM148B	rs11071657	A/G	A	A	Near gene	Chambers *et al*. (2008) and Dupuis *et al*. (2010)
FLJ39370	rs17044137	A/T	T	A	Near gene	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007)
FTO	rs8050136	**A**/C[c]	C	A	Intronic	Wellcome Trust Case Control (2007), Zeggini *et al*. (2007) and Mohlke *et al*. (2008)
G6PC2	rs560887	G/A	G	G	Intronic	Bouatia-Naji *et al*. (2008, 2009), Chen *et al*. (2008), Prokopenko *et al*. (2009) and Dupuis *et al*. (2010)
GCK	rs1799884	G/A	G	A	Near gene	Bouatia-Naji *et al*. (2008, 2009), Chen *et al*. (2008), Prokopenko *et al*. (2009) and Dupuis *et al*. (2010)
GCKR	rs1260326	C/T	C	T	L445P	Bouatia-Naji *et al*. (2009), Dupuis *et al*. (2010) and Saxena *et al*. (2010)
HHEX	rs1111875	G/A	C	C	Near gene	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Florez *et al*. (2007), Scott *et al*. (2007), Sladek *et al*. (2007), Wellcome Trust Case Control (2007), Zeggini *et al*. (2007) and Mohlke *et al*. (2008)
HMGA2	rs1531343	C/G	G	C	Near gene	Voight *et al*. (2010) and Nielsen *et al*. (2011)

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Table 1 *Continued*

Gene name	dbSNP rs#	Nucleotide substitution	Reference allele IMMEnSE	GWAS-identified risk allele for T2D	Location/Aa substitution	References
HNF1A, TCF1	rs7957197	A/T	T	T	Intronic	Voight *et al*. (2010) and Nielsen *et al*. (2011)
IGF1	rs35767	C/**T**[d]	C	C	Near gene	Pechlivanis *et al*. (2007) and Dupuis *et al*. (2010)
IGF2BP2	rs4402960	G/T	C	T	Intronic	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Florez *et al*. (2007), Scott *et al*. (2007), Wellcome Trust Case Control (2007), Zeggini *et al*. (2007), Mohlke *et al*. (2008) and Shu *et al*. (2010)
IL13	rs20541	C/T	C	T	R144Q	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007)
IRS1	rs2943641	C/T	C	C	Near gene	Rung *et al*. (2009), Voight *et al*. (2010) and Tang *et al*. (2013)
JAZF1	rs864745	A/G	A	T	Intronic	Zeggini *et al*. (2008) and Shu *et al*. (2010)
KCNJ11	rs5215	T/**C**[e]	T	C	V337I	Gloyn *et al*. (2003), Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Scott *et al*. (2007), Wellcome Trust Case Control (2007), Zeggini *et al*. (2007), Willer *et al*. (2007) and Mohlke *et al*. (2008)
KCNJ11	rs5219	C/**T**[f]	C	T	K23E	
KCNQ1	rs2237897	C/T	T	C	Intronic	Unoki *et al*. (2008), Yasuda *et al*. (2008), Tsai *et al*. (2010) and Yamauchi *et al*. (2010)
KCNQ1	rs2074196	G/T	G	G	Intronic	
KCNQ1	rs2237892	C/**T**[g]	C	C	Intronic	
KCNQ1	rs2237895	A/C	A	C	Intronic	
KCNQ1OT1	rs231362	C/T	C	G	Intronic	Tsai *et al*. (2010), Voight *et al*. (2010) and Nielsen *et al*. (2011)
LTA	rs1041981	A/**C**[e]	A	A	T60N	Hamid *et al*. (2005)
MADD	rs7944584	A/**T**[d]	A	A	Intronic	Dupuis *et al*. (2010)
MCR4	rs12970134	A/G	G	A	Near gene	Chambers *et al*. (2008)
MTNR1B	rs1387153	C/T	C	T	Near gene	Bouatia-Naji *et al*. (2009), Prokopenko *et al*. (2009) and Voight *et al*. (2010)
NOTCH2	rs10923931	G/**T**[h]	G	T	Intronic	Mohlke *et al*. (2008) and Zeggini *et al*. (2008)
PKN2	rs6698181	C/T	C	T	Intergenic	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007)
PPARG	rs1801282	C/G	C	C	P12A	Altshuler *et al*. (2000), Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Scott *et al*. (2007), Wellcome Trust Case Control (2007), Willer *et al*. (2007), Zeggini *et al*. (2007, 2008) and Mohlke *et al*. (2008)
PRC1	rs8042680	A/C	C	A	Intronic	Voight *et al*. (2010) and Nielsen *et al*. (2011)
PROX1	rs340874	A/G	A	G	Promoter	Dupuis *et al*. (2010)
RBMS1	rs7593730	C/T	C	T	Intronic	Qi *et al*. (2010)
SLC2A2	rs11920090	A/T	A	T	Intronic	Dupuis *et al*. (2010)

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Table 1 *Continued*

Gene name	dbSNP rs#	Nucleotide substitution	Reference allele IMMEnSE	GWAS-identified risk allele for T2D	Location/Aa substitution	References
SLC30A8	rs13266634	C/T	C	C	R325W	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Florez *et al*. (2007), Scott *et al*. (2007), Sladek *et al*. (2007), Steinthorsdottir *et al*. (2007), Wellcome Trust Case Control (2007), Zeggini *et al*. (2007), Mohlke *et al*. (2008), Dupuis *et al*. (2010) and Shu *et al*. (2010)
TCF2	rs7501939	C/T	C	C	Intronic	Gudmundsson *et al*. (2007) and Sandhu *et al*. (2007)
TCF7L2	rs7903146	C/T	C	T	Intronic	Grant *et al*. (2006), Scott *et al*. (2006),
TCF7L2	rs12255372	G/T	G	T	Intronic	Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research *et al*. (2007), Florez *et al*. (2007), Scott *et al*. (2007), Sladek *et al*. (2007), Steinthorsdottir *et al*. (2007), Wellcome Trust Case Control (2007), Zeggini *et al*. (2007), Mohlke *et al*. (2008), Dupuis *et al*. (2010) and Saxena *et al*. (2010)
THADA	rs7578597	T/**C**[e]	T	T	T1187A	Zeggini *et al*. (2008)
TP53INP1	rs896854	A/G	G	G	Intronic	Voight *et al*. (2010) and Nielsen *et al*. (2011)
TSPAN8	rs7961581	C/T	T	C	Near gene	Grarup *et al*. (2008)
VEGFA	rs9472138	C/T	C	T	Near gene	Zeggini *et al*. (2008)
WFS1	rs734312	A/G	A	NS	H611R	Sandhu *et al*. (2007)
WFS1	rs10010131	A/G	G	G	Intronic	Sandhu *et al*. (2007)

NS, not specified; Aa, aminoacid; GWAS, genome-wide association studies. Effect allele in bold and underlined.
[a]C allele was associated with a decreased risk of MM in men whereas an opposite effect was detected in women.
[b]G allele was associated with an increased risk of developing MM.
[c]A/A genotype was associated with a decreased risk of MM (recessive model).
[d]T/T genotype was associated with a decreased risk of MM (recessive model).
[e]C allele was associated with a decreased risk of MM.
[f]T allele was associated with an decreased risk of MM.
[g]T allele was associated with an increased risk of MM.
[h]T allele was associated with a decreased risk of MM in men whereas an opposite effect was detected in women.

was used to determine whether the predictive model including genetic information fitted significantly better the data compared to the reference model. Although the addition of genetic variables to the reference model will almost always make the model fit better, the LR test allowed us to confirm whether the difference in model fit between both models was statistically significant. Besides this suggestive analysis, we also ran a randomization test to confirm whether the improved predictive ability of the model including genetic variants significantly associated with MM was consistent after 10 000 iterations. We compared our full predictive model including significant SNPs, age and gender ('original' model) with 10 000 'randomized' models in which the effect of SNPs on MM risk was neutralized by reassigning randomly all genotypes (null distribution; Supplementary Material). Subsequently, we calculated an empirical $P_{\text{iterations}}$-value by dividing the number of times in which the 'randomized' AUC value was equal or greater than the 'original' AUC value by the number of iterations. Then, we could also calculate the Z score and $P_{Z\ \text{score}}$-value for the original AUC using the 'randomized' AUC average of these 10 000 iterations and their S.D. All analyses were performed using R software (http://www.r-project.org/).

Gender-specific association analysis

We also evaluate gender-specific associations of selected SNPs with MM risk. Logistic regression analyses were corrected for age and country of origin. Of note, to

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evaluate whether a different gender distribution across populations within the IMMEnSE consortium could be responsible for the gender effect modification observed for certain SNPs, we also assessed heterogeneity and index I^2 statistic using Cochran's χ^2 based Q statistic test (Lau *et al*. 1997). Heterogeneity was considered significant when $P<0.1$.

Results

Overall associations of selected SNPs with MM risk

All SNPs were in HWE ($P>0.001$) with the exception of the *COL5A1*$_{rs4240702}$, which was therefore excluded from the statistical analyses. Logistic regression analysis showed that carriers of the *KCNQ1*$_{rs2237892T}$ allele or the *CDKN2A-2B*$_{rs2383208G/G}$, *IGF1*$_{rs35767T/T}$ and *MADD*$_{rs7944584T/T}$ genotypes had an increased risk of MM (odds ratio (OR)=1.32, 95% CI 1.01–1.71, $P=0.039$; OR=1.86, 95% CI 1.12–3.11, $P=0.016$; OR=2.13, 95% CI 1.35–3.37, $P=0.0012$ and OR=1.33, 95% CI 1.06–1.67, $P=0.014$, respectively) whereas those harbouring the *KCNJ11*$_{rs5215C}$, *KCNJ11*$_{rs5219T}$ and *THADA*$_{rs7578597C}$ alleles or the *FTO*$_{rs8050136A/A}$ and *LTA*$_{rs1041981C/C}$ genotypes showed a decreased risk for the disease (OR=0.85, 95% CI 0.73–0.99, $P=0.038$; OR=0.84, 95% CI 0.72–0.99, $P=0.034$; OR=0.81, 95% CI 0.68–0.98, $P=0.032$; OR=0.78, 95% CI 0.64–0.95, $P=0.013$ and OR=0.76, 95% CI 0.58–0.99, $P=0.042$, respectively; Table 2). When we corrected for multiple testing (with a threshold of $P=0.00022$), none of the reported associations remained statistically significant. The strongest association observed was for the *IGF1*$_{rs35767}$ SNP with an increased risk of developing MM (OR=2.13, 95% CI 1.35–3.37, $P=0.0012$). In spite of these results, the QQ plot showed an early deviation of identity line, which suggested a high proportion of true associations for a given *P* value (Fig. 1). Therefore, the data suggest that the effect attributed to SNPs in T2D-related loci (*FTO*, *MADD*, *CDKN2A-2B*, *LTA*) might represent true associations.

Predictive value of T2D-related variants

In order to determine whether there was a joint effect of SNPs significantly associated with MM, we built a prediction model including gender and those nine SNPs showing overall significant associations with MM. After excluding the variables that did not remain significant in the model, the final model included six SNPs that increased the discriminatory ability to predict the risk of MM when compared with a reference model including age and gender as covariates (AUC=0.645 95% CI 0.624–0.666; Table 3). The LR test showed that the model including genetic variants fitted better the data than the reference model, and that the difference in model fit between both models was statistically significant ($P=4.05\times10^{-06}$). In addition, when we evaluated whether the model including genetic variants was consistent in predicting better the MM risk, we found that it showed an AUC value systematically higher than those of the 10 000 randomized models (null distribution; *Z* score=6.42, $P=6.81\times10^{-11}$; Supplementary Material), emphasizing the importance of considering genetic variants significantly associated with MM when building predictive models.

Gender-specific associations with MM risk

Interestingly, a gender-stratified analysis also revealed significant gender effect modifications for *ADAM30*$_{rs2641348}$ and *NOTCH2*$_{rs10923931}$ SNPs ($P_{interaction}=0.001$ and 0.0004 respectively). For *ADAM30*$_{rs2641348C}$ and *NOTCH2*$_{rs10923931T}$ alleles, a significantly reduced risk for the disease was observed in men (per-allele OR=0.71, 95% CI 0.54–0.94, $P=0.015$ and per-allele OR=0.66, 95% CI 0.50–0.86, $P=0.0019$, respectively) whereas a non-significant opposite effect was seen in women (per-allele OR=1.22, 95% CI 0.93–1.60 and per-allele OR=1.15, 95% CI 0.89–1.50 respectively). A statistically significant heterogeneity, considering $P<0.05$ as a threshold, was also confirmed for these two SNPs ($P_{HET}=0.0039$ and $I^2=87.99\%$ and $P_{HET}=0.0024$ and $I^2=89.12\%$, respectively), which supports the notion suggesting a role of gender in modulating the effect of these SNPs on MM risk. Although there was not a significant interaction with gender, we observed additional gender-specific associations for *WFS1*$_{rs10010131}$, *THADA*$_{rs7578597}$, *EXT2*$_{rs1113132}$ and *GCK*$_{rs1799884}$ SNPs according to dominant or recessive models of inheritance (Table 2 and Supplementary Material).

When we took account of multiple testing (with a threshold of $P=0.00022$), we found that the effect of the *IGF1*$_{rs35767}$ variant was stronger in women than men with an association approaching significance with an increased risk of developing MM (OR=3.13, 95% CI 1.46–6.71, $P=0.0026$ vs OR=1.69, 95% CI 0.94–3.02, $P=0.079$ respectively). In addition, we found that the association of *NOTCH2*$_{rs10923931}$ SNP with a decreased risk of MM in men was close to significance according to dominant and log-additive models (OR=0.66, 95% CI 0.50–0.86,

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Table 2 Association of T2D-related variants and risk of developing MM

Variant_dbSNP	Gene	Overall ($n=3278$)		Men ($n=1657$)		Women ($n=1621$)		
		OR (95% CI)[a]	*P* value	OR (95% CI)[b]	*P* value	OR (95% CI)[b]	*P* value	$P_{interaction}$
rs2641348[cd]	*ADAM30*	0.94 (0.78–1.14)	0.53	**0.71 (0.54–0.94)**	**0.015**	1.22 (0.93–1.60)	0.15	**0.001**
rs4607103	*ADAMTS9*	1.00 (0.86–1.16)	1.00	0.97 (0.79–1.20)	0.80	1.05 (0.84–1.30)	0.69	0.803
rs11708067	*ADCY5*	1.02 (0.88–1.20)	0.77	1.05 (0.85–1.31)	0.64	0.97 (0.77–1.22)	0.79	0.425
rs10885122	*ADRA2A*	1.08 (0.91–1.28)	0.40	1.16 (0.92–1.47)	0.21	0.99 (0.77–1.28)	0.96	0.494
rs1552224[e]	*ARAPI, CENTD2*	1.16 (0.72–1.88)	0.54	1.78 (0.96–3.29)	0.066	0.58 (0.25–1.37)	0.20	0.090
rs10490072	*BCL11A*	1.01 (0.87–1.17)	0.92	1.01 (0.82–1.25)	0.91	1.01 (0.81–1.25)	0.94	0.691
rs12779790	*CDC123, CAMK1D*	0.87 (0.74–1.02)	0.075	0.82 (0.66–1.03)	0.083	0.90 (0.71–1.13)	0.35	0.758
rs7754840	*CDKAL1*	0.98 (0.85–1.14)	0.84	1.05 (0.86–1.29)	0.63	0.91 (0.74–1.13)	0.40	0.376
rs564398	*CDKN2A-2B*	0.94 (0.80–1.10)	0.42	0.90 (0.72–1.11)	0.33	0.97 (0.78–1.22)	0.80	0.893
rs10811661	*CDKN2A-2B*	1.02 (0.87–1.20)	0.79	1.11 (0.89–1.38)	0.35	0.93 (0.74–1.17)	0.52	0.358
rs2383208[e]	*CDKN2A-2B*	**1.86 (1.12–3.11)**	**0.016**	**1.92 (1.03–3.58)**	**0.039**	1.68 (0.69–4.10)	0.25	0.585
rs11605924	*CRY2*	0.93 (0.79–1.10)	0.40	0.95 (0.75–1.19)	0.64	0.92 (0.72–1.17)	0.49	0.747
rs1153188	*DCD*	0.91 (0.79–1.06)	0.24	0.83 (0.67–1.02)	0.082	1.05 (0.84–1.30)	0.69	0.072
rs1113132[e]	*EXT2*	0.92 (0.68–1.24)	0.57	1.24 (0.83–1.87)	0.30	0.64 (0.41–1.00)	**0.046**	0.067
rs174550	*FADS1*	1.11 (0.95–1.28)	0.18	1.13 (0.92–1.38)	0.25	1.08 (0.87–1.34)	0.48	0.359
rs11071657	*FAM148B*	1.03 (0.88–1.20)	0.73	0.95 (0.77–1.17)	0.62	1.16 (0.93–1.46)	0.18	0.275
rs17044137	*FLJ39370*	0.91 (0.78–1.05)	0.19	1.05 (0.85–1.29)	0.65	0.89 (0.72–1.11)	0.30	0.357
rs8050136[e]	*FTO*	**0.78 (0.64–0.95)**	**0.013**	**0.70 (0.53–0.93)**	**0.013**	0.88 (0.66–1.17)	0.37	0.420
rs560887[e]	*G6PC2*	1.16 (0.88–1.52)	0.30	0.98 (0.67–1.44)	0.93	1.46 (0.98–2.18)	0.065	0.386
rs1799884[c]	*GCK*	1.10 (0.93–1.30)	0.29	**1.27 (1.01–1.61)**	**0.044**	0.92 (0.72–1.18)	0.51	0.254
rs1260326	*GCKR*	0.92 (0.78–1.08)	0.32	0.93 (0.74–1.17)	0.53	0.90 (0.71–1.13)	0.36	0.926
rs1111875	*HHEX*	1.14 (0.98–1.33)	0.093	1.09 (0.88–1.36)	0.41	1.21 (0.97–1.52)	0.095	0.452
rs35767[e]	*IGF1*	**2.13 (1.35–3.37)**	**0.0012**	1.69 (0.94–3.02)	0.079	**3.13 (1.46–6.71)**	**0.0026**	0.538
rs4402960	*IGF2BP2*	1.05 (0.91–1.22)	0.52	0.95 (0.77–1.16)	0.60	1.16 (0.93–1.44)	0.18	0.304
rs20541	*IL13*	1.01 (0.86–1.18)	0.91	1.14 (0.92–1.42)	0.22	0.88 (0.70–1.10)	0.26	0.147
rs2943641	*IRS1*	1.08 (0.93–1.26)	0.31	1.16 (0.94–1.43)	0.17	1.00 (0.80–1.24)	0.99	0.492
rs864745[e]	*JAZF1*	0.88 (0.74–1.04)	0.14	0.98 (0.77–1.25)	0.85	0.79 (0.61–1.01)	0.060	0.225
rs5215	*KCNJ11*	**0.85 (0.73–0.99)**	**0.038**	0.89 (0.72–1.10)	0.28	0.82 (0.66–1.02)	0.074	0.795
rs5219	*KCNJ11*	**0.84 (0.72–0.99)**	**0.034**	0.92 (0.74–1.14)	0.43	**0.78 (0.62–0.98)**	**0.033**	0.587
rs2237897[c]	*KCNQ1*	1.25 (0.97–1.61)	0.081	1.08 (0.74–1.56)	0.69	1.42 (1.00–2.01)	0.052	0.580
rs2074196	*KCNQ1*	1.01 (0.75–1.37)	0.93	0.80 (0.50–1.27)	0.33	1.21 (0.81–1.81)	0.35	0.456
rs2237892	*KCNQ1*	**1.32 (1.01–1.71)**	**0.039**	1.15 (0.78–1.69)	0.48	**1.47 (1.03–2.10)**	**0.036**	0.741
rs2237895	*KCNQ1*	0.91 (0.77–1.08)	0.28	0.94 (0.75–1.18)	0.59	0.91 (0.71–1.16)	0.44	0.878
rs231362	*KCNQ1OT1*	1.03 (0.87–1.22)	0.71	1.07 (0.85–1.34)	0.59	1.01 (0.80–1.29)	0.92	0.873
rs1041981[e]	*LTA*	**0.76 (0.58–0.99)**	**0.042**	0.85 (0.59–1.21)	0.36	0.68 (0.45–1.01)	**0.050**	0.453
rs7944584[e]	*MADD*	**1.33 (1.06–1.67)**	**0.014**	**1.47 (1.08–2.00)**	**0.015**	1.16 (0.83–1.62)	0.39	0.245
rs12970134	*MCR4*	0.96 (0.82–1.11)	0.58	1.01 (0.82–1.25)	0.89	0.92 (0.72–1.18)	0.51	0.643
rs1387153	*MTNR1B*	1.03 (0.89–1.19)	0.73	1.01 (0.82–1.24)	0.91	1.05 (0.85–1.30)	0.67	0.761
rs10923931[c]	*NOTCH2*	0.88 (0.73–1.06)	0.16	**0.66 (0.50–0.86)**	**0.0019**	1.15 (0.89–1.50)	0.29	**0.0004**
rs7957197[e]	*HNF1A, OASL*	1.33 (0.93–1.92)	0.12	1.60 (0.98–2.60)	0.059	1.04 (0.60–1.81)	0.88	0.388
rs6698181	*PKN2*	1.00 (0.85–1.17)	0.99	1.10 (0.88–1.36)	0.41	0.90 (0.72–1.12)	0.34	0.450
rs1801282	*PPARG*	1.06 (0.89–1.26)	0.52	1.02 (0.80–1.30)	0.88	1.11 (0.86–1.43)	0.43	0.655
rs8042680[e]	*PRC1*	1.24 (0.99–1.55)	0.056	1.21 (0.89–1.64)	0.24	1.37 (0.99–1.89)	0.055	0.777
rs340874	*PROX1*	0.95 (0.80–1.13)	0.55	0.90 (0.71–1.14)	0.37	1.02 (0.79–1.30)	0.89	0.387
rs7593730	*RBMS1*	1.10 (0.95–1.29)	0.20	1.18 (0.95–1.46)	0.13	1.06 (0.85–1.33)	0.58	0.852
rs1531343	*RPSAP52, HMGA2*	0.96 (0.81–1.15)	0.69	1.07 (0.84–1.37)	0.57	0.86 (0.66–1.11)	0.24	0.288
rs11920090	*SLC2A2*	1.02 (0.86–1.20)	0.84	1.17 (0.93–1.47)	0.18	0.88 (0.68–1.12)	0.29	0.257

Table 2 *Continued*

Variant_dbSNP	Gene	Overall ($n=3278$)		Men ($n=1657$)		Women ($n=1621$)		$P_{interaction}$
		OR (95% CI)[a]	*P* value	OR (95% CI)[b]	*P* value	OR (95% CI)[b]	*P* value	
rs13266634	*SLC30A8*	0.91 (0.78–1.05)	0.19	0.95 (0.78–1.17)	0.64	0.86 (0.69–1.07)	0.17	0.727
rs7501939	*TCF2*	1.06 (0.91–1.24)	0.43	1.06 (0.85–1.33)	0.58	1.11 (0.89–1.38)	0.37	0.440
rs7903146	*TCF7L2*	0.99 (0.85–1.15)	0.90	1.10 (0.89–1.36)	0.37	0.88 (0.71–1.10)	0.26	0.487
rs12255372	*TCF7L2*	0.94 (0.81–1.09)	0.43	1.06 (0.86–1.30)	0.60	0.83 (0.67–1.03)	0.088	0.452
rs7578597[c]	*THADA*	**0.81 (0.68–0.98)**	**0.032**	0.91 (0.70–1.18)	0.47	**0.73 (0.56–0.96)**	**0.025**	0.125
rs896854[c]	*TP53INP1*	1.17 (0.99–1.39)	0.072	1.22 (0.96–1.55)	0.01	1.13 (0.88–1.45)	0.33	0.269
rs7961581	*TSPAN8, LGR5*	1.03 (0.89–1.19)	0.71	1.03 (0.84–1.27)	0.78	1.04 (0.84–1.29)	0.71	0.973
rs9472138	*VEGFA*	1.13 (0.97–1.31)	0.11	1.19 (0.97–1.46)	0.10	1.09 (0.88–1.35)	0.43	0.737
rs734312	*WFS1*	0.98 (0.83–1.16)	0.84	1.05 (0.83–1.33)	0.67	0.90 (0.71–1.15)	0.39	0.593
rs10010131	*WFS1*	0.94 (0.80–1.10)	0.42	1.11 (0.89–1.38)	0.34	**0.77 (0.62–0.96)**	**0.022**	0.056

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NS, not specified. Estimates were adjusted for age, sex, country of origin. $P<0.05$ in bold.
[a]Estimates calculated according to a dominant model of inheritance and adjusted for age, gender and region.
[b]Estimates calculated according to a dominant model of inheritance and adjusted for age and region.
[c]*ADAM30*$_{rs2641348}$ (per-allele $OR_{MEN}=0.71$, 95% CI 0.55–0.92; $P_{trend}=0.0072$ vs per-allele $OR_{WOMEN}=1.29$, 95% CI 1.00–1.65; $P_{trend}=0.046$). *GCK*$_{rs1799884}$ (per-allele $OR_{MEN}=1.24$, 95% CI 1.00–1.54; $P_{trend}=0.050$ vs per-allele $OR_{WOMEN}=0.94$, 95% CI 0.75–1.18; $P_{trend}=0.58$). *KCNQ1*$_{rs2237897}$ (per-allele $OR_{MEN}=1.09$, 95% CI 0.77–1.54; $P_{trend}=0.63$ vs per-allele $OR_{WOMEN}=1.41$, 95% CI 1.02–1.97; $P_{trend}=0.041$). *KCNQ1*$_{rs2237892}$ (per-allele $OR_{MEN}=1.13$, 95% CI 0.77–1.66; $P_{trend}=0.52$ vs per-allele $OR_{WOMEN}=1.47$, 95% CI 1.04–2.08; $P_{trend}=0.030$). *NOTCH2*$_{rs10923931}$ (per-allele $OR_{MEN}=0.66$, 95% CI 0.52–0.84; $P_{trend}=0.0007$ vs per-allele $OR_{WOMEN}=1.22$, 95% CI 0.96–1.56; $P_{trend}=0.010$). *THADA*$_{rs7578597}$ (per-allele $OR_{MEN}=0.96$, 95% CI 0.75–1.22; $P_{trend}=0.73$ vs per-allele $OR_{WOMEN}=0.75$, 95% CI 0.58–0.96; $P_{trend}=0.021$). *TP53INP1*$_{rs896854}$ (per-allele $OR_{MEN}=1.17$, 95% CI 1.01–1.35; $P_{trend}=0.04$ vs per-allele $OR_{WOMEN}=1.03$, 95% CI 0.89–1.20; $P_{trend}=0.68$).
[d]*ADAM30*$_{rs2641348}$ ($OR_{RECESSIVE\text{-}MEN}=0.36$, 95% CI 0.12–1.13; $P=0.060$ vs $OR_{RECESSIVE\text{-}WOMEN}=4.40$, 95% CI 1.44–13.40; $P=0.0059$).
[e]Estimates calculated according to a recessive model of inheritance.

$P=0.0019$ and $P_{trend}=0.0007$), which may suggest a gender-specific allele-dosage effect for this variant to modulate the disease risk ($P_{interaction}=0.0004$; Table 2). According to a log-additive model, we also found that the association of the *ADAM30*$_{rs2641348C}$ allele with a decreased risk of MM in men showed a slight trend to be significant considering multiple testing (OR=0.71, 95% CI 0.55–0.92, $P=0.0072$) whereas, according to a recessive model, the association of the *ADAM30*$_{rs2641348C/C}$ genotype was also close to survive multiple testing correction (OR=4.40, 95% CI 1.44–13.40, $P=0.00059$; Table 2 and Supplementary Table 2, see section on supplementary data given at the end of this article).

Discussion

In the present study, we report for the first time evidence of significant associations between GWAS-identified T2D genetic variants and MM risk. We found that carriers of the *KCNQ1*$_{rs2237892T}$ allele, *CDKN2A-2B*$_{rs2383208G/G}$, *IGF1*$_{rs35767T/T}$ and *MADD*$_{rs7944584T/T}$ genotypes were at increased risk of MM, whereas those carrying the *KCNJ11*$_{rs5215C}$, *KCNJ11*$_{rs5219T}$ and *THADA*$_{rs7578597C}$ alleles or the *FTO*$_{rs8050136A/A}$ and *LTA*$_{rs1041981C/C}$ genotypes showed a decreased risk for the disease. The associations for the *KCNQ1*, *CDKN2A-2B*, *IGF1*, *MADD*, *KCNJ11*, and *THADA* gene variants with the risk of MM showed an opposite direction to those previously reported in the GWAS for T2D (i.e., the risk allele was the opposite for MM and T2DM), which points towards a non-diabetogenic mechanism underlying the effect of these variants to modulate the risk of the disease. In support of this hypothesis, several studies have suggested that, besides their influence on pancreatic function and insulin secretion through a wide variety of biological mechanisms, some of these genes may also act as tumour suppressor genes (Koh *et al.* 1995, Kim & Sharpless 2006, Than *et al.* 2013) and have an impact in the modulation of cell survival (Butt *et al.* 1999, Ortega *et al.* 2002, Sharifi *et al.* 2013), differentiation (Pancewicz *et al.* 2010), proliferation (Grimberg 2003, Pancewicz *et al.* 2010) and apoptosis (LeRoith *et al.* 1995, Li *et al.* 2008, Pancewicz *et al.* 2010). Interestingly, a recent study demonstrated that T2D status was not implicated in the relationship between *HNF1B* and *JAZF1* variants and prostate cancer risk (Stevens *et al.* 2010), which is in line with our hypothesis suggesting that T2D-related variants may determine the risk of MM through non-diabetogenic mechanisms.

When we took into account multiple testing corrections, only the association of the *IGF1*$_{rs35767}$ promoter

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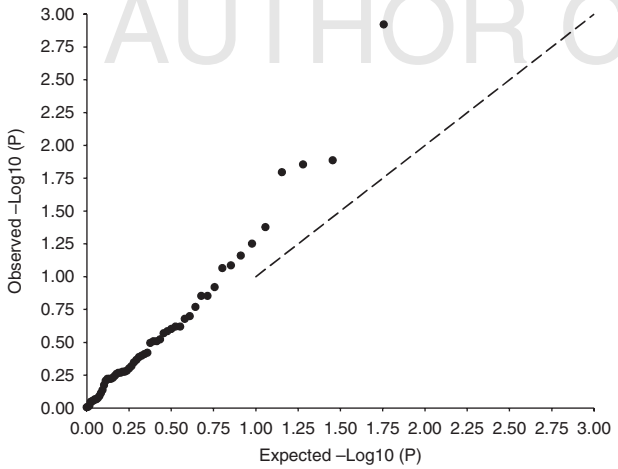

Figure 1
QQ plot used to evaluate the magnitude of observed associations of T2D-related variants with risk of MM. QQ plot was calculated assuming a recessive model of inheritance. Deviation from the expected distribution is observed above an expected X^2 of 0.75. The x-axis is -log10 of the expected *P* values (under a null hypothesis of no effects) whereas the y-axis is -log10 values of the actual *P* values.

polymorphism with an increased risk of developing MM remained close to significance ($P=0.0012$), which suggested that the *IGF1* locus may play an important role in triggering cell proliferation in malignant plasma cells. In support of the hypothesis, it has been observed that *IGF1* acts as a major growth factor in MM that, directly or in cooperation with other growth factors, induces MM cell growth and proliferation (Ferlin *et al.* 2000, Bommert *et al.* 2006, Sprynski *et al.* 2009) and can eventually lead to chemoresistance (Xu *et al.* 1997, Kuhn *et al.* 2012). Likewise, it has been also reported that treatment with metformin, an anti-diabetic drug that inhibits *IGF1* signaling pathway, significantly reduces the risk of transformation from MGUS to symptomatic MM (American Society of Clinical Oncology Annual Meeting 2014; abstract 1532) and that constant use of this treatment may induce cell apoptosis (Rattan *et al.* 2012) and enhance the effectiveness of chemotherapeutic regimes in blood and solid cancers (Feng *et al.* 2011, Pan *et al.* 2012, Watson 2013). Interestingly, several authors have also reported that *IGF1* and its analogues are associated with an increased death in patients with progressive MM (Standal *et al.* 2002, Chou *et al.* 2012, Wu *et al.* 2014), whereas the administration of metformin results in the reduction of deaths in patients with progressive disease (Wu *et al.* 2014).

In fact, Chen *et al.* (2013) recently demonstrated that the $IGF1_{rs35767}$ SNP together with two neighbour SNPs constitutes a haplotype that efficiently regulates transcriptional activity (Chen *et al.* 2013). Similarly, several studies have consistently reported that carriers of the $IGF1_{rs35767T}$ allele showed significantly higher levels of circulating *IGF1* than those harbouring the WT allele (Mannino *et al.* 2013, Sesti *et al.* 2014) and that the presence of this variant is associated with an increased risk of developing several types of cancer (Ollberding *et al.* 2012, Qian *et al.* 2014).

Although it is tempting to speculate that the $IGF1_{rs35767}$ SNP may be responsible for the effect attributed to diabetes on the risk of MM, we believe that rather than

Table 3 Discriminative value AUC for models including T2D-related variants

SNPs	*P* value	OR 95% CI	AUC 95% CI[ab]
Reference model[c]			
Gender	0.731	0.972 (0.828–1.141)	
Age	$<2.00\times10^{-16}$	1.036 (1.030–1.042)	0.629 (0.607–0.650)[e]
Predictive model built with six significant SNPs[d]			
$IGF1_{rs35767}$	0.004	2.076 (1.258–3.426)	
$FTO_{rs8050136}$	0.002	0.723 (0.586–0.892)	
$MADD_{rs7944584}$	0.094	1.218 (0.967–1.535)	
$PRC1_{rs8042680}$	0.061	1.261 (0.989–1.607)	
$KCNJ11_{rs5215}$	0.027	0.832 (0.706–0.980)	
$KCNQ1_{rs2237892}$	0.008	1.468 (1.106–1.950)	
Gender	0.776	1.024 (0.871–1.204)	
Age	$<2.00\times10^{-16}$	1.037 (1.031–1.043)	0.645 (0.624–0.666)[ef]

[a]Including age and gender as variables never dropped from models.
[b]Compared with a baseline model with AUC=0.5.
[c]Including age and gender as covariates.
[d]SNPs showing a significant association with MM ($P<0.05$). After removing missing values, 2460 subjects were available for prediction capacity analysis.
[e]A LR test showed that the model including genetic variants fitted the data better than the reference model and that the difference in model fit between both models was statistically significant (−2log likelihood ratio test, df=6, $P=4.05\times10^{-06}$). residual deviance (reference model): 3380.4. residual deviance (significant SNPs model): 3345.2.
[f]A sort analysis revealed that this model showed an AUC value systematically higher than those observed in 10 000 randomized models (null distribution; *Z* score=6.42, $P=6.81\times10^{-11}$; Supplementary Material).

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acting separately to modulate the risk of the disease, this genetic variant acts along with additional variants within *KCNQ1*, *CDKN2A-2B*, *MADD*, *KCNJ11*, *THADA*, *LTA* and *FTO* genes to modulate the disease risk. In order to test this hypothesis, we decided to evaluate the predictive value of T2D-related polymorphisms for prediction of MM using stepwise logistic and Cox regression analyses. Interestingly, we found that adding genetic factors to a model without covariates (including only age and gender) substantially improved the prediction of disease development. A predictive model including six SNPs significantly associated with MM in the single analysis showed an adjusted concordance statistic AUC of 64.5% for MM. The consistency of this result was confirmed through a randomization test that showed that none of the 10 000 randomized models showed a higher AUC value than our 'original' model including six genetic variants, age and gender. The addition of genetic variants associated with MM at $P<0.10$ level did not improve the discriminatory ability to predict MM, which pointed towards a joint contribution of *IGF1*, *FTO*, *MADD*, *PRC1*, *KCNJ11* and *KCNQ1* polymorphisms to predict the risk of the disease. Although the prediction capacity of these models could be considered relatively small when compared with a reference model, the relative absence of current diagnostic factors for MM suggest that the use of genetic variants could be a good option to improve the prediction of the disease risk.

The association of the non-diabetogenic alleles or genotypes for the polymorphisms within *KCNQ1*, *CDKN2A-2B*, *MADD*, *KCNJ11*, *THADA* and *FTO* genes with the risk of MM suggests that these genes, rather than acting through an insulin-dependent mechanism, may modulate the risk of MM by acting as tumour suppressor genes (Duro *et al*. 1995) or through mechanisms promoting cancer cell apoptosis (Rippe *et al*. 2003, Turner *et al*. 2013). In support of this idea, it has been recently reported that most of these genes are highly expressed in tumours and that genetic polymorphisms in these loci are also associated with cancer development (Sauroja *et al*. 2000, Cander *et al*. 2014) and tumour progression (Chen *et al*. 2009).

The association of $LTA_{rs1041981}$ SNP with a decreased risk of MM showed a similar direction to that observed in the GWAS for T2D ($LTA_{rs1041981A}$ as risk allele) suggests a diabetogenic effect of this SNP to modify the risk of MM. However, we could not dismiss the idea suggesting that observed association could be due to a different distribution of diabetics between MM cases and controls. Similarly, although the direction of the association for the $FTO_{rs8050136}$ SNP with the risk of MM was opposite to the one observed in the GWAS for T2D, we could not rule out the possibility that the observed effect for this obesogenic SNP (Scuteri *et al*. 2007) could be due to significant differences in BMI between MM cases and controls. Further studies using well-characterized cohorts are needed to confirm these latter associations.

Although it was not the primary objective of this study, we also performed gender-stratified analysis to assess whether there was a gender effect modification of selected SNPs to modulate the risk of developing MM. Interestingly, we found a significant gender effect modification for $ADAM30_{rs2641348}$, and $NOTCH2_{rs10923931}$ SNPs, which suggested a gender-specific effect of these loci to modulate the risk of MM. We observed that, according to a log-additive model of inheritance, the association of the $NOTCH2_{rs10923931}$ SNP with a decreased risk of MM in men and the association of $ADAM30_{rs2641348C/C}$ genotype with an increased risk of MM in women showed a marginal level of significance after correction for multiple testing. Recently, it has been demonstrated that *NOTCH2* is highly expressed in MM cells and that it is a key regulator of MM pathogenesis (Colombo *et al*. 2013). In particular, it has been reported that the activation of the *NOTCH2*, which interacts with Wnt components, induces an exacerbated growth of MM cells and accelerated the course of the disease by promoting cancer stem cell self-renewal (Xu *et al*. 2012*a*) and resistance to chemotherapeutic agents (Xu *et al*. 2012*b*). Considering that $NOTCH2_{rs10923931}$ and $ADAM30_{rs2641348}$ are neighbour SNPs in strong linkage disequilibrium (Zeggini *et al*. 2008) and that they showed gender-specific associations with the risk of MM, we hypothesize that *NOTCH2-ADAM30* might represent a gender-specific susceptibility region for MM. In support of this idea, it has been reported that gender-specific variants within *NOTCH* and *WNT* signalling pathways, which are involved in determining cell proliferation and differentiation, may lead to important gender-specific differences in tumour recurrence and chemoresistance (Paez *et al*. 2014).

Our study has both strengths and weaknesses. The major strength is the large sample size. To our knowledge, this is the first study to evaluate the overall and gender-specific associations of T2D-related variants with the risk of developing MM and to assess their predictive value for MM. Although the influence of diabetogenic variants on the risk of the disease was expected to be very modest, our study was sufficiently powered to detect such small effects. Based on the genotype frequencies observed in our study cohort, we had 80% of power (dominant model) to detect an OR of 1.29 at $\alpha=0.00022$ (multiple testing threshold) for a polymorphism with a minor allele frequency of 0.25. Although the gender-stratified analysis reduced the

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statistical power to detect effect of SNPs, we still had 80% of power to detect ORs of 1.43 and 1.44 for men and women respectively. It is important to realise, however, that although the present study involves data on over 3334 individuals, the retrospective and multicentre study design places inevitable limitations on clinical data availability. T2D status and BMI were not available for a substantial subset of MM cases, which did not allow us to adjust our analyses for these variables and, consequently, to rule out the possibility that some of the reported associations could arise as a result of a different distribution of diabetics and/or obese subjects between MM cases and controls. Nonetheless, considering that most of the reported associations with MM risk showed a different direction to those previously published in the GWAS for T2D and given that most of these genes are not linked to obesity, we could not expect to find false positive associations due to these confounding factors.

In conclusion, our study indicate that T2D-related variants within *IGF1*, *KCNJ11*, *CDKN2A-2B*, *MADD*, *THADA*, *LTA*, *FTO*, *ADAM30* and *NOTCH2* genes may influence the risk of MM through insulin-independent mechanisms and that genotyping of specific T2D-related variants may be useful to improve the prediction of MM development. Additional work is needed to replicate our findings in independent and well-characterized populations and functional studies are also warranted to elucidate the biological mechanisms underlying the observed effects.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-15-0029.

Declaration of interest

V Andersen is receiving compensation as a consultant for MSD (Merck) and Janssen. The rest of the authors have nothing to disclose.

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Resumen artículo 5

Ríos-Tamayo R, Lupiañez CB, Campa D, Hielscher T, Weinhold N, Martínez-López J, et al. A common variant within the HNF1B gene is associated with overall survival of multiple myeloma patients: Results from the IMMEnSE consortium and meta-analysis. Oncotarget 2016 [Epub ahead of print].

Este trabajo responde al objetivo 5.

Introducción. Es bien conocido que determinados polimorfismos de un solo nucleótido (SNPs) están asociados al riesgo de desarrollar MM. Recientemente se ha demostrado que algunas variantes genéticas en *MTHFD1L, AKAP12 y FOPNL* también pueden tener impacto en la supervivencia global de los pacientes con MM.

Específicamente, en relación con variantes genéticas identificadas mediante GWAS asociadas a DM2, hemos comprobado que determinadas variantes pueden influir en el riesgo de presentar MM. Sin embargo, su potencial impacto en términos de supervivencia global es desconocido.

Métodos. Estudio de cohorte multicéntrico en el contexto del consorcio internacional IMMEnSE. Un total de 58 variantes genéticas identificadas mediante GWAS asociadas a DM2 fueron evaludadas analizando su impacto en la supervivencia global de pacientes con MM. Todos los pacientes incluidos en el consorcio para los que se disponía de datos de supervivencia fueron incluidos en el análisis. Para la replicación, se usó la cohorte de pacientes con MM de la Clínica Universitaria de Heidelberg, con información genética disponible para 53 de las 58 variantes del estudio.

Para valorar la asociación de cada polimorfismo con la supervivencia global se utilizaron *Hazard Ratios* estimadas mediante modelos de Cox multivariantes, ajustando por edad, sexo y estadío Durie-Salmon (IMMEnSE) o edad, sexo y participación en

ensayo clínico (Heidelberg). Las asociaciones fueron estimadas de acuerdo con modelos de herencia dominante, recesivo y log-aditivo. Para el análisis de supervivencia se usó el método de Kaplan-Meier, estimando las diferencias entre grupos mediante el test *log-rank*.

Para confirmar las asociaciones significativas, se realizó un meta-análisis combiando ambas cohortes. Se usó el estadístico I^2 para valorar heterogeneidad entre ambos estudios. El HR global fue estimado usando un modelo de efectos aleatorios.

Resultados. De los 1420 pacientes de la cohorte IMMEnSE, 936 (65.9%) fueron incluidos en el estudio por disponer de datos de supervivencia (454 mujeres y 482 hombres). La cohorte de Heidelberg incluyó 700 pacientes (296 mujeres y 404 hombres), de los cuales 389 participaron en los ensayos clínicos GMMG-HD3 y HD4, y 311 correspondieron a pacientes sometidos a trasplante fuera de ensayo.

En la población IMMEnSE, 6 SNPs mostraron asociación con la supervivencia global. El efecto observado más relevante fue para $HNF1B_{rs7501939,}$ que se asoció a una pobre supervivencia global cuando se asume un modelo de herencia recesivo o un modelo log-aditivo (HR_{REC} = 1.49, IC 95% 1.11–2.00, p = 0,008 y HR_{AD} = 1.34 IC 95% 1,13–1,59, P = 0,001), respectivamente. Los pacientes con el genotipo no diabetogénico $HNF1B_{rs7501939T/T}$ tuvieron una mediana de supervivencia global de 81,91 vs. 101,42 meses para $HNF1B_{rs7501939C/C+C/T}$. El resultado fue confirmado en la cohorte Heidelberg, HR_{REC} = 1.40, IC 95% 1.06-1.84 y supervivencia global de 74.4 vs. 97.2 meses, respectivamente. El resultado del meta-análisis para este SNP permanece estadísticamente significativo tras corregir por múltiples ensayos ($HR_{Meta\text{-}Rec}$ = 1.44, IC 95% 1.18-1.76, p = 0.0001, I^2 =0.0%).

El estudio también mostró una asociación de SNPs en *BCL11A, MADD, PRC1, PROX1, SCL30A8, SLC2A2 y TCF7L2* con la supervivencia global. La asociación de *SCL30A8*

rs13266634 es específica de género. La presencia de cada copia adicional de este alelo menor se asoció a pobre supervivencia global en hombres ($HR_{Hombres\text{-}Ad}$ = 1.32, IC 95% 1.13–1.54, p = 0.0003).

Interpretación. El estudio sugiere que *HNF1B*$_{rs7501939}$ se asocia con peor supervivencia global en MM tanto en hombres como en mujeres, a través de un mecanismo probablemente independiente de insulina.

Así mismo, un SNP en *SCL30A8* tiene un impacto negativo en la supervivencia global de los pacientes de sexo masculino. Este hecho podría explicar, al menos parcialmente, las diferencias en supervivencia en función del sexo.

A common variant within the HNF1B gene is associated with overall survival of multiple myeloma patients: Results from the IMMEnSE consortium and meta-analysis

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ABSTRACT

Diabetogenic single nucleotide polymorphisms (SNPs) have recently been associated with multiple myeloma (MM) risk but their impact on overall survival (OS) of MM patients has not been analysed yet. In order to investigate the impact of 58 GWAS-identified variants for type 2 diabetes (T2D) on OS of patients with MM, we analysed genotyping data of 936 MM patients collected by the International Multiple Myeloma rESEarch (IMMENSE) consortium and an independent set of 700 MM patients recruited by the University Clinic of Heidelberg. A meta-analysis of the cox regression results of the two sets showed that rs7501939 located in the *HNF1B* gene negatively impacted OS (HR_{Rec}= 1.44, 95% CI = 1.18–1.76, *P* = 0.0001). The meta-analysis also showed a noteworthy gender-specific association of the $SLC30A8_{rs13266634}$ SNP with OS. The presence of each additional copy of the minor allele at rs13266634 was associated with poor OS in men whereas no association was seen in women ($HR_{Men\text{-}Add}$ = 1.32, 95% CI 1.13–1.54, *P* = 0.0003). In conclusion, these data suggest that the $HNF1B_{rs7501939}$ SNP confers poor OS in patients with MM and that a SNP in SLC30A8 affect OS in men.

INTRODUCTION

Multiple myeloma is an incurable and heterogeneous plasma cell neoplasm that affects about 6.3 per 100.000 people per year worldwide (i.e., 25.850 new cases only in 2015) and represents 1.6% of all cancers and 2% of all cancer deaths [1]. In spite of the widespread use of proteasome inhibitors and immunomodulatory drugs, which has dramatically improved the life expectancy of MM patients over the last few decades [2, 3], MM survival still remains poor with a 5-year survival of 46.6% (SEER Cancer Statistics Review, http://seer.cancer.gov/csr/1975_2012/).

Epidemiological and observational studies have consistently identified several factors that affect MM patient survival such as age at diagnosis [4, 5], stage at diagnosis (coded by either the Durie-Salmon staging system (DSS) [6] or the International Staging System (ISS)) [7], Eastern Cooperative Oncology Group (ECOG) performance status [8], renal failure [9, 10], high plasma cell proliferative rate [11, 12], high lactate deshydrogenase (LDH) levels [13] and chromosomal abnormalities [14–18]. Increasing evidences point towards a positive correlation of pre-existing type 2 diabetes (T2D) with MM risk [19] but also with the appearance of severe clinical complications [20–23] and patient survival [24, 25]. In this regard, Chiu et al. (2006) reported that high level of postload glucose was associated with increased risk of mortality in hematological malignancies [24] whereas Chou et al. (2012) reported that MM patients with pre-existing T2D have 50% higher all-cause mortality compared with non-diabetic patients [25]. These observations might be explained, at least in part, by the stimulatory effects of T2D-associated hyperglycaemia, insulin resistance and resulting hyperinsulinemia on MM cell growth [26, 27] but also by the deregulation of tumour-suppressor genes linked to T2D (such as CDKN2A-2B, KCNQ1, HNF1B) [28–30] that might lead to uncontrolled cell proliferation, cell differentiation and disease progression and, consequently, to shorter survival periods. In support of this notion, CDKN2A-2B genes have been found to be frequently hypermethylated in MM [31–33] whereas loss of expression of KCNQ1 has been associated with poor overall survival in cancer patients [34]. Furthermore, emerging evidences also suggest that the activation of certain T2D-related genes (such as NOTCH2) may induce MM cell migration from the infiltrated site to different bone marrow districts [35] and promote osteoclast formation [36], which is a process intimately related to proliferation and long-term survival of MM cells [37].

Although germline variants may influence the susceptibility of MM [38–43] and survival [39, 44–46], the knowledge regarding the role of diabetogenic variants in modulating the risk of MM and survival remains scarce. We have recently reported that diabetogenic variants influence MM risk [47] and recent genome-wide association studies (GWAS) have also suggested the involvement of genetic variants within the *MTHFD1L*, *AKAP12* and *FOPNL* loci in determining MM patient survival [39, 44, 45] but also an indirect implication of diabetogenic genes such as *TCF7L2* [45]. Johnson *et al.* (2016) reported in their GWAS a strong association of rs12374648, which maps to a binding site for the transcription factor *TCF7L2*, with MM overall survival (OS) and proposed a functional mechanism of this variant to modulate the synthesis of purines and the regulation of cell cycle [45].

Based on these findings, we explored for the first time the relationship between diabetogenic variants and OS of MM patients in a study developed in the context of the International Multiple Myeloma rESEarch (IMMENSE) consortium. We attempted to confirm our findings by analysing GWAS data on an independent set of German MM patients (Heidelberg cohort) [45].

RESULTS

The demographic and clinical characteristics of the MM patients included in the IMMENSE (n = 939) and Heidelberg (n = 700) cohorts are listed in Table 1. The median age at diagnosis was similar in both populations (59.73 ± 10.08 vs. 55.85 ± 8.33) but the male/female ratio was higher in the Heidelberg cohort (1.36 vs. 1.06). Durie-Salmon stage was available for IMMENSE and Heidelberg cohorts and included patients at stages I, II and III (11.83%, 23.54% and 64.63% vs. 0.8% , 12.4% and 86.8% , respectively).

All SNPs tested showed genotype frequencies consistent with the HWE ($P > 0.001$) and the observed allele frequency for all selected SNPs was in accordance with Hapmap data. When we evaluated the effect of selected polymorphisms on MM OS in the IMMENSE population, we found that 6 SNPs showed a noteworthy association with OS. The most relevant effect was observed for the *HNF1B*$_{rs7501939}$ SNP that was associated with poor OS when recessive and log-additive models of inheritance were assumed (HR_{REC} = 1.49, 95% CI 1.11–2.00, P = 0.008 and HR_{ADD} = 1.34 95% CI 1.13–1.59, P = 0.001, respectively; Table 2). Patients harbouring the non-diabetogenic *HNF1B*$_{rs7501939T/T}$ genotype showed a median survival time (MST) significantly shorter than those carrying the C allele ($MST_{T/T}$ = 81.91 months vs. $MST_{C/C+C/T}$ = 101.42 months; Figure 1A). This result was confirmed with the 700 MM patients recruited from the University Clinic of Heidelberg (HR_{REC} = 1.40, 95% CI 1.06–1.84 and $MST_{T/T}$ = 74.4 months vs. $MST_{C/C+C/T}$ = 97.2 months; Table 3 and Figure 1B). The result of the meta-analysis for this SNP remained significant after correction for multiple testing ($HR_{Meta-Rec}$ = 1.44, 95% CI 1.18–1.76, P = 0.0001, I^2 = 0.0% , P_{Het} = 0.74; Table 3). According to publicly available eQTL data for human peripheral blood mononuclear cells, the risk allele (T) was associated with higher *HNF1B* mRNA expression levels (Z score = 3.31, $P = 9.23 \cdot 10^{-4}$ and FDR = 0.23). However, we could not validate this finding using eQTL data on plasma cells from 665 German MM patients (P = 0.60; Supplementary Table S1). Nonetheless, according to Haploreg and ENCODE annotation data, the rs7501939 SNP resides near of a poised promoter in many cell lines including a lymphoblastoid and human stem cell lines (GM12878 and H1-HSCs) that might be rapidly activated upon specific stimuli. In addition, this SNP was predicted to change binding motifs for 2 regulatory transcription factors (CEBPB and p300) and mapped among enhancer histone marks in primary naïve and memory forms of cytotoxic T cells (CD_8^+) and helper T cells (CD_4^+) from peripheral blood.

We also observed significant associations at $P < 0.05$ for SNPs within *CDKN2A-2B*, *GCKR*, *KCNQ1* and *SLC30A8* genes with OS in the IMMENSE population. Thus, patients carrying the *KCNQ1*$_{rs2074196T}$ and *SLC30A8*$_{rs13266634T}$ alleles or the *GCKR*$_{rs1260326T/T}$ genotype had an increased risk of death whereas subjects bearing the *CDKN2A-2B*$_{rs564398C/C}$ genotype showed longer OS (Table 2 and Supplementary Tables S2–S4). The association of the *SLC30A8*$_{rs13266634T}$ allele with OS was confirmed in the Heidelberg population and the meta-analysis showed that the presence of each additional copy of the *SLC30A8*$_{rs13266634T}$ allele was associated with poor OS ($HR_{Meta-Add}$ = 1.22, 95% CI 1.09-1.37; Table 3). Although the association of the *SLC2A2*$_{rs11920090}$ SNP with OS was not significant in the IMMENSE population, we observed a significant association of this variant with MM survival in the Heidelberg population that remained significant in the pooled analysis. Patients harbouring the *SLC2A2*$_{rs11920090T}$ allele showed a better survival compared with those carrying the A/A genotype ($HR_{Meta-Dom}$ = 0.80, 95% CI 0.66-0.97; Table 3). The meta-analysis also showed a weak association of the *TCF7L2*$_{rs7903146T}$ allele with better survival that was neither significant in the IMMENSE population nor in the Heidelberg cohort ($HR_{Meta-Dom}$ = 0.84, 95% CI 0.71–0.98). Based on Haploreg data, the missense rs13266634 SNP was predicted to change binding motifs for transcription factors implicated in tumorigenesis (AP1 and PAX5) and mapped on enhancer histone marks in several human embrionic stem cell lines. In addition, this polymorphism affects binding to 5 proteins implicated in cancer development (CCNT2, GATA2, TAL1, KAP1 and CTCF). On the other hand, the rs11920090 and rs7903146 SNPs mapped among enhancer and promoter histone marks in bone marrow- and/or adipose-derived mesenchymal stem cells. In addition, the rs7903146 SNP was predicted to alter the binding site of 7 transcription factors. However, despite the consistency and the potential interest of these findings, none of the associations of the *SLC2A2*, *SLC30A8*, and *TCF7L2* SNPs with OS remained significant after correction for multiple testing and, therefore, require further confirmation. Given the lack of genetic information in the GWAS conducted in the Heidelberg population for SNPs within *MADD and KCNQ1* genes, we imputed genotypes to test whether the preliminary associations observed in the IMMENSE population could be validated. Although there was no imputed data available for the *KCNQ1* SNP, the meta-analysis of IMMENSE data with imputed genotypes of *MADD* variants in the Heidelberg cohort suggested a link between this locus and MM survival ($HR_{Meta-Rec}$ = 0.75, 95% CI 0.57–0.99; Table 3).

Based on the evidences that point toward the existence of gender-associated differences in survival for patients with MM [48], we decided to carry out a gender-stratified analysis. This analysis revealed gender-specific associations for SNPs within or near the *ADAMTS9*, *KCNJ11, PROX1* and *SLC30A8* genes with OS. We found that men carrying the *KCNJ11*$_{rs5215C}$ or *SLC30A8*$_{rs13266634T}$ alleles had poorer OS compared with those harbouring the wild type genotype whereas

Table 1: Clinical characteristics of IMMEnSE and Heidelberg cohorts

IMMENSE population			
	MM patients (n = 936)		
Country of origin	Gender M/F (Total)	Mean Age (± STD)	Median Age (Range)
Italy	69/69 (138)	61.31 ± 9.47	51.0 (35–86)
Poland	145/163 (308)	62.48 ± 10.50	52.0 (34–86)
Spain	49/55 (104)	62.44 ± 11.45	66.0 (22–88)
France	42/33 (75)	55.80 ± 9.04	41.0 (34–75)
Portugal	14/22 (36)	65.22 ± 9.54	35.0 (45–80)
Denmark	163/112 (275)	55.18 ± 7.32	51.0 (29–69)
Demographic variables			
Age (years, average ± SD)		59.73 ± 10.08	
Sex ratio (male/female)		1.06 (482/454)	
Overall survival (months)		99.69 [92.98,106.39]	
Number of deaths		323	
Median follow-up time (months)		100 (52–111)	
Disease stage (Durie-Salmon)*			
Stage I		93 (11.83)	
Stage II		185 (23.54)	
Stage III		508 (64.63)	

Heidelberg population			
	MM patients (n = 700)		
Country of origin	Gender M/F (Total)	Mean Age (± STD)	Median Age (Range)
Germany (HD3 trial)	56/42 (98)	55.42 ± 7.47	56.5 (38–65)
Germany (HD4 trial)	170/121 (291)	55.02 ± 7.30	57.0 (27–65)
Germany (non-trial)	178/133 (311)	56.76 ± 9.37	57.2 (24–73)
Demographic variables			
Age (years, average ± SD)		55.85 ± 8.33	
Sex ratio (male/female)		1.36 (404/296)	
Overall survival (months)$^{\partial}$		91.2 [85.5,105]	
Number of deaths		326	
Median follow-up time (months)		84.3 (80–88)	
Disease stage (Durie-Salmon)			
Stage I		5 (0.8)	
Stage II		82 (12.4)	
Stage III		575 (86.8)	

Abbreviations: IMMENSE, International Multiple Myeloma rESEarch; MM, Multiple Myeloma; SD, standard deviation.
*Durie-Salmon data was not available for 150 MM patients.
$^{\partial}$Median overall survival after diagnosis (IMMENSE) or the 1st autotransplant (Heidelberg cohort) (KM estimators).

an opposite but not significant effect was seen in women ($P_{Interaction}$ = 0.022 and $P_{Interaction}$ = 0.057, respectively; Table 2 and Supplementary Table S2–S4). We also observed that women carrying the $PROX1_{rs340874G}$ allele or the $ADAMTS9_{rs4607103T/T}$ genotype experienced a poorer survival with an opposite but not significant effect in men ($P_{Interaction}$ = 0.016 and $P_{Interaction}$ = 0.024). In order to confirm these gender-specific associations, we performed a

Table 2: Association of T2D–related variants and overall survival (OS) of MM patients

Variant_ dbSNP	Gene	OVERALL (*N* = 936)		MEN (*N* = 482)		WOMEN (*N* = 454)		
		OR (95% CI)[a]	P_{value}	OR (95% CI)[b]	P_{value}	OR (95% CI)[b]	P_{value}	$P_{Interaction}$
rs2641348	*ADAM30*	0.94 (0.69–1.28)	0.69	0.99 (0.67–1.48)	0.97	0.87 (0.53–1.41)	0.56	0.70
rs4607103†	*ADAMTS9*	1.23 (0.76–2.00)	0.40	0.83 (0.42–1.63)	0.58	**2.53 (1.26–5.07)**	**0.009**	0.024
rs11708067	*ADCY5*	0.87 (0.68–1.11)	0.25	1.02 (0.75–1.39)	0.89	**0.63 (0.42–0.95)**	**0.028**	0.08
rs10885122	*ADRA2A*	1.03 (0.79–1.33)	0.83	1.01 (0.72–1.40)	0.97	1.07 (0.71–1.62)	0.73	0.89
rs1552224	*ARAPI, CENTD2*	0.88 (0.68–1.15)	0.35	0.90 (0.64–1.26)	0.55	0.83 (0.54–1.25)	0.37	0.77
rs10490072	*BCL11A*	1.05 (0.83–1.32)	0.70	1.22 (0.90–1.65)	0.19	0.85 (0.59–1.23)	0.39	0.11
rs12779790	*CDC123, CAMK1D*	0.86 (0.67–1.11)	0.24	1.06 (0.77–1.46)	0.72	**0.64 (0.43–0.97)**	**0.035**	0.06
rs7754840	*CDKAL1*	1.14 (0.90–1.44)	0.27	1.22 (0.90–1.65)	0.20	1.06 (0.74–1.53)	0.75	0.52
rs564398†	*CDKN2A–2B*	**0.64 (0.42–0.98)**	**0.042**	0.62 (0.36–1.07)	0.084	0.71 (0.34–1.47)	0.36	0.88
rs10811661	*CDKN2A–2B*	0.93 (0.72–1.19)	0.55	0.93 (0.67–1.30)	0.68	0.92 (0.63–1.35)	0.67	0.92
rs2383208	*CDKN2A–2B*	0.94 (0.73–1.21)	0.64	0.92 (0.66–1.29)	0.64	0.95 (0.65–1.41)	0.81	0.87
rs4240702	*COL5A1*	0.88 (0.68–1.15)	0.35	0.85 (0.60–1.21)	0.36	0.95 (0.63–1.44)	0.82	0.65
rs11605924	*CRY2*	0.91 (0.70–1.18)	0.47	0.81 (0.58–1.14)	0.23	1.09 (0.71–1.69)	0.68	0.29
rs1153188	*DCD*	1.18 (0.94–1.48)	0.16	1.25 (0.92–1.69)	0.15	1.09 (0.76–1.57)	0.63	0.58
rs1113132	*EXT2*	1.02 (0.81–1.28)	0.86	0.96 (0.71–1.30)	0.79	1.08 (0.75–1.56)	0.66	0.57
rs174550	*FADS1*	1.00 (0.79–1.26)	1.00	0.97 (0.72–1.30)	0.82	1.05 (0.73–1.51)	0.80	0.71
rs11071657	*FAM148B*	0.86 (0.68–1.09)	0.22	0.96 (0.70–1.32)	0.81	0.77 (0.53–1.12)	0.17	0.44
rs17044137	*FLJ39370*	1.06 (0.84–1.33)	0.64	1.07 (0.79–1.44)	0.67	1.03 (0.71–1.50)	0.87	0.92
rs8050136	*FTO*	0.91 (0.70–1.18)	0.46	0.81 (0.58–1.13)	0.21	1.10 (0.72–1.67)	0.66	0.23
rs560887†	*G6PC2*	1.11 (0.74–1.66)	0.61	0.78 (0.44–1.39)	0.40	1.76 (1.00–3.09)	0.050	0.045
rs1799884	*GCK*	0.99 (0.76–1.28)	0.92	1.10 (0.80–1.52)	0.57	0.81 (0.52–1.26)	0.36	0.39
rs1260326†	*GCKR*	1.36 (1.01–1.82)	0.043	1.24 (0.84–1.83)	0.28	1.53 (0.97–2.42)	0.068	0.47

rs1111875	*HHEX*	1.00 (0.78–1.27)	0.97	0.92 (0.67–1.26)	0.61	1.19 (0.80–1.77)	0.38	0.33
rs7957197	*HNF1A (TCF1)*	1.17 (0.92–1.48)	0.20	1.18 (0.87–1.59)	0.30	1.16 (0.80–1.70)	0.43	0.89
rs7501939†	*HNF1B (TCF2)*	**1.49 (1.11–2.00)**	**0.008**	**1.49 (1.03–2.17)**	**0.036**	1.49 (0.91–2.45)	0.11	0.99
rs35767	*IGF1*	0.87 (0.67–1.12)	0.27	0.78 (0.56–1.10)	0.16	1.00 (0.68–1.48)	1.00	0.40
rs4402960	*IGF2BP2*	0.90 (0.71–1.13)	0.36	0.78 (0.58–1.06)	0.11	1.10 (0.76–1.60)	0.60	0.16
rs20541†	*IL13*	1.42 (0.89–2.27)	0.14	0.93 (0.45–1.90)	0.84	**2.38 (1.24–4.57)**	**0.009**	0.07
rs2943641	*IRS1*	1.13 (0.89–1.43)	0.33	1.01 (0.75–1.38)	0.93	1.38 (0.94–2.02)	0.10	0.30
rs864745	*JAZF1*	0.90 (0.71–1.15)	0.40	1.01 (0.73–1.39)	0.98	0.75 (0.52–1.09)	0.14	0.22
rs5215	*KCNJ11*	1.09 (0.86–1.37)	0.49	**1.39 (1.02–1.90)**	**0.038**	0.79 (0.55–1.13)	0.20	**0.022**
rs5219	*KCNJ11*	1.11 (0.87–1.43)	0.39	1.37 (0.98–1.91)	0.063	0.88 (0.60–1.28)	0.50	0.12
rs2237897	*KCNQ1*	1.25 (0.88–1.77)	0.21	1.28 (0.81–2.01)	0.28	1.22 (0.71–2.10)	0.48	0.83
rs2074196	*KCNQ1*	1.57 (1.03–2.40)	0.036	**1.83 (1.01–3.32)**	**0.047**	1.42 (0.77–2.59)	0.26	0.48
rs2237892	*KCNQ1*	1.38 (0.97–1.97)	0.070	1.53 (0.97–2.41)	0.065	1.24 (0.70–2.18)	0.46	0.53
rs2237895	*KCNQ1*	1.06 (0.82–1.36)	0.66	1.30 (0.94–1.80)	0.11	0.74 (0.50–1.11)	0.15	0.033
rs231362	*KCNQ1OT1*	1.15 (0.88–1.51)	0.31	1.04 (0.73–1.47)	0.84	1.34 (0.87–2.07)	0.19	0.33
rs1041981	*LTA*	0.86 (0.68–1.09)	0.21	0.76 (0.56–1.04)	0.088	1.04 (0.71–1.51)	0.85	0.25
rs7944584†	*MADD*	0.68 (0.46–1.01)	0.058	0.55 (0.32–0.95)	0.031	0.83 (0.46–1.52)	0.55	0.27
rs12970134	*MCR4*	0.89 (0.70–1.12)	0.31	**0.86 (0.64–1.16)**	**0.31**	0.94 (0.65–1.35)	0.74	0.71
rs1387153	*MTNR1B*	0.89 (0.71–1.12)	0.32	1.01 (0.75–1.37)	0.93	0.71 (0.49–1.02)	0.063	0.16
rs10923931	*NOTCH2*	0.98 (0.73–1.31)	0.88	1.05 (0.72–1.53)	0.81	0.88 (0.55–1.41)	0.59	0.58
rs6698181	*PKN2*	1.17 (0.92–1.48)	0.20	1.28 (0.94–1.75)	0.12	1.00 (0.69–1.46)	0.98	0.33
rs1801282	*PPARG*	0.84 (0.65–1.10)	0.21	**0.66 (0.46–0.96)**	**0.030**	1.12 (0.76–1.65)	0.56	0.09
rs8042680†	*PRC1*	0.91 (0.64–1.29)	0.60	1.13 (0.74–1.73)	0.56	0.56 (0.29–1.07)	0.081	0.08
rs340874	*PROX1*	1.03 (0.78–1.36)	0.83	0.75 (0.52–1.07)	0.11	**1.60 (1.02–2.50)**	**0.041**	**0.016**
rs7593730	*RBMS1*	0.92 (0.73–1.16)	0.48	0.77 (0.56–1.05)	0.10	1.20 (0.83–1.75)	0.33	0.07

rs1531343	*RPSAP52, HMGA2*	1.11 (0.84–1.46)	0.45	1.40 (1.00–1.97)	0.053	0.76 (0.46–1.23)	0.26	0.07
rs11920090	*SLC2A2*	0.88 (0.67–1.14)	0.34	0.87 (0.62–1.22)	0.41	0.91 (0.59–1.40)	0.67	0.91
rs13266634[∂]	*SLC30A8*	**1.24 (1.05–1.47)**	**0.011**	**1.42 (1.14–1.77)**	**0.002**	1.01 (0.77–1.33)	0.94	0.057
rs7903146	*TCF7L2*	0.83 (0.66–1.05)	0.12	**0.72 (0.53–0.97)**	**0.030**	1.04 (0.72–1.50)	0.82	0.13
rs12255372	*TCF7L2*	0.87 (0.69–1.09)	0.23	**0.73 (0.54–0.98)**	**0.039**	1.14 (0.79–1.65)	0.49	0.08
rs7578597	*THADA*	1.18 (0.88–1.58)	0.27	0.97 (0.67–1.41)	0.88	1.59 (0.99–2.55)	0.054	0.12
rs896854[†]	*TP53INP1*	0.76 (0.58–1.00)	0.050	0.74 (0.52–1.06)	0.10	0.77 (0.50–1.19)	0.24	0.81
rs7961581	*TSPAN8, LGR5*	1.09 (0.87–1.37)	0.47	1.08 (0.80–1.47)	0.62	1.14 (0.79–1.63)	0.49	0.77
rs9472138	*VEGFA*	1.03 (0.82–1.30)	0.80	0.93 (0.69–1.26)	0.63	1.19 (0.83–1.71)	0.33	0.32
rs10010131	*WFS1*	1.00 (0.78–1.28)	0.99	1.15 (0.81–1.61)	0.44	0.85 (0.59–1.23)	0.38	0.28

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; n/s, not specified.
Estimates were adjusted for age, sex, country of origin and Durie–Salmon stage. $P < 0.05$ in bold.
[a]Estimates calculated according to a dominant model of inheritance.
[b]Estimates calculated according to a dominant model of inheritance and adjusted for age, region and Durie–Salmon stage.
[†]Estimates calculated according to a recessive model of inheritance.
[∂]Estimates calculated according to an additive model of inheritance.

meta-analysis with available GWAS data of the Heidelberg population. Although there was a partial overlapping of SNPs between both populations that limited our ability to validate some potentially interesting gender-associated effects on OS, we could confirm the strong association of the *SLC30A8*$_{rs13266634}$ SNP with OS in men that could not be detected in women (per-allele HR_{Men} = 1.32, 95% CI 1.13-1.54; Supplementary Table S5). This gender-specific association remained significant at the experiment-wide significance threshold. On the other hand, although it was not statistically significant in the analysis of the IMMENSE population, the pooled analysis also showed that men carrying the *BCL11A*$_{rs10490072C}$ allele had a poorer OS compared with those carrying the wild type genotype whereas no effect was seen in women (HR_{Men} = 1.37, 95% CI 1.10-1.70). Finally, we observed in the pooled analysis that women bearing the *PRC1*$_{rs8042680A}$ allele or men carrying the *PROX1*$_{rs340874G}$ allele or the *MADD*$_{rs7944584T/T}$

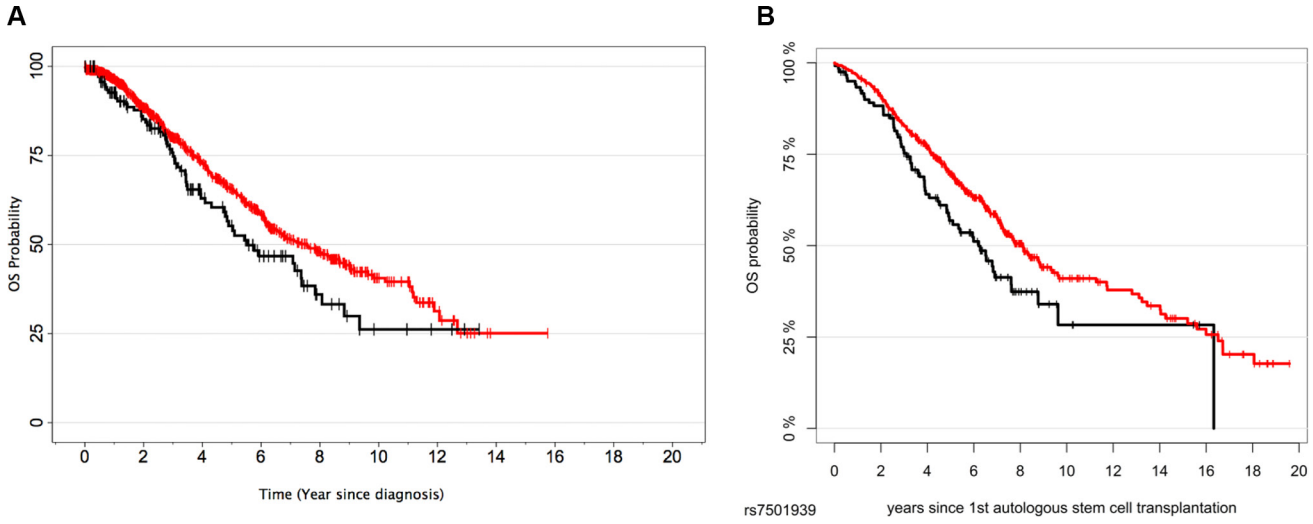

Figure 1: Kaplan-Meier plots for the *HNF1B*$_{rs7501939}$ SNP in the IMMENSE (A) and Heidelberg (B) populations.

Table 3: Meta–analysis for the association of T2D–related variants and overall survival (OS) of MM patients

		IMMENSE (*N* = 936)		GWAS (*N* = 700)		META-ANALYSIS (*N* = 1636)	
Variant_ dbSNP	Gene	OR (95% CI)[a]	P_{value}	HR (95% CI)[b]	P_{value}	HR (95% CI)[c]	P_{value}
rs2641348	*ADAM30*	0.94 (0.69–1.28)	0.69	1.12 (0.86–1.45)	0.41	1.04 (0.85–1.27)	0.69
rs4607103†	*ADAMTS9*	1.23 (0.76–2.00)	0.40	0.99 (0.63–1.55)*	0.98	1.10 (0.79–1.52)*	0.59
rs11708067	*ADCY5*	0.87 (0.68–1.11)	0.25	0.89 (0.71–1.11)*	0.31	0.88 (0.75–1.04)*	0.13
rs10885122	*ADRA2A*	1.03 (0.79–1.33)	0.83	0.85 (0.65–1.12)	0.24	0.94 (0.78–1.13)	0.52
rs1552224	*ARAPI, CENTD2*	0.88 (0.68–1.15)	0.35	1.12 (0.89–1.42)	0.34	1.00 (0.79–1.27)	1.00
rs10490072	*BCL11A*	1.05 (0.83–1.32)	0.70	**1.30 (1.04–1.62)***	**0.019**	1.17 (0.95–1.44)*	0.14
rs12779790	*CDC123, CAMK1D*	0.86 (0.67–1.11)	0.24	ND	ND	ND	ND
rs7754840	*CDKAL1*	1.14 (0.90–1.44)	0.27	1.15 (0.92–1.43)	0.22	1.14 (0.98–1.35)	0.10
rs564398†	*CDKN2A–2B*	**0.64 (0.42–0.98)**	**0.042**	1.17 (0.88–1.55)	0.29	0.88 (0.49–1.59)	0.68
rs10811661	*CDKN2A–2B*	0.93 (0.72–1.19)	0.55	ND	ND	ND	ND
rs2383208	*CDKN2A–2B*	0.94 (0.73–1.21)	0.64	ND	ND	ND	ND
rs4240702	*COL5A1*	0.88 (0.68–1.15)	0.35	1.12 (0.87–1.44)	0.39	1.00 (0.79–1.26)	0.97
rs11605924	*CRY2*	0.91 (0.70–1.18)	0.47	1.05 (0.82–1.35)*	0.70	0.98 (0.82–1.18)*	0.83
rs1153188	*DCD*	1.18 (0.94–1.48)	0.16	0.85 (0.68–1.07)*	0.16	1.00 (0.73–1.38)*	0.99
rs1113132	*EXT2*	1.02 (0.81–1.28)	0.86	1.01 (0.81–1.26)	0.93	1.02 (0.87–1.19)	0.86
rs174550	*FADS1*	1.00 (0.79–1.26)	1.00	1.10 (0.88–1.37)	0.39	1.05 (0.90–1.24)	0.54
rs11071657	*FAM148B*	0.86 (0.68–1.09)	0.22	1.01 (0.81–1.27)	0.91	0.94 (0.80–1.10)	0.42
rs17044137	*FLJ39370*	1.06 (0.84–1.33)	0.64	1.04 (0.83–1.31)*	0.71	1.05 (0.89–1.23)*	0.56
rs8050136	*FTO*	0.91 (0.70–1.18)	0.46	1.02 (0.81–1.29)	0.84	0.97 (0.82–1.15)	0.73
rs560887†	*G6PC2*	1.11 (0.74–1.66)	0.61	0.91 (0.61–1.35)	0.63	1.01 (0.77–1.33)	0.94
rs1799884	*GCK*	0.99 (0.76–1.28)	0.92	1.04 (0.83–1.31)*	0.74	1.02 (0.86–1.21)*	0.84
rs1260326†	*GCKR*	**1.36 (1.01–1.82)**	**0.043**	1.10 (0.83–1.47)	0.51	1.22 (0.99–1.50)	0.061
rs1111875	*HHEX*	1.00 (0.78–1.27)	0.97	1.00 (0.79–1.25)*	0.97	1.00 (0.85–1.18)*	1.00
rs7957197	*HNF1A (TCF1)*	1.17 (0.92–1.48)	0.20	1.07 (0.85–1.35)*	0.56	1.12 (0.95–1.32)*	0.19
rs7501939†	*HNF1B (TCF2)*	**1.49 (1.11–2.00)**	**0.008**	**1.40 (1.06–1.84)**	**0.016**	**1.44 (1.18–1.76)**	**0.0001**
rs35767	*IGF1*	0.87 (0.67–1.12)	0.27	1.08 (0.85–1.37)	0.53	0.98 (0.79–1.20)	0.81
rs4402960	*IGF2BP2*	0.90 (0.71–1.13)	0.36	0.90 (0.72–1.12)	0.34	0.90 (0.77–1.06)	0.20
rs20541†	*IL13*	1.42 (0.89–2.27)	0.14	0.82 (0.48–1.41)	0.47	1.10 (0.64–1.88)	0.73
rs2943641	*IRS1*	1.13 (0.89–1.43)	0.33	1.08 (0.86–1.36)	0.49	1.10 (0.94–1.30)	0.24
rs864745	*JAZF1*	0.90 (0.71–1.15)	0.40	1.01 (0.79–1.30)*	0.94	0.95 (0.80–1.13)*	0.58
rs5215	*KCNJ11*	1.09 (0.86–1.37)	0.49	0.98 (0.78–1.23)	0.85	1.03 (0.88–1.22)	0.70
rs5219	*KCNJ11*	1.11 (0.87–1.43)	0.39	0.98 (0.78–1.23)	0.85	1.04 (0.88–1.23)	0.67
rs2237897	*KCNQ1*	1.25 (0.88–1.77)	0.21	ND	ND	ND	ND
rs2074196	*KCNQ1*	**1.57 (1.03–2.40)**	**0.036**	ND	ND	ND	ND
rs2237892	*KCNQ1*	1.38 (0.97–1.97)	0.070	1.09 (0.80–1.49)	0.59	1.21 (0.96–1.53)	0.11
rs2237895	*KCNQ1*	1.06 (0.82–1.36)	0.66	0.94 (0.75–1.19)	0.62	0.99 (0.84–1.18)	0.93
rs231362	*KCNQ1OT1*	1.15 (0.88–1.51)	0.31	1.04 (0.80–1.35)	0.76	1.09 (0.91–1.32)	0.36
rs1041981	*LTA*	0.86 (0.68–1.09)	0.21	0.95 (0.76–1.18)	0.64	0.91 (0.77–1.07)	0.24

rs7944584[†]	*MADD*	0.68 (0.46–1.01)	0.058	0.83 (0.56–1.24)*	0.37	**0.75 (0.57–0.99)***	**0.044**
rs12970134	*MCR4*	0.89 (0.70–1.12)	0.31	1.11 (0.89–1.39)	0.34	1.00 (0.80–1.24)	0.98
rs1387153	*MTNR1B*	0.89 (0.71–1.12)	0.32	1.12 (0.90–1.30)	0.30	1.01 (0.81–1.26)	0.94
rs10923931	*NOTCH2*	0.98 (0.73–1.31)	0.88	1.10 (0.85–1.44)*	0.48	1.04 (0.86–1.27)*	0.66
rs6698181	*PKN2*	1.17 (0.92–1.48)	0.20	0.97 (0.78–1.21)	0.77	1.06 (0.88–1.27)	0.54
rs1801282	*PPARG*	0.84 (0.65–1.10)	0.21	0.90 (0.70–1.16)*	0.41	0.87 (0.73–1.05)*	0.14
rs8042680[†]	*PRC1*	0.91 (0.64–1.29)	0.60	1.05 (0.75–1.47)	0.77	0.98 (0.77–1.25)	0.87
rs340874	*PROX1*	1.03 (0.78–1.36)	0.83	0.81 (0.64–1.03)*	0.08	0.90 (0.71–1.14)*	0.40
rs7593730	*RBMS1*	0.92 (0.73–1.16)	0.48	1.09 (0.87–1.36)*	0.44	1.00 (0.85–1.19)*	0.96
rs1531343	*RPSAP52, HMGA2*	1.11 (0.84–1.46)	0.45	0.88 (0.66–1.17)*	0.39	0.99 (0.79–1.25)*	0.94
rs11920090	*SLC2A2*	0.88 (0.67–1.14)	0.34	**0.73 (0.56–0.95)**	**0.022**	**0.80 (0.66–0.97)**	**0.020**
rs13266634[∂]	*SLC30A8*	**1.24 (1.05–1.47)**	**0.011**	**1.20 (1.02–1.41)**	**0.025**	**1.22 (1.09–1.37)**	**0.001**
rs7903146	*TCF7L2*	0.83 (0.66–1.05)	0.12	0.84 (0.67–1.05)	0.12	**0.84 (0.71–0.98)**	**0.028**
rs12255372	*TCF7L2*	0.87 (0.69–1.09)	0.23	0.83 (0.67–1.04)	0.10	0.85 (0.73–1.00)	**0.043**
rs7578597	*THADA*	1.18 (0.88–1.58)	0.27	0.95 (0.73–1.24)	0.72	1.05 (0.85–1.30)	0.66
rs896854[†]	*TP53INP1*	0.76 (0.58–1.00)	0.050	0.97 (0.75–1.25)*	0.82	0.86 (0.68–1.10)*	0.23
rs7961581	*TSPAN8, LGR5*	1.09 (0.87–1.37)	0.47	0.92 (0.74–1.14)	0.44	1.00 (0.85–1.18)	0.98
rs9472138	*VEGFA*	1.03 (0.82–1.30)	0.80	1.10 (0.88–1.37)	0.39	1.07 (0.91–1.25)	0.43
rs10010131	*WFS1*	1.00 (0.78–1.28)	0.99	1.07 (0.86–1.35)	0.54	1.04 (0.88–1.23)	0.66

Abbreviations: SNP, single nucleotide polymorphism; HR, hazard ratio; CI, confidence interval. ND, not determined. Estimates were adjusted for age, sex, country of origin and Durie–Salmon stage. $P < 0.05$ in facebold.

[a]Estimates calculated according to a dominant model of inheritance and adjusted for age, gender, region and Durie–Salmon stage.

[b]Estimates calculated according to a dominant model of inheritance and adjusted for age, gender and clinical trial.

[c]Meta–analyses were performed assuming a random effect model.

[†]Estimates calculated according to a recessive model of inheritance.

[∂]Estimates calculated according to an additive model of inheritance.

[*]Estimates based on imputed genotypes.

genotype showed a significantly better OS when compared with those patients carrying the corresponding wild type allele or genotype (HR_{Women} = 0.62, 95% CI 0.39–0.98; HR_{Men} = 0.74, 95% CI 0.59–0.94 and HR_{Men} = 0.59, 95% CI 0.39–0.87; Supplementary Table S5). The regulatory characteristics of the rs10490072, rs8042680 and rs7944584 SNPs were changes in transcription binding motifs for transcription factors involved in tumorigenesis and T- and B-cell malignancies. The rs10490072 changed sites for HNF4B, Pou2f2 and Pou5f1 whereas the rs8042680 altered sites for GR, PAX5 and TAL1. The rs7944584 was found to modify regulatory motifs for AP1, AP4, IRF and KAP1. Finally, the rs340874 mapped among promoter and enhancer histone marks in primary naïve and memory forms of helper T cells (CD_4^+) and regulatory T cells from peripheral blood.

DISCUSSION

Previous population-based studies have demonstrated the impact of GWAS-identified variants for T2D on cancer susceptibility [47, 49–52] and patient survival [53]. However, despite these important research advances, there is still a noticeable lack of information regarding the role of T2D-related variants in modulating patient survival especially in hematological malignancies. In this scenario, we decided to investigate for the first time to our knowledge the relationship between 58 genetic variants associated with T2D identified by GWAS and OS of MM patients.

The analysis of the IMMENSE consortium data revealed a significant association of the intronic $HNF1B_{rs7501939}$ SNP with poor OS. We successfully replicated this association in a large and independent population recruited by the University Clinic of Heidelberg. However, although a positive correlation between this variant and eQTL data on PBMCs has been reported [54], we failed to find correlation between the risk allele and *HNF1B* mRNA expression levels on plasma cells from a large cohort of MM patients. This suggested that the effect of this variant on overall survival is not mediated by changes in transcriptional activity of the

gene. Nonetheless, given that *HFN1B* contains multiple independent SNPs or haplotypes that have been associated with *HNF1B* mRNA expression [55, 56] and methylation [57] levels but also with the risk of developing several types of cancer [55–57], it seems to be reasonable to consider the possibility that other SNPs within this locus and showing a stronger association with OS could explain better the link between the *HNF1B* and clinical outcome. However, when we analysed imputed common SNPs from the GWAS conducted in the Heidelberg cohort, we could not find any stronger association signals with OS in the region, which suggested that the $HNF1B_{rs7501939}$ SNP or perhaps a rare SNP in LD with it might be responsible of the observed effect. Future fine-mapping studies encompassing common but also rare variants within or near the *HNF1B* locus are needed to elucidate whether a rare variant or haplotype might account for the observed effect.

HNF1B contains 9 exons and expands over 58 kb on chromosome 17p21 [58, 59]. It encodes for a transcription factor that has been associated with multiple clinical features including early-onset of T2D [56]. In line with this, it has been also suggested that *HNF1B* may induce impaired glucose tolerance and attenuated insulin sensitivity in a miRNA-dependent manner [60], which might lead to an enhanced insulin secretion and the activation of the *IGF1* pathway, an important factor mediating myeloma cell growth, proliferation and cell maturation [27, 59, 61]. Alternatively, it has been postulated that *HNF1B* is able to influence cancer cell survival by promoting the activation of NFkB pathway or through the inhibition of mitochondria-associated apoptotic signals [62]. In support of the tumorogenic effect of HFN1B, it has also been reported that it may act as an oncogene [63] and that the HNF1B gene is amplified in 23% of all cancers and in about 5% of all haematological malignancies (http://broadinstitute.org/tumorscape). On the contrary, it has also been reported that HFN1B may act as tumour suppressor gene [56] and that its expression may largely vary depending on the target tissue. Whereas HNF1B has been found to be overexpressed in ovarian clear cell carcinomas [58] and prostate [64] or endometrial [65] cancers and its silencing induces apoptosis of cancer cells [58], it has been found to be down-regulated in serous epithelial ovarian cancer [57] and colorectal, gastric and pancreatic cancer cell lines [66]. In addition, it has been reported that the down-regulation of HFN1B gene is associated with progression in hepatocarcinoma [67] and poor prognosis in renal [68] and prostate [69] cancers. Considering all the above but also the fact that the association of the HNF1Brs7501939 SNP with OS was driven by a non-diabetogenic (T) allele that does not affect HNF1B mRNA expression, we hypothesize that the effect of this variant to contribute to tumour progression in MM might be mediated by a non-insulin-dependent mechanism. There was a reasonable amount of regulatory data for the HNF1Brs7501939 SNP that supported evidence of the active role of the HNF1B locus. However, whether elevated HNF1B levels lead to tumour transformation and disease progression is not yet understood and functional studies to examine whether HNF1B variants influence cancer prognosis are lacking.

Another interesting finding of this study was the association of the *BCL11A, MADD, PRC1, PROX1, SCL30A8*, *SLC2A2* and *TCF7L2* SNPs with OS. We found an overall association of the $SLC2A2_{rs11920090T}$ and $TCF7L2_{rs7903146T}$ alleles with better OS whereas the association of the $SLC30A8_{rs13266634T}$, $BCL11A_{rs10490072C}$, $PRC1_{rs8042680A}$ and $PROX1_{rs340874G}$ alleles or the $MADD_{rs7944584T/T}$ genotype with OS was restricted to male or female genders. Despite the potential interest of the associations observed for these SNPs with OS, only the association of the $SLC30A8_{rs13266634}$ SNP with poor OS in men reached significance at experiment-wide significance threshold. This result suggested a key role of the *SLC30A8* locus in the modulation of overall survival. However, given the consistency of the overall or gender-specific associations observed for *BCL11A, MADD, PRC1, PROX1, SLC2A2* and *TCF7L2* SNPs with OS across the populations tested and considering that gender-specific genetic alterations might influence MM survival [48], we suggest that these variants might also exert a modest effect to modulate patient survival.

SCL30A8 gene encodes a zinc transporter involved in the control of insulin processing and secretion [70]. Although no previous studies have reported a link between this locus and MM, there are evidences that suggest that zinc transporters might contribute to cancinogenesis [71, 72] through a gender-dependent mechanism [73]. The association of the coding $SLC30A8_{rs13266634}$ SNP with OS was due to a non-diabetogenic allele suggesting that, rather than modulating glucose homeostasis and insulin secretion [74], the effect attributed to the *SLC30A8* locus on MM survival might be driven by a direct effect of Zinc in biological processes such as DNA and RNA stabilization [75], binding of protooncogenes to DNA [75–77] and the activation of IGF1 [26, 27, 61] or telomerase [78]. The $SLC30A8_{rs13266634C}$ allele has been consistently associated with decreased rates of Zinc transport activitiy and reduced intragranular Zinc levels [79]. However, eQTL data on plasma cells from MM patients did not reveal correlation between this variant and *SLC30A8* mRNA levels suggesting that, rather than regulating gene expression, the T allele affect transporter activity in an allele-dose-dependent manner causing increased Zinc concentration and thereby promoting unlimited proliferation of MM cells, disease progression and poor survival. In addition, regulatory data suggest that the *SLC30A8* locus might play a role in survival through the modulation of specific transcription factors implicated in tumour promotion and dissemination.

As for the *HNF1B* and *SLC30A8* SNPs, the association of the *BCL11A*$_{rs10490072}$ and *MADD*$_{rs7944584}$ SNPs with OS was determined by non-diabetogenic alleles. *BCL11A* functions as a myeloid and B-cell proto-oncogen and has been associated with the development of B-cell malignancies [80, 81] whereas *MADD* encodes for a MAP-kinase activating cell domain involved in the control of physiological cell death through TNF- and caspase-dependent apoptosis [82]. In contrast to these associations, the association of the *TCF7L2*$_{rs7903146,}$ *SLC2A2*$_{rs11920090}$ *PRC1*$_{rs8042680}$, and *PROX1*$_{rs340874}$ SNPs with OS was driven by diabetogenic alleles, which suggested that the effect of these variants on OS might be explained by their regulatory effect on insulin secretion and, consequently, on cell proliferation and tumour cell growth. Whereas *SLC2A2* encodes a highly efficient glucose transport that is expressed in pancreatic cells and regulates insulin secretion by modulating entry of glucose into the pancreatic cell [83], TCF7L2, PRC1 and PROX1 are proteins that have been involved in β-cell survival and function [84] and in glucose and nonesterified fatty acids or branched-chain amino acids metabolism in liver [85, 86]. Despite these interesting results and the regulatory data observed for all these SNPs, the lack of information regarding T2D status among MM patients did not allow us to ensure that the observed effect of the TCF7L2, SLC2A2, PRC1, and PROX1 SNPs on OS could not be due to a different distribution of diabetic patients when grouping by genotype or gender.

This study had both strengths and limitations. Strengths include the use of relative large discovery and replication populations that allowed us to validate the most interesting associations. Limitations include lack of information regarding the classical genetic prognostic factors (chromosomal abnormalities, etc.), T2D status and a relatively small statistical power to detect modest associations with OS, especially when gender-stratified analysis were performed. Another limitation was the partial overlapping of genetic information between studies and the use of imputed genotypes that did not allow to perform a reliable validation of the association observed for genetic variants within *ADAMTS9, BCL11A, KCNQ1, MADD* and *PROX1* genes with overall survival.

In conclusion, this study reports the first evidence of an association between the *HNF1B*$_{rs7501939}$ SNP and OS for MM and suggests that the *HNF1B* locus might, likely through a non-insulin-dependent mechanism, play an important role in modulating MM prognosis. Likewise, this study shows a strong association of the *SLC30A8*$_{rs13266634}$ SNP with poor OS in men that might, at least in part, account for gender differences in OS. Additional studies using larger and well-characterized populations are needed to further replicate these findings but also those involving the *BCL11A, MADD, PRC1, PROX1, SLC2A2* and *TCF7L2* loci on OS.

MATERIALS AND METHODS

Patients, clinical data collection and survival endpoint definition

A total of 1420 Caucasian MM patients were ascertained through the IMMEnSE consortium. Full details of this consortium have been published elsewhere [87]. In brief, inclusion criteria were newly diagnosed MM with Salmon & Durie stage I, II and III, age 18–90 years inclusive and Caucasian origin. DNA was purified from blood specimens using the QIAamp DNA Blood Mini Kit (Qiagen) and clinicophathological characteristics including age, gender, country of origin and disease stage (Durie-Salmon) were retrospectively gathered from medical records in each participant institution (Table 1). Diagnosis of patients with symptomatic MM was carried out by hematologists according to the International Myeloma Working Group (IMWG) criteria [88, 89]. All patients within the IMMENSE consortium for whom survival information was available were included in the study (936 MM cases, 454 women and 482 men) (Table 1). All participants gave their written informed consent to participate in the study.

SNP selection and genotyping

Fifty-eight variants were selected based on the GWAS for T2D [84, 90–126] and were genotyped in the IMMEnSE consortium population (Table 4). We considered only SNPs that were replicated in large and independent populations or which came up in several GWAS or their meta-analyses. Additional criteria were potential functionality and linkage disequilibrium (LD) between the reported SNPs. The genotyping of the selected polymorphisms was carried out at GENYO (Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain) using KASPar® assays (LGC Genomics, Hoddesdon, UK) according to manufacturer's instructions. For internal quality control, 5% of samples were randomly selected and included as duplicates. Concordance between the original and the duplicate samples for the 58 SNPs was ≥ 99.0% . Call rates for all SNPs were ≥ 90.0% with the exception of the WFS1$_{rs734312}$ SNP that was excluded from further analyses.

Replication

For replication purposes, seven hundred MM patients (296 women and 404 men) were provided by the University Clinic of Heidelberg (Germany). This cohort consists of 98 GMMG-HD3 trial patients, 291 GMMG-HD4 trial patients and 311 patients transplanted in Heidelberg but not enrolled in clinical trials (Table 1). Ethical approval for these patients and written informed

Table 4: Selected type-2 diabetes-related SNPs

Gene name	dbSNP rs#	Nucleotide substitution	Reference allele IMMENSE	GWAS-identified risk allele for T2D	Location/Aa substitution	References
ADAM30	rs2641348	T/C	T	C	L359P	[103, 124]
ADAMTS9	rs4607103	**T**/C[1]	C	C	Near gene	[84, 104, 124]
ADCY5	rs11708067	T/**C**[2]	T	T	Intronic	[96, 111]
ADRA2A	rs10885122	G/T	G	G	Near ADRA2A	[96]
ARAPI, CENTD2	rs1552224	G/T	T	T	Near gene	[105, 120]
BCL11A	rs10490072	C/T	T	T	Near gene	[124]
CDC123, CAMK1D	rs12779790	A/**G**[3]	A	G	Near gene	[84, 104, 124]
CDKAL1	rs7754840	C/G	G	C	Intronic	[95, 97, 113]
CDKN2A-2B	rs564398	T/**C**[4]	T	T	Near gene	[84, 95, 104, 113, 116, 122, 124]
CDKN2A-2B	rs10811661	T/C	T	T	Near gene	
CDKN2A-2B	rs2383208	A/G	A	A	Near gene	
COL5A1	rs4240702	C/T	C	n/s	Intronic	[91]
CRY2	rs11605924	A/C	C	A	Intronic	[96]
DCD	rs1153188	A/T	A	A	Near gene	[124]
EXT2	rs1113132	C/G	C	C	Intronic	[97, 114]
FADS1	rs174550	C/T	T	T	Intronic	[96]
FAM148B	rs11071657	A/G	A	A	Near gene	[93, 96]
FLJ39370	rs17044137	A/T	T	A	Near gene	[95]
FTO	rs8050136	A/C	C	A	Intronic	[104, 125, 126]
G6PC2	rs560887	G/A	G	G	Intronic	[91, 92, 94, 96, 107]
GCK	rs1799884	G/A	G	A	Near gene	[91, 92, 94, 96, 107]
GCKR	rs1260326	C/**T**[5]	C	T	L445P	[91, 96, 111]
HHEX	rs1111875	G/A	G	G	Near gene	[95, 97, 104, 113, 114, 125, 126]
HMGA2	rs1531343	C/G	G	C	Near gene	[105, 120]
HNF1A (TCF1)	rs7957197	A/T	T	T	Intronic	[105, 120]
HNF1B (TCF2)	rs7501939	C/**T**[6]	C	C	Intronic	[101, 110]
IGF1	rs35767	C/T	C	C	Near gene	[96, 106]
IGF2BP2	rs4402960	G/T	G	T	Intronic	[84, 95, 97, 104, 113, 125, 126]
IL13	rs20541	C/**T**[7]	C	T	R144Q	[95]
IRS1	rs2943641	C/T	C	C	Near gene	[109, 117, 120]
JAZF1	rs864745	A/G	A	A	Intronic	[84, 124]
KCNJ11	rs5215	T/**C**[8]	T	C	V337I	[95, 98, 104, 113, 121, 125, 126]
KCNJ11	rs5219	C/T	C	T	K23E	
KCNQ1	rs2237897	C/T	T	C	Intronic	[118, 119, 122, 123]
KCNQ1	rs2074196	G/**T**[9]	G	G	Intronic	
KCNQ1	rs2237892	C/T	C	C	Intronic	
KCNQ1	rs2237895	A/C	A	C	Intronic	
KCNQ1OT1	rs231362	G/A	G	G	Intronic	[105, 118, 120]

LTA	rs1041981	A/C	A	A	T60N	[102]
MADD	rs7944584	A/**T**[10]	A	A	Intronic	[96]
MCR4	rs12970134	A/G	G	A	Near gene	[93]
MTNR1B	rs1387153	C/T	C	T	Near gene	[91, 107, 120]
NOTCH2	rs10923931	G/T	G	T	Intronic	[104, 124]
PKN2	rs6698181	C/T	C	T	Intergenic	[95]
PPARG	rs1801282	C/**G**[11]	C	C	P12A	[90, 95, 104, 113, 121, 124–126]
PRC1	rs8042680	A/C	C	A	Intronic	[105, 120]
PROX1	rs340874	A/**G**[12]	A	G	Promoter	[96]
RBMS1	rs7593730	C/T	C	T	Intronic	[108]
SLC2A2	rs11920090	A/T	A	T	Intronic	[96]
SLC30A8	rs13266634	C/**T**[13]	C	C	R325W	[84, 90, 95–97, 104, 113, 114, 125, 126]
TCF7L2	rs7903146	C/**T**[14]	C	T	Intronic	[95–97, 99, 104, 11–115, 125, 126]
TCF7L2	rs12255372	G/**T**[15]	G	T	Intronic	
THADA	rs7578597	T/C	T	T	T1187A	[124]
TP53INP1	rs896854	A/G	G	G	Intronic	[105, 120]
TSPAN8	rs7961581	C/T	T	C	Near gene	[100]
VEGFA	rs9472138	C/T	C	T	Near gene	[124]
WFS1	rs734312	A/G	A	n/s	H611R	[110]
WFS1	rs10010131	A/G	G	G	Intronic	[110]

n/s, not specified; Aa, Aminoacid; GWAS, genome-wide association studies; OS, overall survival.
References are listed in Supplementary Material. Effect allele in bold and underlined.

[1]T/T genotype was associated with poor OS in women with an opposite but not significant effect in men.
[2]C allele was associated with better OS in women with no effect in men.
[3]G allele was associated with better OS in women with no effect in men.
[4]C/C genotype was associated with better OS. No gender-specific effect was observed.
[5]T/T genotype was associated with poor OS. No gender-specific effect was observed.
[6]T allele was associated with poor OS. No gender-specific effect was observed.
[7]T/T genotype was associated with poor OS in women with an opposite but not significant effect in men.
[8]C allele was associated with poor OS in men with an opposite but not significant effect in women.
[9]T allele was associated with poor OS. No gender-specific effect was observed.
[10]T/T genotype was associated with better OS in men with no significant effect in women.
[11]G allele was associated with better OS in men with an opposite but not significant effect in women.
[12]G allele was associated with poor OS in women with an opposite but not significant effect in men.
[13]The presence of each additional copy of the T allele was associated with poor OS in men with no effect in women (additive effect).
[14]T allele was associated with better OS in men with no effect in women.
[15]T allele was associated with better OS in men with an opposite but not significant effect in women.

consent of trial patients was also obtained. Clinical and survival data were prospectively collected for trial patients on case report forms and retrospectively gathered from medical records for none-trial patients. Genetic information of 53 SNPs (36 genotyped SNPs and 17 imputed SNPs) was extracted from the GWAS conducted in the Heidelberg cohort. After imputation, no information was available for 5 SNPs.

In silico functional analysis

Haploreg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) and ENCODE annotation data (https://genome.ucsc.edu/ENCODE/) were used to predict the functional role of potentially interesting SNPs.

eQTL analysis

We also assessed whether selected SNPs correlated with mRNA expression levels in a public eQTL browser for peripheral blood mononuclear cells (http://genenetwork.nl/bloodeqtlbrowser/) [54]. Expression quantitative trait loci (eQTL) data on malignant plasma cells of 658 patients from the University Clinic of Heidelberg (Germany) were also available for this study. Detailed information on sample collection and clinico-pathological characteristics of MM patients as well as technical details of gene expression analysis have been published elsewhere [127].

Statistical analysis

We used chi-square tests to assess Hardy–Weinberg Equilibrium (HWE) for each SNP among IMMEnSE patients. The primary outcome was OS and the endpoint was defined as death from any cause. Survival time was calculated as the time from MM diagnosis (discovery population) or the first stem cell transplantation (replication population) until the occurrence of the study endpoint, censoring at the date of death or the last observed follow-up time. Association with OS defined as hazard ratio (HR) was calculated for each SNP using Cox regression multivariate analysis adjusted for age, gender, country of origin and Durie-Salmon stage (IMMENSE cohort) or for age, gender and clinical trial (Heidelberg cohort). Association estimates were calculated according to dominant, recessive and log-additive models of inheritance with the major allele as reference for regression analyses (Table 4). We also performed gene-gender interaction analyses to determine whether the association between SNPs and MM OS was of similar magnitude in men and women. Survival function was displayed using the Kaplan-Meier method [128] and survival differences across genotypic groups were analysed using the log-rank test.

In order to account for multiple comparisons, we used the Meff/MeffLi method [129], which calculates the effective number of independent genetic markers analysed ($N = 54$) on the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNPs (http://neurogenetics.qimrberghofer.edu.au/SNPSpDlite). In addition, we also considered the number of genetic inheritance models tested (dominant, recessive and log-additive). This resulted in a study-wide significance threshold of 0.00031 ([0.05/54]/3) to keep type I error rate at 5% .

Finally, in order to confirm significant associations, a meta-analysis combining genetic data obtained in the IMMENSE population with those extracted from the GWAS conducted in the Heidelberg cohort was also performed following dominant, recessive and additive models of inheritance. The I^2 statistic was used to assess heterogeneity between both studies and the pooled HR was computed using the random-effect model (assuming that between-study variation might depend on chance or random variation and an individual study effect). Random-effects models are more conservative than fixed-effects models and give rise to wider confidence intervals (CI), which ensures the reliability of the results even though the data come from studies with a relatively different design. All statistics were calculated using SPSS (v.20) and STATA (v.12) for MAC.

CONFLICTS OF INTEREST

All authors have nothing to disclose.

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VI. DISCUSIÓN

1. Introducción

A pesar de los avances acontecidos en las últimas décadas, el MM sigue siendo una de las neoplasias hematológicas con peor pronóstico (De Angelis R *et al*, 2014) y una de las enfermedades que presenta un mayor impacto negativo en la calidad de vida relacionada con la salud (Allart-Vorelli P *et al*, 2015).

El MM presenta varios niveles de heterogeneidad. En primer lugar existe heterogeneidad clínica, que abarca numerosos factores pronósticos inherentes al huésped, de los cuales algunos no forman parte de la valoración estándar del pronóstico en la práctica clínica habitual, o simplemente son desconocidos. Por otra parte, en la medida en que los estudios de genómica se aplican tanto al diagnóstico como a la evolución de los pacientes, existe una enorme heterogeneidad molecular. Se ha señalado una relación entre el evento definitorio de MM y las anomalías citogenéticas primarias (Greenberg AJ *et al*, 2014), hasta el punto de considerar al MM como un grupo de trastornos con pronóstico distinto, más que como una única entidad. También se ha observado una asociación entre las anomalías citogenéticas primarias y la raza (Greenberg AJ *et al*, 2015).

Además, el MM, como otros tipos de cáncer, puede estar constituido por varias clonas que compiten por los recursos de tal forma que provocan progresión de la enfermedad y resistencia al tratamiento. En este contexto de heterogeneidad intraclonal (Brioli A *et al*, 2014), el tratamiento puede ser considerado como una presión selectiva que actúa de manera diferencial en las diferentes clonas impactando en su probabilidad de supervivencia. Todo ello conduce a diversos patrones de evolución clonal (Keats JJ *et al*, 2012; Egan JB *et al,* 2012; Bolli N *et al,* 2014).

Esta heterogeneidad clínica y molecular se traslada en una enorme heterogeneidad a nivel de resultados, existiendo pacientes con una larga supervivencia y otros que

fallecen a los pocos meses del diagnóstico. Los actuales sistemas de estratificación del riesgo (Palumbo A *et al*, 2015b) han representado un gran avance en la evaluación general del pronóstico de los pacientes, permitiendo diseñar una estrategia de tratamiento adaptado al riesgo. Se han reportado otros sistemas más sofisticados incluyendo información molecular (Kuiper *et al*, 2015; Weinhold N *et al*, 2016; Chng WJ *et al*, 2016). Sin embargo, estos sistemas son incapaces de predecir de forma fiable la gran variabilidad en los resultados, ni el pronóstico individualizado de cada paciente, ya que no tienen en cuenta toda la información clínica y molecular relevante.

A nivel clínico, una análisis minucioso de la comorbilidad basal puede ayudar a explicar parte de la variabilidad observada en los resultados, en términos de supervivencia y mortalidad precoz.

A nivel molecular, el análisis de determinadas variantes genéticas puede también aportar información relevante para predecir el comportamiento de los pacientes en términos de supervivencia global (Ziv E *et al*, 2015; Johnson DC *et al*, 2016).

La información clínica y genómica debe por tanto ser integrada para disminuir el nivel de incertidumbre en los resultados. La comorbilidad se puede convertir en un interesante vínculo que permite integrar información clínica y molecular. Los trabajos presentados pretenden avanzar en este doble sentido para intentar mejorar la valoración del pronóstico de los pacientes con MM.

A pesar del indudable valor de los ensayos clínicos, existe también un creciente interés por conocer los resultados clínicos de los pacientes con MM de la vida real (Yong K *et al*, 2016).

2. Discusión del diseño

2.1 Metodología genérica

2.1.1 Estudios de base poblacional. Los resultados en salud del MM deben ser necesariamente enmarcados en un marco temporal, debido al vertiginoso cambio acontecido en años recientes en el paradigma terapéutico, tanto en el tratamiento de base como en los cuidados de soporte. Los estudios de base poblacional de un solo centro, con un registro clínico contrastado con un registro de cáncer, permiten analizar la tendencia temporal de los resultados, asegurando la inclusión de todos los nuevos casos incidentes, incluyendo los pacientes unfit, no susceptibles de tratamiento activo.

En onco-hematología, el *end-point* primario más importante en la mayoría de los estudios, ya sean ensayos clínicos diseñados con suficiente seguimiento o estudios de base poblacional, sigue siendo la supervivencia global. Los estudios de base poblacional se convierten así en un complemento necesario de los ensayos clínicos, describiendo los resultados de los pacientes (todos los pacientes) de la vida real en un marco geográfico y temporal definido, en contraposición a los ensayos clínicos, que en general reflejan lo ocurrido en un grupo muy seleccionado de pacientes.

2.1.2 Análisis de la comorbilidad a nivel clínico y genómico. Las herramientas pronósticas disponibles para los pacientes con MM no pueden justificar la enorme variabilidad en los resultados clínicos, en términos de supervivencia global y mortalidad precoz. Un estudio exhaustivo de la comorbilidad basal, así como un análisis de determinadas variantes genéticas, pueden ayudar a explicar parte de esta heterogeneidad, y ayudar en definitiva a adaptar, individualizar y optimizar el manejo terapéutico.

Un *end-point* de especial interés es la supervivencia libre de progresión, que en muchos casos se asocia a la calidad de vida relacionada con la salud. Sin embargo, la

supervivencia libre de progresión no se ha utilizado en los presentes trabajos por ser determinadas variables de interés pronóstico (ACAR), la variable respuesta y la confirmación de la progresión, elementos con criterios cambiantes en el tiempo. De igual manera, los cambios tecnológicos en años recientes, tanto en las técnicas de imagen (RNM, PET/TC, etc) como en la valoración de la respuesta (EMR), han modulado el manejo y monitorización de los pacientes con MM.

Los estudios de genes candidato y GWAS han dado un importante salto cualitativo reciente demostrando que la presencia de determinadas variantes genéticas está asociada de forma significativa a la supervivencia global en los pacientes con MM. Un ejemplo de los pocos estudios de genes candidato existentes hasta la fecha con impacto en la supervivencia general se presenta en esta tesis.

2.2 Metodología específica

2.2.1 Registros de cáncer. No cabe duda de que los avances más relevantes en el manejo clínico de los pacientes con MM se ha derivado de los ensayos clínicos habitualmente multicéntricos. Sin embargo, sus resultados no siempre son generalizables a la población general debido a que se trata generalmente de pacientes muy seleccionados. Esta "debilidad" se podría subsanar diseñando ensayos que incluyan a pacientes con criterios de inclusión y exclusión más amplios, y en particular, pacientes mayor número de comorbilidades y comorbilidades más severas. Otra opción más plausible es complementar los resultados de los ensayos con los registros de cáncer de base poblacional, que idealmente deben llevarse a cabo de forma prospectiva y en el contexto de registros de cáncer bien establecidos, que cuentan con la infraestructura necesaria para garantizar la fiabilidad en los resultados en base al uso de estándares internacionales en su metodología (IARC). Estos registros tradicionalmente han carecido de información clínica, por lo que su misión fundamental ha sido valorar la

incidencia del cáncer, su tendencia temporal y la supervivencia global. El Registro de Cáncer de Granada comenzó su andadura en 1985 y en la actualidad cuenta con un volumen de información muy importante para cada tipo de cáncer en la provincia de Granada. Además, está inmerso en la Escuela Andaluza de Salud Pública, por lo que permite su interacción con los profesionales sanitarios promoviendo la investigación epidemiológica aplicada, con una sólida base metodológica. Los registros usan la Clasificación Internacional de Enfermedades (CIE) para garantizar la comparabilidad a nivel global, en base a un sistema de códigos estándar. Por ejemplo, el código para el MM es C90.0 y es muy importante no incluir pacientes con neoplasias de células plasmáticas afines, como la leucemia de células plasmáticas primaria (C90.1), el plasmocitoma extramedular (C90.2) o el plasmocitoma solitario (C90.3), con criterios diagnósticos y pronóstico diferentes. La CIE en el contexto de las neoplasias hematológicas es compleja y el personal de los registros debe estar muy cualificado para codificar correctamente cada caso. Por otra parte, los criterios diagnósticos del MM (y de las patologías afines) son cambiantes en el tiempo y es, por tanto, imprescindible definir y establecer los criterios diagnósticos (y la codificación secundaria) en su marco temporal adecuado.

2.2.2. Registros clínicos de base poblacional. Los registros de cáncer de base poblacional se nutren de diversas fuentes, para asegurar que todo caso incidente es incluido, pero la fuente primaria fundamental son los propios centros sanitarios, donde los pacientes en este caso con MM son diagnosticados y tratados. En los hospitales la fuente de datos básica es la propia historia clínica, que ha pasado recientemente en el caso de Granada del formato papel al formato electrónico. A pesar de la informatización creciente en los sistemas de gestión hospitalarios, la información de la historia clínica digital debe ser procesada, matizada, ampliada, criticada y complementada por un

profesional especialista en cada tipo de cáncer. Además, es el profesional sanitario quien debe establecer qué variables de interés clínico deben recogerse de forma prospectiva en cada momento. El aprovechamiento de la información clínica contenida en la historia médica electrónica representa una fuente de datos infrautilizada, con un potencial interés en investigación para revelar elementos de estratificación del riesgo y correlaciones desconocidas. La integración de los datos clínicos con los datos genéticos permitirá un conocimiento más profundo de la relación genotipo-fenotipo (Jensen PB *et al,* 2012).

Un problema habitual en los registros observacionales, a pesar de ser prospectivos, son los datos faltantes. Sólo una recogida sistemática de todas las variables de interés basales permitirá una adecuada monitorización del MM y de la respuesta al tratamiento (Rifkin RM *et al*, 2015). Para asegurar la consistencia y comparación entre los estudios, se requiere un estricto control de calidad antes de estimar la supervivencia (Li R *et al*, 2014).

La complejidad creciente en el manejo del MM hace necesaria una sub-especialización y la creación de unidades específicas para atender a los pacientes con gammapatías monoclonales y con MM en particular. Uno de los objetivos fundamentales de estas unidades específicas debe ser la creación de un registro clínico del máximo rigor. Por tanto, en nuestro contexto, en el año 2011 se creó la Unidad de Gammapatías Monoclonales y el Registro Clínico de MM (RCMMBP) uniendo las ventajas del Registro de Cáncer que garantiza la inclusión de todos los casos (algunos pacientes no llegan a ser valorados por el clínico por diferentes motivos) y las ventajas de la inclusión de todas las variables clínicas de interés establecido y/o potencial.

2.2.3 Participación en consorcios. La coordinación y gestión de la recogida de muestras para los estudios genéticos es de vital importancia para garantizar la

integración de la información clínica y genómica, en el contexto habitualmente de consorcios como el IMMEnSE, en el que nuestro grupo participa desde su inicio (Martino A *et al*, 2012). Esta estrategia es necesaria para garantizar el tamaño muestral necesario para llevar a cabo estudios tanto de genes candidato como de GWAS, cuyo objetivo es demostrar el efecto, habitualmente modesto, de determinadas variantes genéticas, sobre el riesgo de desarrollar MM o el impacto en la supervivencia.

3. Discusión de los resultados

3.1 Supervivencia global

La supervivencia global es el *end-point* más importante en la evaluación de los resultados clínicos del MM, tanto en los ensayos clínicos diseñados a tal efecto, como en los estudios de base poblacional. Nuestro registro clínico nos ha permitido medir la supervivencia global en una cohorte de pacientes con MM de nuevo diagnóstico a lo largo de más de tres décadas.

En general, hemos asistido a un incremento progresivo en la supervivencia en los últimos años para todos los grupos de edad (Pulte D *et al*, 2016). La Tabla 8 resume los datos de los estudios recientes más relevantes sobre supervivencia en MM. Como es previsible, los estudios de base poblacional, ya sean de centro único (Turesson I *et al*, 2010; Ríos-Tamayo R *et al*, 2015) o multicéntricos (Kristinsson SY *et al*, 2007; Pozzi S *et al*, 2013; Oortgiesen B *et al* 2014) , muestran unos resultados más modestos, ya que reflejan la realidad de una cohorte no seleccionada de pacientes. En cambio, los estudios que no son de base poblacional, de centro único (Kumar S *et al*, 2008; Geng C *et al*, 2013; Kumar S *et al*, 2014) o multicéntricos (Kastritis E *et al*, 2009; Ludwig H *et al*, 2010; Dimopoulos MA *et al*, 2014; Liwing J *et al*, 2014; Ozaki S *et al*, 2014), hacen referencia a cohortes de pacientes seleccionados, tratándose en ocasiones de centros internacionales de referencia como la Clínica Mayo. En algunos estudios se refleja solamente la supervivencia en un período selectivo del total del marco temporal del estudio, lo que puede conducir a confusión. Nuestro estudio presenta una mediana de supervivencia global de 26 meses para toda la cohorte, sin embargo si tomamos en cuenta sólo los pacientes diagnosticados a partir de 2010, la mediana de supervivencia no se ha alcanzado y superará los 60 meses. En cualquier caso, nuestra supervivencia es algo mejor que la del único estudio de similares características (centro único, base

poblacional) publicado por Turesson *et al* (22.2 meses), probablemente debido a que nuestra cohorte incluye un marco temporal más reciente y refleja por tanto, la tendencia a la mejoría global en supervivencia de los últimos años.

Tabla 8. Estudios recientes sobre SG en MM de nuevo diagnóstico

Primer autor	Tipo de estudio	Periodo	Año publicación	Nº pacientes	Mediana SG (meses)
Kristinsson et al	Nacional BP (Suecia)	1973-2003	2007	14381	Mejor RSR a 5 años: 0.36
Kumar et al	Centro único	1971-2006	2008	2981	44.8 (a partir de 1996)
Kastritis et al	Multicéntrico (Grecia)	1985-?	2009	1376	48.0 (a partir de 2000)
Turesson et al	Centro único PB	1950-2005	2010	773	22.2
Ludwig et al	Multicéntrico	1982-2002	2010	10549	44.4
Pozzi et al	Multicéntrico BP (Italia)	1988-2009	2013	1206	50.9 % (a 5 años)
Geng et al	Centro único	2006-2011	2013	264	61.0
Kumar et al	Centro único	2001-2010	2014	1038	62.4
Dimopoulos et al	Multicéntrico (Grecia)	1990-2011	2014	1773	54.0 (a partir de 2005)
Liwing et al	Multicéntrico (Suecia)	2000-2011	2014	1638	33.6 vs 82.8 (TAPH)
Ozaki et al	Multicéntrico (Japón)	2004-2009	2014	318	55.2 (ISS III)
Oortgiesen et al	Multicéntrico BP (Holanda)	2005-2013	2014	270	49.5
Ríos-Tamayo et al	Centro único PB	1985-2014	2015	582	26.0

Abreviaturas: BP= Base poblacional, RSR= Ratio de supervivencia relativa, SG= Supervivencia Global, TAPH= trasplante autólogo de progenitores hematopoyéticos.

3.1.1 Factores pronósticos clásicos

Nuestro estudio señala que, además de la importancia de la edad, los factores pronósticos asociados de manera independiente a la supervivencia son: ISS, LDH, insuficiencia renal, TAPH y la presencia basal de amiloidosis.

La edad es una variable pronóstica fundamental en MM (Ludwig H *et al*, 2010; Bringhen S *et al*, 2013; Chretien ML *et al*, 2014). La supervivencia también está mejorando en los pacientes mayores de 65 años, pero en esta población la eficacia ha de

ser balanceada con la toxicidad y la calidad de vida (Wildes TM *et al*, 2014). En el estudio de Turesson *et al* (2010) no se encontró una tendencia significativa a la mejora en la supervivencia en pacientes mayores de 65 años. En nuestro estudio, tras excluir del análisis a los pacientes *unfit* o subsidiarios solamente de cuidados paliativos, se obtiene una mejoría progresiva de la supervivencia con significación estadística marginal en pacientes mayores de 65 años.

El ISS (Greipp PR *et al*, 2005) es un instrumento de estratificación del riesgo consolidado y validado, por lo que se ha convertido en la base para diseñar otras herramientas que mejoren aún más su poder discriminatorio pronóstico. Así, si se combina ISS con las ACAR detectadas por FISH, se mejora la valoración del riesgo (Avet-Loiseau H *et al*, 2013). Añadiendo LDH y ACAR nace el R-ISS (Palumbo A *et al*, 2015b). Si se combina el ISS con PEG nace el EMC92-ISS (Kuiper *et al*, 2012; Kuiper *et al*, 2015). Combinando ISS con un set de mutaciones y cambios en el número de copias nace el ISS-MUT (Walker BA *et al*, 2015). En nuestra cohorte se ha confirmado la utilidad del ISS en la estratificación del riesgo.

La LDH es una enzima cuya elevación, aunque ocurre con poca frecuencia, se correlaciona con la presencia de alta masa tumoral. Por su valor pronóstico y facilidad de medida, ha sido incorporada al R-ISS. En nuestro estudio no resultó ser un factor pronóstico independiente, al ajustar por las otras variables predictoras.

La insuficiencia renal es un factor pronóstico consolidado y crucial en el MM (Dimopoulos MA *et al*, 2010; Gonsalves WI *et al*, 2015; Dimopoulos MA *et al*, 2016). El 52% de los pacientes en nuestro estudio presenta una TFGe basal <60%. Nuestro estudio confirmó su importancia como factor pronóstico independiente.

El TAPH es una estrategia terapéutica consolidada cuya eficacia está bien demostrada incluso en la era de los nuevos agentes. Por todo ello, la tendencia actual es a realizar

este procedimiento sin que la edad cronológica sea una contraindicación. Un 19,2% de los pacientes de nuestra cohorte fueron sometidos al menos a un TAPH a lo largo del periodo de estudio. Debido a su eficacia, la tendencia general es a aumentar el número de pacientes candidatos. En nuestro centro, de los pacientes diagnosticados en el último quinquenio se han trasplantado el 36%.

3.1.2 Comorbilidad como factor pronóstico

Hasta donde conocemos, este es el primer estudio de un único centro de base poblacional que valora el impacto de la comorbilidad basal en la supervivencia global de los pacientes con MM de nuevo diagnóstico. De todo el panel de comorbilidades registrado, sólo la presencia de amiloidosis asociada al MM en el momento del diagnóstico, que ocurre sólo en un 2,1% en nuestra cohorte, se asocia a la supervivencia como un factor pronóstico independiente, al ajustar por las variables anteriormente señaladas. Es muy probable que otras comorbilidades influyan también en la supervivencia de los pacientes, pero en nuestro estudio no alcanzaron significación estadística en el modelo de regresión multivariante. Las razones pueden ser varias, en ocasiones el escaso número de pacientes como en el caso de la infección por el virus de la inmunodeficiencia humana, en otras porque no se realizó un análisis específico para determinadas entidades, como la cirrosis hepática, o para subgrupos con comorbilidades de especial intensidad, como la enfermedad pulmonar obstructiva crónica grave.

El registro de la comorbilidad está aceptablemente estandarizado en el contexto del TAPH (Sorror ML *et al*, 2005; Raymondi R *et al*, 2012; Sorror ML *et al*, 2014; Saad A *et al*, 2015), pero en la actualidad no existe una aproximación estandarizada para medir la comorbilidad en los pacientes con MM no candidatos (Safarti D, 2012), que son los más frecuentes y donde la comorbilidad se observa de forma más patente debido al acúmulo de patologías asociadas con el envejecimiento. No existe consenso para tomar

determinadas decisiones clínicas basadas en la comorbilidad. A pesar de sus interaccioes, la comorbilidad, el estado funcional y la calidad de vida relacionada con la salud son entidades separadas con efectos independientes en los resultados (Safarti D *et al,* 2016).

3.2 Mortalidad precoz y comorbilidad

Un aspecto que ha generado gran interés recientemente en los pacientes con MM de nuevo diagnóstico es la mortalidad precoz. La mortalidad precoz refleja la primera fase de la curva en el análisis de supervivencia y las variables implicadas no necesariamente son las mismas que inciden en la supervivencia global, y esto es particularmente cierto para la comorbilidad.

Nuestro estudio de un solo centro, de base poblacional, analiza sistemáticamente el impacto diferencial y dependiente del tiempo, de la comorbilidad en la mortalidad precoz de los pacientes con MM de nuevo diagnóstico. En nuestra cohorte, tras excluir los pacientes *unfit* no candidatos a recibir tratamiento antimieloma, el porcentaje de mortalidad a los 2, 6 y 12 meses fue 10.6%, 20% y 28.6%, respectivamente. El análisis temporal muestra una tendencia progresiva a disminuir la mortalidad precoz en los últimos años. La edad y la insuficiencia renal son factores pronósticos asociados a la mortalidad precoz en los tres puntos de corte analizados (2, 6 y 12 meses tras el diagnóstico). Junto a ellos, la presencia de enfermedad respiratoria es un factor asociado de forma independiente a la mortalidad a los dos meses, mientras que la enfermedad hepática es un factor predictor de mortalidad antes de los 6 meses. Finalmente, a los 12 meses, el ISS y la infección por virus de hepatitis C, son los predictores independientes, junto a la edad y la insuficiencia renal.

La Tabla 9 refleja los resultados de los escasos estudios enfocados al estudio de la mortalidad precoz en el MM de nuevo diagnóstico.

Tabla 9. Estudios recientes sobre MP en MM de Nuevo diagnóstico							
Primer autor	**Tipo de estudio**	**Periodo**	**Año publicación**	**Nº pacientes**	**MP 2**	**MP 6**	**MP 12**
Augustson et al	Multicéntrico (RU)	1980-2002	2005	3107	10.0	-	-
Kastritis et al	Centro único	1994-2012	2013	509	6.0	13.0	18.0
Terebelo et al	Multicéntrico (USA)	2009-2013	2013	1494	-	7.0	-
Kumar et al.	Centro único	2001-2010	2014	1038	-	-	13.0
Dimopoulos et al	Multicéntrico (Grecia)	1990-2011	2014	1773	12.0/7.0/ 3.0[a]	-	-
Holmström et al	Multicéntrico (Dinamarca)	2005-2012	2015	1497	-	22.0	-
Costa et al	Multicéntrico (SEER) BP	1993-2010	2015	30324	-	-	28.6
O'Donnell et al	Centro único	2005-2015	2015	836	-	-	32.0[b]
Hsu et al	Centro único	2002-2015	2015	451	12.6	-	-
Chen et al	Centro único	2007-2013	2016	122	-	22.9	-
Ríos-Tamayo et al	Centro único BP	1985-2015	2016	621	10.6	20.0	28.6

Abreviaturas: BP= Base poblacional, MP2=Mortalidad precoz a los 2 meses, MP6= Mortalidad precoz a los 6 meses , MP12= Mortalidad precoz a los 12 meses , [a] % MP según TFGe (<30, 30-59 or >30 ml/min), [b] % MP a los 24 meses. SEER: *Surveillance Epidemiology and End Result Registry*.

Augustson *et al* reportaron en 2005 una mortalidad precoz a los 2 meses del 10% tras analizar los resultados de 5 ensayos clínicos, siendo la causa principal de mortalidad la infección en el 45% de los pacientes. Desde ese momento no hemos localizado ningún trabajo relevante en mortalidad precoz hasta 2013, momento en el que por primera vez

se utilizan los 3 puntos de corte indicados, por el grupo de Atenas (Kastritis E *et al*, 2013), con unos resultados espectaculares, destacando una mortalidad precoz a los dos meses de sólo un 6%, la más baja reportada hasta la fecha. Otros trabajos de un único centro (Kumar SK *et al*, 2014; O'Donnell EK *et al*, 2015; Hsu P *et al*, 2015; Chen YK *et al*, 2016) o multicéntricos (Terebelo HR *et al*, 2013; Dimopoulos MA *et al*, 2014; Holmström MO *et al*, 2015; Costa LJ *et al*, 2015) reflejan resultados muy heterogéneos indicando el valor para un solo punto de corte. Destaca una mortalidad precoz a los 12 meses de sólo un 13% en el estudio de la Clínica Mayo (Kumar SK *et al*, 2014), el mejor dato en este punto de corte hasta el momento. El estudio de Holmström *et al* es de ámbito nacional pero sólo hace referencia a pacientes no candidatos a TPH.

La naturaleza del estudio puede condicionar los resultados. Ninguno de los estudios indicados de un solo centro es de base poblacional, salvo el nuestro. De los estudios multicéntricos, sólo el de Costa *et al* es de base poblacional reflejando los resultados del SEER, y su mortalidad a los 12 meses es curiosamente idéntica a la nuestra (28.6%) (Costa LJ *et al*, 2015) . Recientemente se han publicado una serie de recomendaciones para intentar reducir la mortalidad precoz (Gonsalves WI *et al*, 2016).

La prevención de la infección es un punto crucial. Los pacientes con MM presentan un riesgo basal de infección 7 veces mayor que la población normal (Blimark C *et al*, 2015). Al igual que ocurre con otros trabajos, en nuestro estudio la infección sigue siendo la primera causa de mortalidad en los primeros 12 meses. Este dato enfatiza aún más el papel de la comorbilidad, ya que es conocido que varias de las comorbilidades más frecuentemente asociadas al MM, como la DM2 (Pearson-Stuttard J *et al*, 2016) o la obesidad (Kaspersen KA *et al*, 2015; Harpsøe MC *et al*, 2016) aumentan el riesgo de infección, que ya está aumentado de forma basal por el propio trastorno inmunitario que caracteriza al MM.

3.3 Supervivencia global comparada

Un tercer aspecto relevante para analizar es la posible influencia del tipo de centro donde los pacientes con MM son atendidos en los resultados en salud, en términos de supervivencia global. En general, existe una asociación entre el nivel de complejidad del hospital y mejores resultados en una serie de situaciones clínicas, en particular el cáncer (Gruen RL *et al*, 2009). En el caso del MM, los estudios son escasos y no concluyentes. Un estudio italiano de 231 pacientes no mostró diferencias significativas en la supervivencia entre hospitales de distintos niveles (Patriarca F *et al*, 1998). En un estudio finlandés (Oivanen T *et al*, 1999) no se encontraron diferencias ni en la tasa de respuestas, ni en la supervivencia libre de progresión ni en la supervivencia global entre diferentes hospitales con diversas tasas de inclusión en los ensayos. El estudio MICORE intentaba responder a esta pregunta en el ámbito del Sistema Sanitario Público Andaluz, analizando los resultados de 5 hospitales públicos. En nuestro estudio, el nivel de complejidad del hospital no se asocia con la mortalidad bruta; sin embargo, tras ajustar por edad, sexo, estadío de Durie-Salmon, insuficiencia renal y tipo de MM, se observa una ligera tendencia a presentar menor riesgo en los hospitales comarcales con respecto al hospital de referencia, en el marco temporal del estudio. El estudio confirma la edad, el estadío y la insuficiencia renal como factores pronósticos independientes asociados a mortalidad. Igualmente, el estudio avala el modelo organizativo para la atención de pacientes con MM en nuestro entorno, en el que los hospitales comarcales tienen un papel importante en el diagnóstico y tratamiento del MM. No obstante, estamos asistiendo a una escalada progresiva en el nivel de complejidad en el manejo de los pacientes con MM, que hace muy aconsejable la creación de unidades de referencia para la atención de las gammapatías monoclonales desde un abordaje multidisciplinar, donde una serie de especialistas (internistas,

inmunólogos, nefrólogos, cardiólogos, patólogos) deben de estar coordinados con el hematólogo para ofrecer una atención integral.

3.4 DM2 y riesgo de MM

Hemos resaltado en los estudios previos el impacto pronóstico de la comorbilidad en términos de supervivencia global y mortalidad precoz, a nivel clínico. No obstante, tanto el MM como muchas de las enfermedades con las que se asocia de forma primaria (obesidad, diabetes, etc) son enfermedades complejas, con una base genética cada vez mejor conocida. En nuestra cohorte, un 32,3% de los pacientes con MM de nuevo diagnóstico son obesos y un 18,9% presentan DM2. Es por tanto muy interesante analizar el papel potencial de determinadas variantes genéticas asociadas con DM2 y obesidad para valorar por un lado su influencia en el riesgo de desarrollar MM, y por otro su impacto pronóstico potencial en términos de supervivencia.

Los portadores del alelo $KCNQ1_{rs2237892T}$ o los genotipos $CDKN2A\text{-}2B_{rs2383208G/G}$, $IGF1_{rs35767T/T}$ y $MADD_{rs7944584T/T}$ mostraban un riesgo mayor de desarrollar MM cuando se comparaban con los portadores de los alelos o genotipos de referencia, mientras que los portadores de los alelos $KCNJ11_{rs5215C}$, $KCNJ11_{rs5219T}$ y $THADA_{rs7578597C}$ o los genotipos $FTO_{rs8050136A/A}$ y $LTA_{rs1041981C/C}$ mostraban un riesgo significativamente menor de desarrollar MM. Las asociaciones de las variantes *KCNQ1, CDKN2A-2B, IGF1, MADD, KCNJ11, y THADA* mostraron una dirección opuesta a la reportada previamente en el GWAS para DM2 (el alelo de riesgo fue el opuesto para MM y DM2) lo que apunta hacia un mecanismo subyacente no diabetogénico para modular el riesgo de MM. Varios estudios han sugerido que, además de su influencia en la función pancreática y en la secreción de insulina a través de varios mecanismos biológicos, estos genes también pueden actuar como genes supresores de tumores (Kim WY *et al*, 2006; Tang Y *et al*, 2013) y tener un impacto en la supervivencia (Sharifi S *et al*, 2013),

diferenciación (Pancewicz J *et al*, 2010), proliferación (Grimberg A, 2003; Pancewicz J *et al*, 2010) y apoptosis (Li H *et al*, 2008; Pancewicz J *et al*, 2010). Como ha sido reportado en otros tipos de cáncer, como el de próstata (Stevens VL *et al*, 2010), nuestros datos sugieren que las variantes genéticas relacionadas con la DM2 pueden determinar el riesgo de MM a través de mecanismos no diabetogénicos.

Al corregir por el test de comparaciones múltiples, sólo la asociación de la variante $IGF1_{rs35767}$ permanecía cercana a la significación, lo que sugiere que el locus *IGF1* podría jugar un importante papel en la proliferación de las CPc. En apoyo de esta hipótesis, ha sido demostrado que *IGF1* actúa como un importante factor de crecimiento en MM, que directa o indirectamente en colaboración con otros factores de crecimiento, induce crecimiento y proliferación celular (Bommert K *et al*, 2006; Sprynski AC *et al*, 2009) y puede eventualmente conducir a resistencia al tratamiento (Kuhn DJ *et al*, 2012). Además el tratamiento antidiabético con metformina, que inhibe la vía de señalización del *IGF1*, ha demostrado que reduce el riesgo de transformación de GMSI a MM y que puede inducir apoptosis (Rattan R *et al,* 2012) y aumentar la efectividad de los regímenes terapéuticos (Feng YH *et al*, 2011). De forma interesante, otros autores han demostrado que *IGF1* se asocia a un aumento de mortalidad en pacientes con MM en progresión (Chou YS *et al*, 2012), mientras que la administración de metformina reduce la mortalidad en este contexto (Wu W *et al*, 2014). Por otra parte, los portadores del alelo $IGF1_{rs35767T}$ muestran mayores niveles circulantes de *IGF1* que los portadores del alelo WT (Mannino GC *et al*, 2013) y esta variante se asocia a otros tipos de cáncer (Ollberding NJ *et al,* 2012). Aunque se podría especular que el SNP $IGF1_{rs35767}$ podría ser responsable del efecto atribuido a la DM2 en el riesgo de MM, pensamos que más que actuar aisladamente, esta variante interacciona con otros genes modulando el riesgo de MM. Para probar esta hipótesis evaluamos el valor predictivo de los polimorfismos

asociados a DM2 en un modelo de regresión y comprobamos que al añadir los polimorfismos genéticos a un modelo con covariables demográficas (sólo edad y sexo) se mejoraba sustancialmente la capacidad predictiva, mostrando un área bajo la curva del 64,5% para MM.

La principal fortaleza del estudio realizado en el marco del consorcio IMMEnSE es su tamaño muestral, lo que permite valorar el efecto generalmente modesto de las variantes genéticas. No obstante, y debido a la naturaleza retrospectiva del estudio, la no disponibilidad del índice de masa corporal y del conocimiento de la DM2 como comorbilidad en los pacientes en un porcentaje importante de casos nos impidió ajustar por estas variables en el análisis. Hasta donde conocemos, nuestro estudio es el primero en evaluar la asociación entre variantes genéticas relacionadas con la DM2 y el riesgo de desarrollar MM. Su genotipado podría ayudar a estimar el riesgo de MM mediante la utilización de modelos predictivos.

3.5 DM2 y supervivencia global en MM

Tras comprobar que determinados SNPs relacionados con DM2 están asociados con el riesgo de desarrollar MM, el paso siguiente fue analizar, también por primera vez según la información disponible por nuestra parte, si alguno de estos SNPs podía tener un impacto significativo en la supervivencia global. Este análisis se planteó porque se ha demostrado recientemente que variantes relacionadas con DM2 han sido asociadas con la supervivencia global en otros tipos de cáncer (Bao PP *et al*, 2015). En el caso del MM, otras variantes no relacionadas con DM2 ya han sido asociadas con la supervivencia global (Andersen NF *et al*, 2015; Ziv E *et al*, 2015; Johnson DC *et al*, 2016).

En nuestro estudio se ha comprobado que el SNP $HNF1B_{rs7501939}$ está asociado con una supervivencia global significativamente menor, tanto en la población IMMEnSE como

en la población Heidelberg. Aunque previamente se describió la existencia de una correlación positiva entre esta variante y los niveles de expresión génica en sangre periférica (Westra HJ *et al*, 2013), nuestros datos de eQTL en CPc no confirmaron una correlación positiva entre el alelo de riesgo y los niveles de expresión de ARNm de *HFN1B*. Estos datos sugieren que el efecto de esta variante en la supervivencia no está mediado por cambios en la actividad transcripcional del gen. Por otra parte, dado que *HNF1B* contiene múltiples SNPs que han sido asociados con la expresión de ARNm y procesos de metilación, así como con el riesgo de desarrollo de varios tipos de cáncer (Harries LW *et al*, 2010; Shen H *et al*, 2013; Painter JN *et al*, 2015), parece razonable considerar que otros SNPs en el mismo locus pudieran explicar mejor el vínculo entre *HNF1B* y los resultados clínicos adversos.

HNF1B contiene 9 exones y se localiza en una región de 58 kb en el cromosoma 17p21, codificando para un factor de transcripción que ha sido asociado con múltiples características clínicas, incluyendo DM2 (Painter JN *et al*, 2015). Se ha sugerido que *HNF1B* induce intolerancia a la glucosa y disminución de la sensibilidad a la insulina, con aumento de la secreción de insulina y activación de la vía *IGF1* (Bommert K *et al*, 2006), vía implicada en la patogénesis del MM. Alternativamente, *HNF1B* puede influir en la supervivencia de las células tumorales activando la vía NFkB o a través de la inhibición de señales de apoptosis (Suzuki E *et al*, 2015). También ha sido reportado que *HNF1B* puede actuar como un oncogen (Shao DD *et al*, 2013), encontrándose amplificado en el 23% de todos los tipos de cáncer y en el 5% de las neoplasias hematológicas, e incluso *HNF1B* puede actuar como un gen supresor con una expresión variable según el tipo de tejido. Considerando todo lo anterior y que la asociación de $HNF1B_{rs7501939}$ con la menor supervivencia estaba determinada por un alelo no diabetogénico (T) que no afecta a la expresión de ARNm, consideramos que el efecto de

esta variante en el MM podría ser mediado por un mecanismo independiente de insulina.

Otro hallazgo interesante de este estudio fue la asociación de SNPs en *BCL11A, MADD, PRC1, PROX1, SCL30A8, SLC2A2 y TCF7L2* con la supervivencia global en pacientes con MM. De todas ellas, sólo la asociación de $SCL30A8_{rs13266634}$ alcanzaba significación estadística al nivel determinado en el estudio y estaba restringida al sexo masculino.

El gen *SCL30A8* codifica para un transportador de zinc implicado en el control del procesamiento y secreción de la insulina (Chimienti F *et al*, 2006). Aunque no existen estudios previos relacionando este locus con el MM, existen evidencias de que determinados transportadores pueden contribuir a la carcinogénesis a través de un mecanismo dependiente del género (Pound LD *et al*, 2012). La asociación de $SCL30A8_{rs13266634}$ con la supervivencia también está mediada por un alelo no diabetogénico lo que sugiere que el efecto podría estar asociado a un efecto directo del transportador en procesos biológicos tales como estabilización del RNA y DNA, unión de protooncogenes al DNA y activación de *IGF1* o telomerasa. El alelo $SCL30A8_{rs13266634C}$ ha sido asociado a tasas reducidas de actividad de transporte de zinc y niveles intragranulares de zinc reducidos. Sin embargo, los datos eQTL en CPc de pacientes con MM no revelaron correlación entre esta variante y los niveles de ARNm lo que sugiere que el alelo T afecta a la actividad transportadora causando aumento de la concentración de zinc y promoviendo la proliferación de las CPc, la progresión de la enfermedad y la peor supervivencia. No obstante, sus mecanismos de acción deberían ser analizados y confirmados mediante estudios específicos.

Como limitaciones del estudio, en parte ya comentadas para la relación entre variantes diabetogénicas y MM, relacionadas con la calidad de la información, hay que señalar que carecemos de información pronóstica clínica como las ACAR mediante FISH y el

diagnóstico de DM2. Además, el poder estadístico es relativamente pequeño para detectar asociaciones modestas, en particular cuando se realiza estratificación por sexo. Por todo ello, son necesarios estudios adicionales en poblaciones bien caracterizadas de mayor tamaño para replicar estos hallazgos.

VII. CONCLUSIONES

1. La supervivencia global de los pacientes con MM está mejorando progresivamente. Los factores pronósticos asociados a una supervivencia pobre son la edad avanzada, los estadíos ISS 2 y 3, el nivel elevado de LDH sérica, la insuficiencia renal, y la presencia de amiloidosis asociada al diagnóstico. Por el contrario, la realización de TAPH se asocia con un aumento de la supervivencia.
2. La mortalidad precoz de los pacientes con MM está descendiendo paulatinamente. Además de la edad y la insuficiencia renal, que son factores pronósticos asociados a un mayor riesgo de mortalidad durante el primer año, con independencia del momento en que se mida, otras variables predictoras independientes fueron la enfermedad respiratoria para la mortalidad en los dos primeros meses, la hepatopatía para un mayor riesgo de mortalidad en los 6 primeros meses, y finalmente, para la mortalidad durante el primer año, el ISS y la infección por virus de hepatitis C. El análisis de la mortalidad precoz no está estandarizado. Se sugiere una valoración a los 2, 6 y 12 meses.
3. La presencia de comorbilidades basales en los pacientes con MM tiene impacto en los resultados. La insuficiencia renal y la amiloidosis influyen negativamente en la supervivencia global, mientras que la insuficiencia renal, la presencia de enfermedad respiratoria, hepatopatía e infección por el virus de la hepatitis C se asocian a mayor riesgo de mortalidad precoz. Un adecuado control de estas comorbilidades podría ayudar a mejorar los resultados de los pacientes con MM.
4. El tipo de hospital donde se diagnostican y son tratados los pacientes con MM no se asocia con la mortalidad. No obstante, y dada la creciente complejidad en el manejo de estos pacientes, se sugiere que el tratamiento sea coordinado con un centro de referencia.

5. Algunas variantes genéticas asociadas a DM2, en concreto $IGF1_{rs35767}$, pueden aumentar el riesgo de desarrollar MM. El genotipado de dichas variables podría ayudar a valorar de forma más precisa el riesgo de MM en modelos predictivos.
6. Algunas variantes genéticas asociadas a DM2, en particular $HNF1B_{rs7501939}$ (en ambos sexos) y $SCL30A8_{rs13266634}$ (en hombres), pueden tener un impacto negativo en la supervivencia global de los pacientes con MM, probablemente a través de mecanismos independientes de la insulina.

VIII. REFERENCIAS BIBLIOGRÁFICAS

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TRABAJOS RELACIONADOS CON ESTA TESIS

COMUNICACIONES A CONGRESOS NACIONALES

LIII Reunión Nacional de la SEHH. Zaragoza, Octubre-2011.

-R Ríos, et al. Prevalencia e impacto clínico del exceso de peso en pacientes con mieloma múltiple. Haematologica (ed. Esp.)2011;96, supl.2:67-8.

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