

**ENFERMEDAD MÍNIMA
RESIDUAL EN MIELOMA
ESTUDIO DEL GREMI**

ASPECTOS A TRATAR

- ▶ EVOLUCIÓN DE LA TÉCNICA DE LA EMR EN MM
- ▶ IMPACTO PRONÓSTICO DE LA EMR EN MM
- ▶ ESTUDIO DEL GREMI

TÉCNICA DE LA EMR EN MM

- ▶ 2008 (EMN):
 - ▶ CD38/CD138/CD45 definen CP.
 - ▶ CD56 positivo y la ausencia de CD19 se consideró un IF aberrante típico en la CPP (aplicable en más del 90% de los pacientes).
 - ▶ CD117+, CD20+, CD28++, CD27-, CD81-, CD200++ marcadores más útiles para diferenciar CPP/CPN

TÉCNICA DE LA EMR EN MM

► 2012 (EUROFLOW):

<i>Tube</i>	<i>PacB</i>	<i>PacO</i>	<i>FITC</i>	<i>PE</i>	<i>PerCPCy5.5</i>	<i>PECy7</i>	<i>APC</i>	<i>APCH7</i>
1	CD45	CD138	CD38	CD56	β2micro	CD19	CyIgκ	CyIgλ
2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81
<i>Marker</i>	<i>Fluorochrome</i>	<i>Clone</i>	<i>Source</i>	<i>Catalogue number</i>	<i>(μl/test)</i>			
CD19	PECy7	J3-119	Beckman Coulter	IM3628	5			
CD27	PerCPCy5.5	L128	BD Biosciences	649805	10			
CD28	PE	L293	BD Biosciences	348047	20			
CD38	FITC	LD38	Cytognos	CYT-38F	3	} 5 ^a		
CD38	Pure	LD38	Cytognos	CYT-38P1	2			
CD45	PacB	T29/33	Dako	PB986	5			
CD56	PE	C5.9	Cytognos	CYT-56PE	5			
CD81	APCH7	JS-81	BD Biosciences	646791	5			
CD117	APC	104D2	BD Biosciences	333233	5			
CD138	PacO	B-A38	Exbio	PO-520	4			
β2micro	PerCPCy5.5	Tü99	BD Biosciences	646781	4.75	} 5 ^a		
β2micro	Pure	Tü99	BD Biosciences	555550	0.25			
CyIgκ	APC	Polyclonal rabbit serum	Dako	C0222	2.5			
CyIgλ	APCH7	1-155-2	BD Biosciences	646792	4			

^aMixture of fluorochrome-conjugated and -unconjugated antibodies is used to reduce signal intensity, while retaining saturating conditions to avoid unpredictable variation in staining patterns. The 19:1 ratio for the two β2micro antibodies is caused by the five fold higher antibody concentration of the unconjugated antibody.

TÉCNICA DE LA EMR EN MM

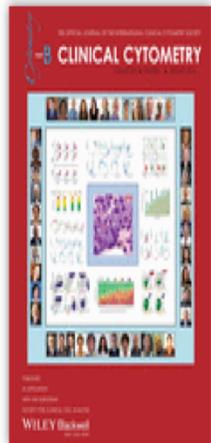
► EMR: GRAN HETEROGENEIDAD: PRIMERA GENERACIÓN

COMBINACIÓN	REFERENCIA
1. CD38-FITC/CD56-PE/CD19-PerCPCy5.5/CD45-APC 2. CD138-FITC/CD28-PE/CD33-PerCPCy5.5/CD38-APC 3. CD20-FITC/CD117-PE/CD138-PerCPCy5.5/CD38-APC	Sarasquete y cols. Haematologia 2005
1. Cylgλ-FITC/CD19-PE/Cylk-PECy5/CD38-PECy7/CD138-APC/ CD45-APC Cy7	De Tute y cols. Leukemia 2007.
1. CD19-FITC/CD56-PE/CD38-PerCP Cy5.5/CD138-APC 2. CD45-FITC/CD52-PE/CD38-PerCPCy5.5/CD138-APC 3. CD20-FITC/CD117-PE/CD38-PerCP Cy5.5/CD138-APC	Gupta y cols. Am J Clin Pathol 2009
1. CD38-FITC/CD56-PE/CD19-PerCPCy5.5/CD45-APCa 2. CD38-FITC/CD27-PE/CD45-PerCPCy5.5/CD28-APC 3. b2 micro-FITC/CD81-PE/CD38-PerCP Cy5.5/CD117-APC	Paiva y cols. J Clin Oncol 2011
1. CD27-FITC/CD56-PE/CD19-PerCPCy5.5/CD38-PE Cy7/ CD138-APC/CD45-APCCy7 2. CD81-FITC/CD117-PE/CD52-FITC/CD200-PE (en algunos casos dudosos)	Rawstron y cols. J Clin Oncol 213
1. CD38-HV450/Cylgk-FITC/CD56-PE/CD28-PE/CD138-PE Cy5/ CD19-PECy7/Cylgj-APC/CD45-APC H7	Robillard y cols. J Blood Cancer J 2013

- 4-6 colores
- 1-5.10⁵ células adquiridas (S:10⁻⁴)
- NO ESTANDARIZACIÓN

TÉCNICA DE LA EMR EN MM

► EMR: GRAN CONSENSO. SEGUNDA GENERACIÓN



Volume 90, Issue 1

Special Issue: Multiple Myeloma Special Issue: The Flow Cytometric Detection of Minimal Residual Disease

Pages: 1-100

January 2016

- 8 colores
- $2 \cdot 10^6$ células adquiridas(S: 10^{-5})
- ESTANDARIZACIÓN

2. Eight Color Panel: EuroFlow Consortium, also used by PETHEMA

	FITC	PE	PerCP Cy5.5	PC7	APC	APCC750	V450	BV510
1	CD38 (L38, Cytognos)	CD56 (C5.9, Cytognos)	CD45 (HI30, EBioscience)	CD19 (J3-119, Beckman-Coulter)	CD117 (104D2 BD Biosciences)	CD81 (M38 Cytognos)	CD138 (MI15 BD Biosciences)	CD27 (0323 Biolegend)
2 ^a	CD38 (L38, Cytognos)	CD56 (C5.9, Cytognos)	CD45 (HI30, EBioscience)	CD19 (J3-119, Beckman-Coulter)	clgk (polyclonal, Dako)	clgλ (polyclonal, Cytognos)	CD138 (MI15 BD Biosciences)	CD27 (0323 Biolegend)

Reference clone and manufacturer in parenthesis.

^aTube 2 complementary if further demonstration of clonality is needed among cells with phenotypic deviation identified in Tube 1.

TÉCNICA DE LA EMR EN MM

► EMR: RECOMENDACIÓN ACTUAL. NUEVA GENERACIÓN

Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma

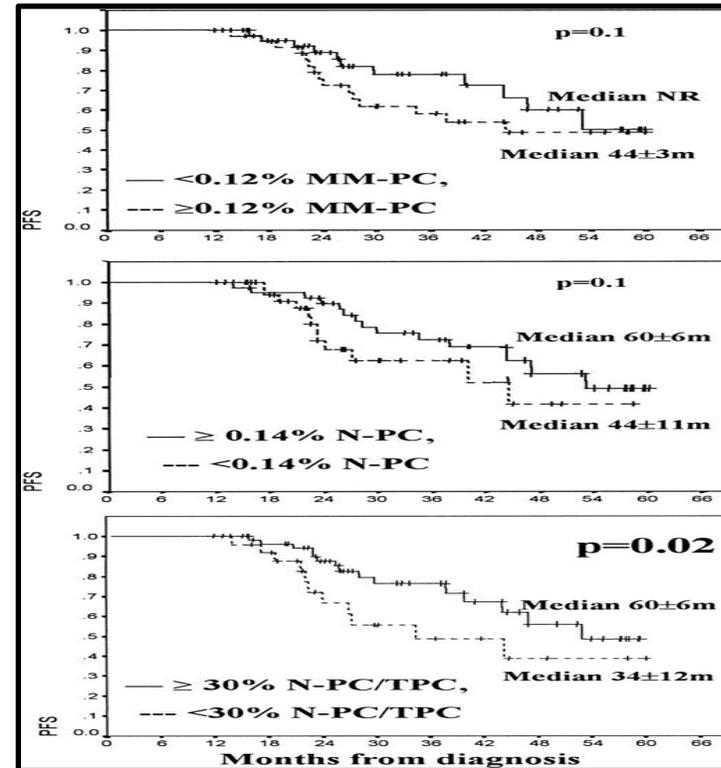
J Flores-Montero^{1,19}, L Sanoja-Flores^{1,19}, B Paiva^{2,19}, N Puig³, O García-Sánchez³, S Böttcher⁴, VHJ van der Velden⁵, J-J Pérez-Morán³, M-B Vidriales³, R García-Sanz³, C Jimenez³, M González³, J Martínez-López⁶, A Corral-Mateos¹, G-E Grigore⁷, R Fluxá⁷, R Pontes⁸, J Caetano⁹, L Sedek¹⁰, M-C del Cañizo³, J Bladé¹¹, J-J Lahuerta⁶, C Aguilar¹², A Báñez¹³, A García-Mateo¹⁴, J Labrador¹⁵, P Leoz¹, C Aguilera-Sanz¹⁶, J San-Miguel^{2,20}, M-V Mateos^{3,20}, B Durie^{17,21}, JJM van Dongen^{5,18,21} and A Orfao^{1,21}

Tube	BV421	BV510	FITC	PE	PerCPCy 5.5	PECy7	APC	APCC750
1							CD117	CD81
2	CD138	CD27	CD38	CD56	CD45	CD19	CyIgκ	CyIgλ

- 10.10⁶ de células adquiridas(S:10⁻⁶).
- Bulk-lysis
- Plantillas
- Calidad de la muestra

IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

- ▶ PETHEMA94
- ▶ 4 COLORES
- ▶ 200.000 CÉLULAS

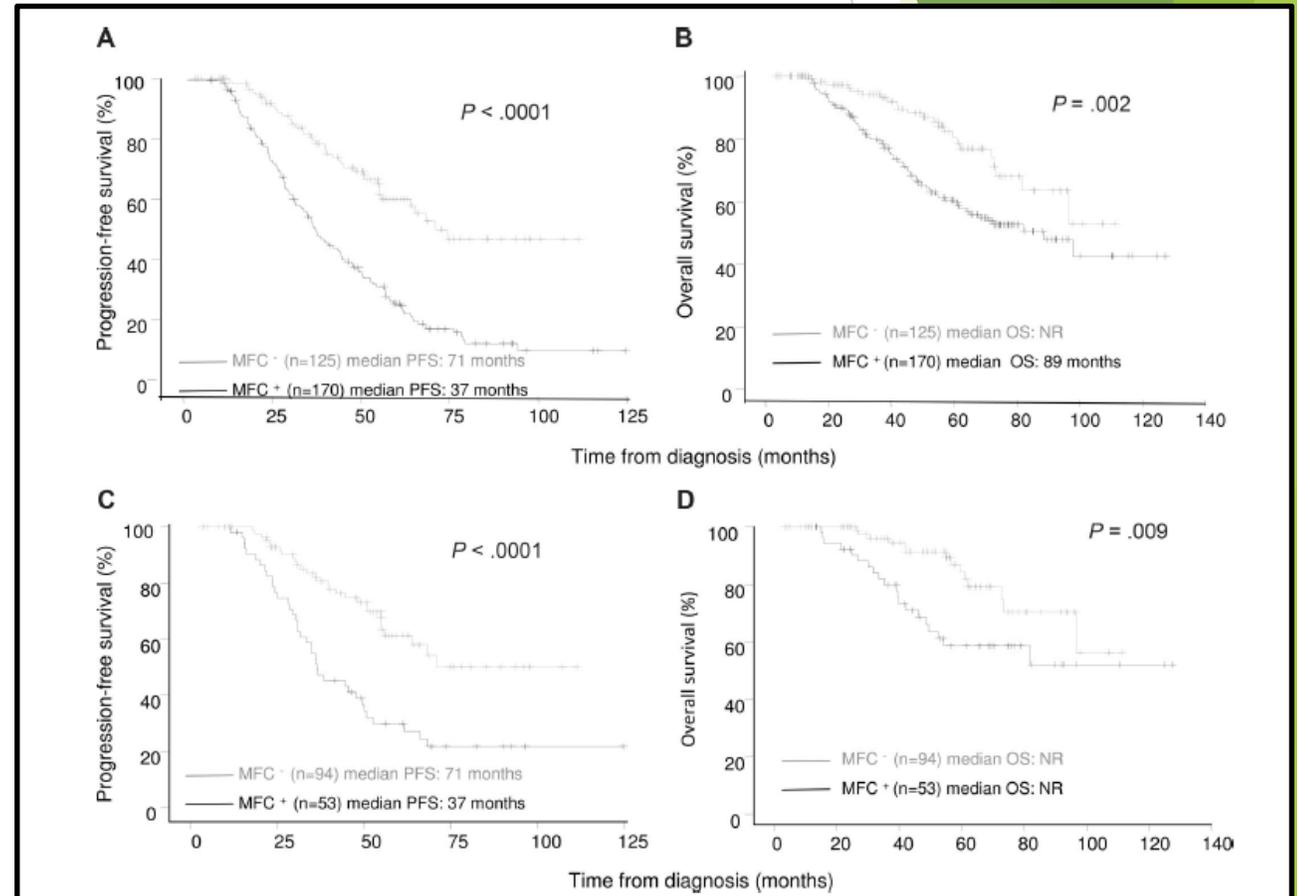


IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

▶ PETHEMA/GEM 2000

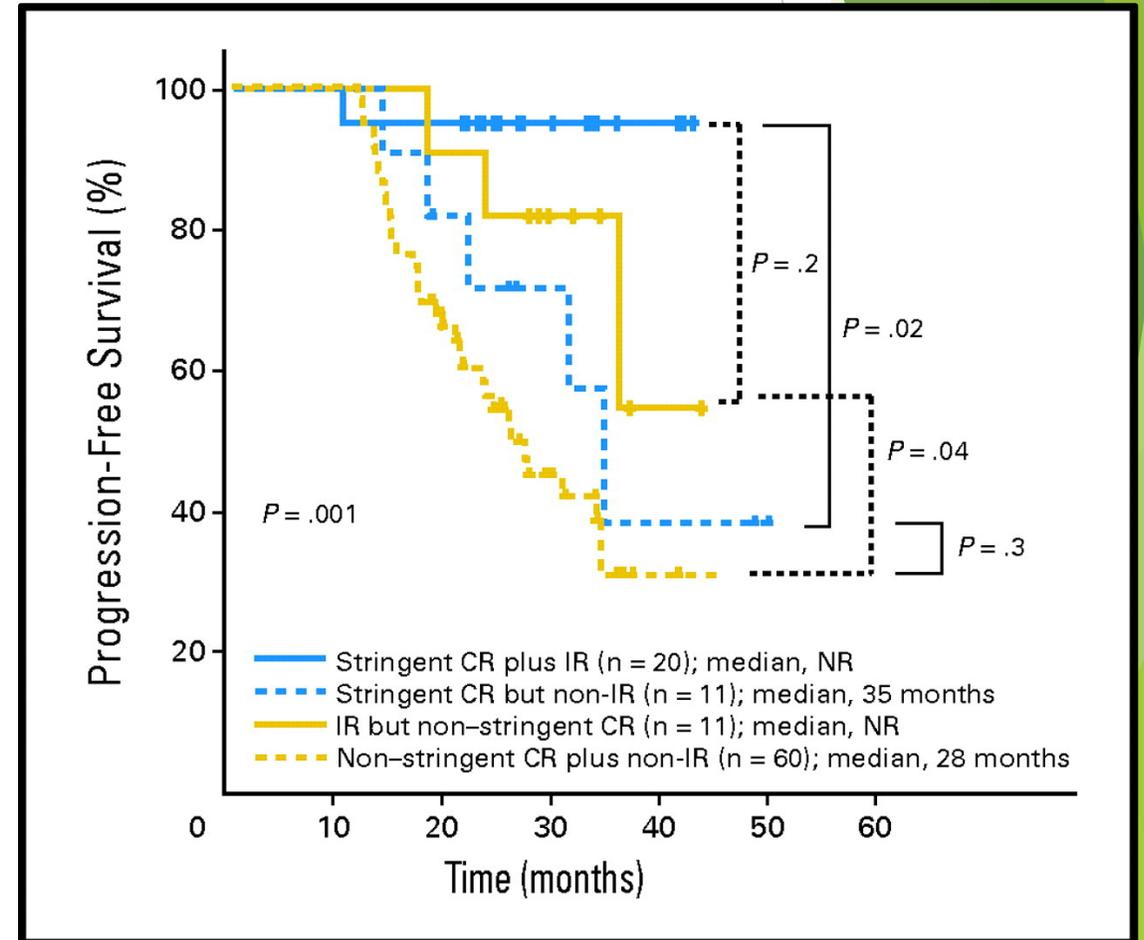
▶ 4 COLORES

▶ 300.000 CÉLULAS



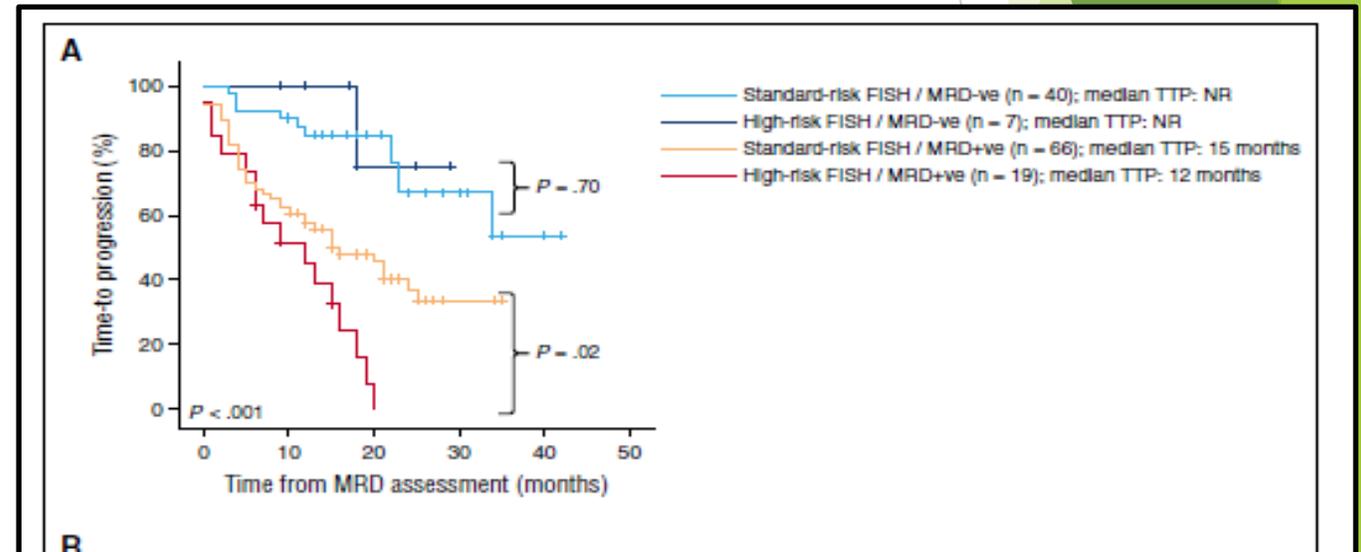
IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

- ▶ GEM 2005 > 65
- ▶ 4 COLORES
- ▶ $2 \cdot 10^6$ CÉLULAS



IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

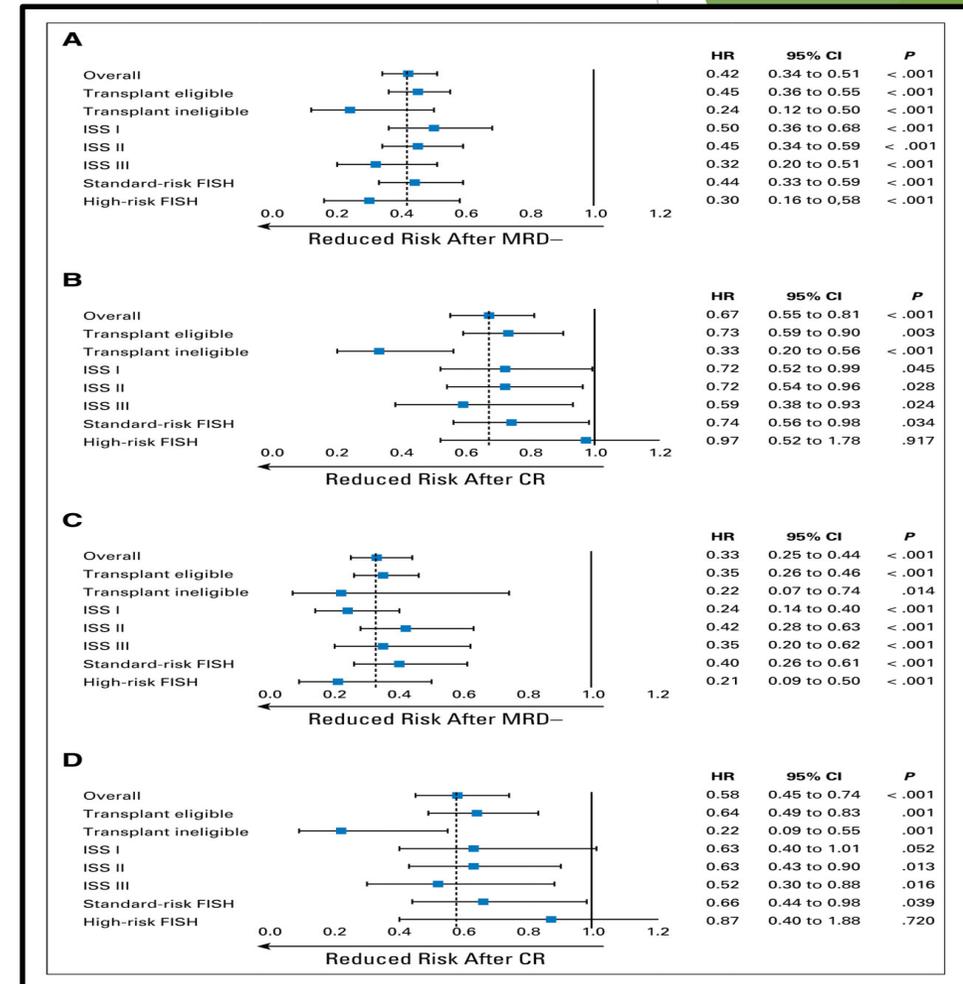
- ▶ PETHEMA/GEM2010MAS65
- ▶ 1 tubo de 8 COLORES
- ▶ $2 \cdot 10^6$ CÉLULAS



IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

► 609 pacientes

► GEM 2000/GEM2005 < 65/
GEM2010 > 65



IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

REVIEW | VOLUME 17, ISSUE 8, E328-E346, AUGUST 01, 2016



Purchase

International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Prof Shaji Kumar, MD • Bruno Paiva, PhD • Prof Kenneth C Anderson, MD • Prof Brian Durie, MD • Ola Landgren, MD • Prof Philippe Moreau, MD • et al. [Show all authors](#)

Published: August, 2016 • DOI: [https://doi.org/10.1016/S1470-2045\(16\)30206-6](https://doi.org/10.1016/S1470-2045(16)30206-6) • Check for updates

Response criteria*

IMWG MRD criteria (requires a complete response as defined below)

Sustained MRD-negative MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†

Flow MRD-negative Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10⁵ nucleated cells or higher

Sequencing MRD-negative Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10⁵ nucleated cells§ or higher

Imaging-positive MRD-negative MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue¶

Standard IMWG response criteria||

IMPACTO DE LA EMR EN EL PRONÓSTICO DEL MM

Review > [Cancer Treat Res. 2016;169:103-122. doi: 10.1007/978-3-319-40320-5_7.](#)

Multiple Myeloma Minimal Residual Disease

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Affiliations + expand

PMID: 27696260 DOI: [10.1007/978-3-319-40320-5_7](#)

antibodies and small molecules. Therefore, conventional serological and morphological techniques have become suboptimal for sensitive evaluation of highly effective treatment strategies. Although, existing data suggests that MRD could be used as a biomarker to evaluate treatment efficacy, help on therapeutic decisions, and act as surrogate for overall survival, the role of MRD in MM is still a matter of extensive debate. Here, we review the different levels of remission used to define depth of

ESTUDIO DEL GREMI: MATERIALES Y MÉTODOS

- ▶ SEPTIEMBRE 2015-MAYO 2018
- ▶ 6 HOSPITALES DE LA CV
- ▶ PACIENTES CON MM CANDIDATOS A TASP EN PRIMERA LÍNEA(VTD)
- ▶ ALCANZAN MBRP O MEJOR
- ▶ EMR CMFNG:
 - ▶ AL INGRESO EN EL ESTUDIO
 - ▶ A LOS 3,12 Y 24 MESES DEL TASP

ESTUDIO DEL GREMI: MATERIALES Y MÉTODOS

44 PACIENTES

DEMOGRÁFICO DE PACIENTES Y CARACTERÍSTICAS EN MBRP

Total	15
Hombre/Mujer	12/3
Mediana edad	50
TIPO MIELOMA	
IGG	10
IGA	2
NS	0
BJ	3
ISS I/II/III	3/8/4
ISSR I/II/III	3/10/2
Alto riesgo (t(4;14),(t(14:16) o Del 17(p)%	3

DEMOGRÁFICO DE PACIENTES Y CARACTERÍSTICAS EN RC

Total	29
Hombre/Mujer	15/14
Mediana edad	57
TIPO MIELOMA	
IGG	9
IGA	15
NS	3
BJ	2
ISS I/II/III	15/10/4
ISSR I/II/III/ND	10/13/0/6
Alto riesgo (t(4;14),(t(14:16) o Del 17(p)%	4

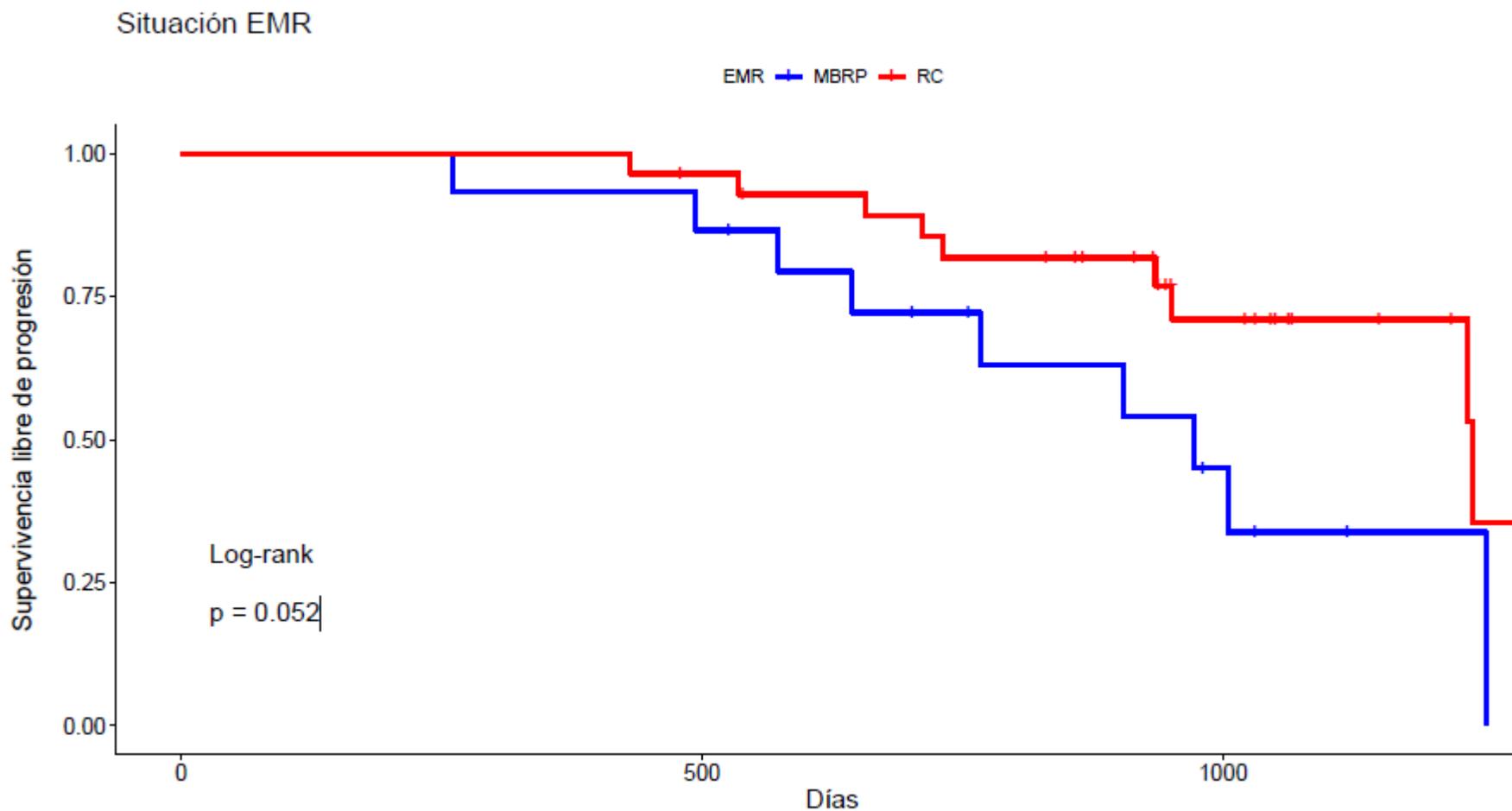
ESTUDIO DEL GREMI: MATERIALES Y MÉTODOS

EMRs	
CON UNA EMR	1
CON DOS EMR	11(22)
CON TRES EMR	23(69)
CON CUATRO EMR	9(36)
TOTAL EMR	128
POS/NEG	62/64/2nv
MOMENTO DE LA PRIMERA EMR	
PRETASP	39
+3M	45
+12M	33
+24 M	11

ESTUDIO DEL GREMI: RESULTADOS SUPERVIVENCIA GLOBAL

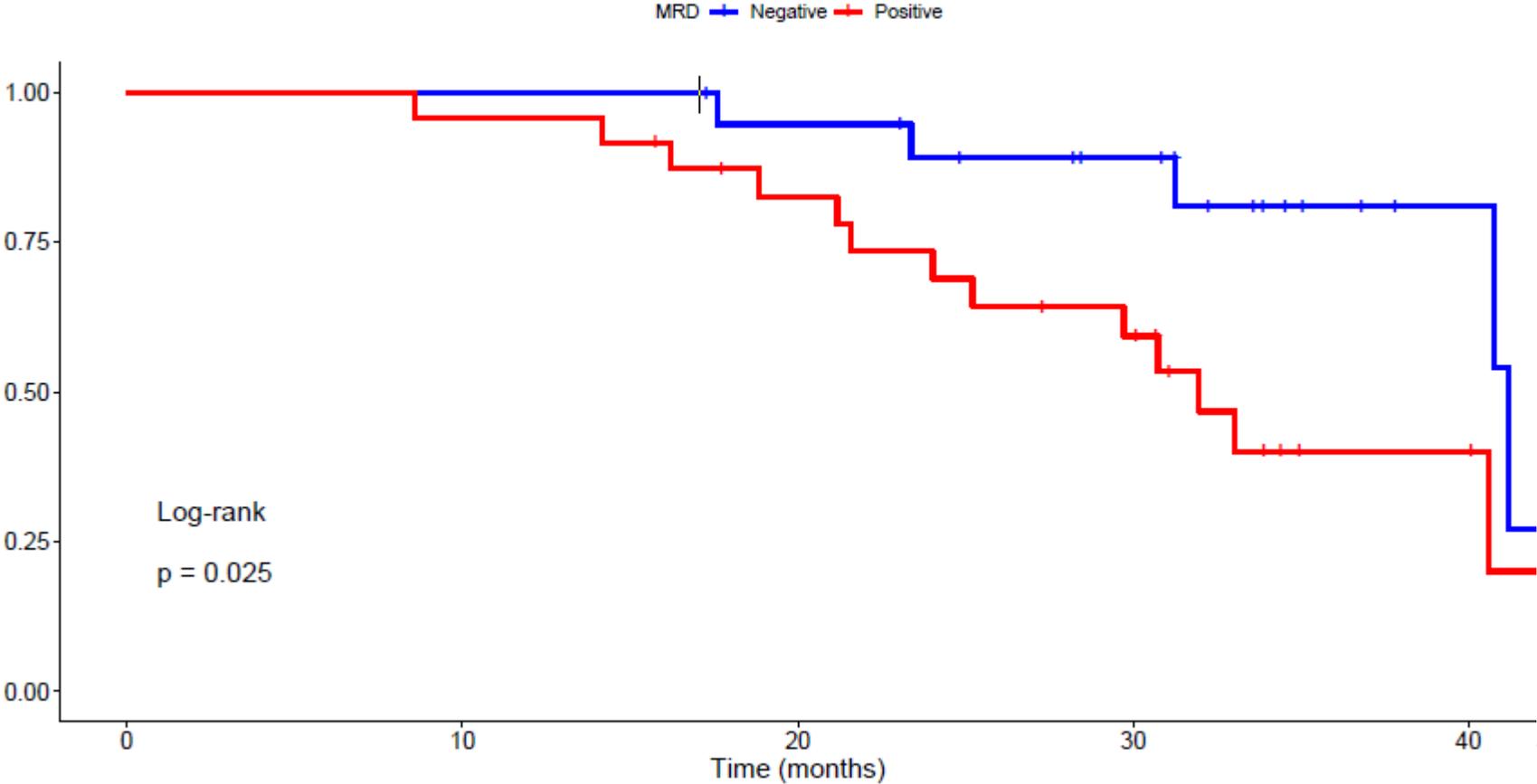
- ▶ **MEDIANA DE SEGUIMIENTO: 37M**
- ▶ **SG: 4 MUERTES DE 44: 9%**
- ▶ **SLP: 18 RECAÍDAS DE 44: 40%**

ESTUDIO DEL GREMI: RESULTADOS SLP SEGÚN RESPUESTA SEROLÓGICA



MBRP: 9 REC/15
RC: 9 REC/29
32 vs 41m

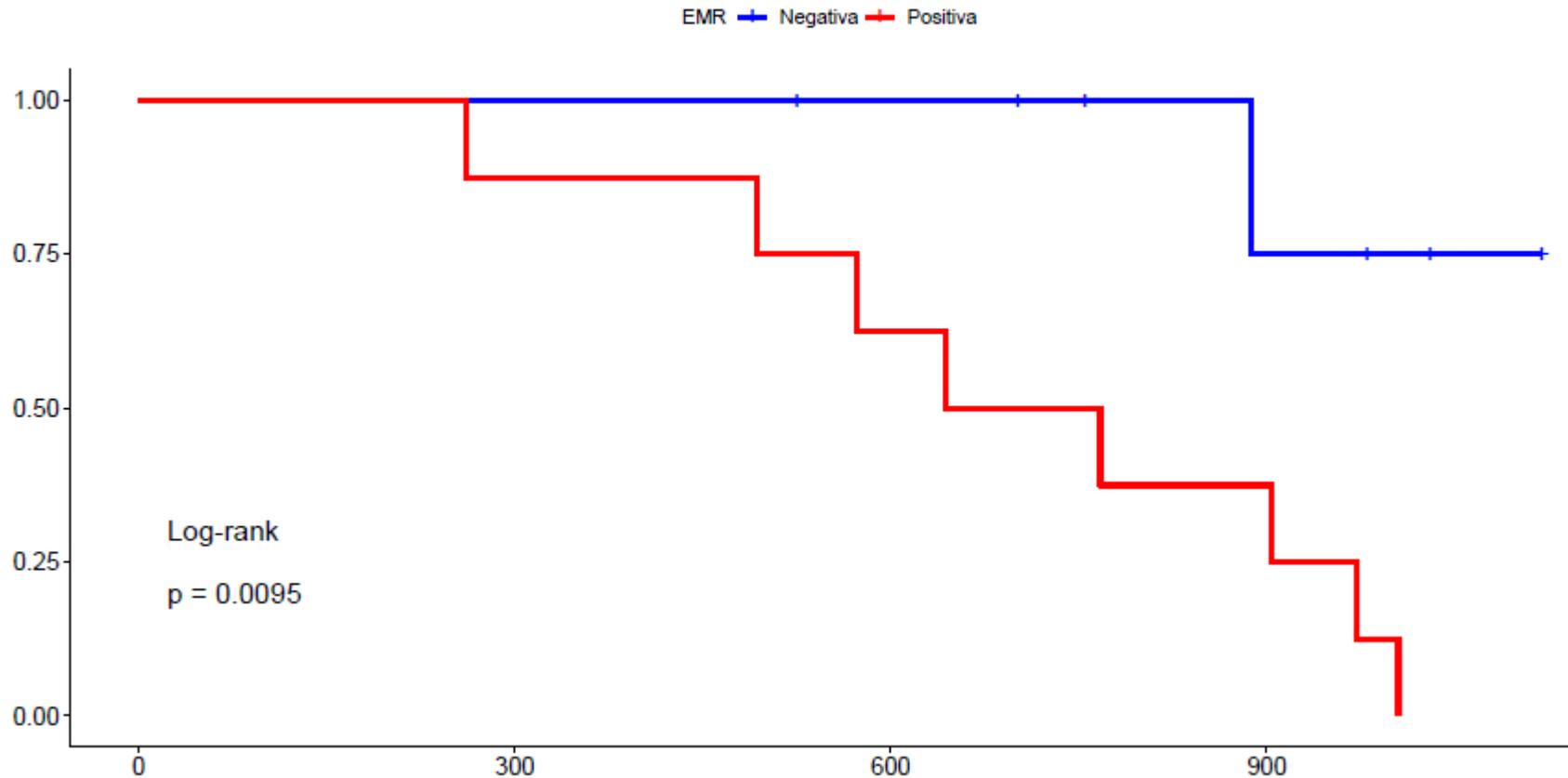
ESTUDIO DEL GREMI: RESULTADOS SLP SEGÚN EMR POSITIVA O NEGATIVA



POS: 13 REC/24
NEG: 5 REC/20
32 vs 42m

ESTUDIO DEL GREMI: RESULTADOS SLP SEGÚN EMR POSITIVA O NEGATIVA EN MBRP

Resultado EMR



POS: 8 REC/8
NEG: 1 REC/7
23m vs NA

ESTUDIO DEL GREMI: RESPUESTA MANTENIDA

	EMR 2 POSITIVA	EMR2 NEGATIVA
EMR 1 POSITIVA	3	5
EMR 1 NEGATIVA	2	10

ESTUDIO DEL GREMI: CALIDAD DE LA MUESTRA

	M CELULARIDAD(FM LEUKEMIA 2017)	M CELULARIDAD (47 MUESTRAS)
MASTOCITOS	0,006(0,002-0,03%)	0,004(0-0,19%)
SERIE ROJA	6,4(2-11,5%)	9,67(0,4-38%)
PRE B27+	0,08(0,004-0,4%)	0(0-1,16%)
PRE B27-	0,4 (0,05-2,2%)	0,05(0-6%)
B MADUROS	1,6 (0,6-3,5%)	0,86(0-11,2%)
PRECURSORES MIELOIDES	1,8(0,2-3,6%)	1,2(0,007-7,7%)

ESTUDIO DEL GREMI: CALIDAD DE LA MUESTRA

(FM Leukemia 2017)

CÉLULAS	% DE NIVELES BAJOS	
	EMR NEGATIVA	EMR POSITIVA
MASTOCITOS	10	5
SERIE ROJA	3	1
PRE B 27+	12	11
PRE B 27-	11	13
B MADUROS	26	23
PRECURSORES MIELOIDES	8	8

NOTA: 5 PACIENTES CON EMR NEGATIVA Y RECAÍDA (1 RECAÍDA EXTRAMEDULAR)

ESTUDIO DEL GREMI: CONCLUSIONES

- ▶ LA EMR NEGATIVA EN MM ES PREDICTIVA DE MEJOR SLP EN PACIENTES JÓVENES CON MM TRATADOS EN PRIMERA LÍNEA CON INDUCCIÓN Y TASP EN SITUACIÓN DE MBRP Y RC.
- ▶ ES POSIBLE INCORPORAR LA ESTANDARIZACIÓN DE LA EMR EN LA PRÁCTICA CLÍNICA HABITUAL.

Gracias