CITOMETRÍA DE FLUJO EN EL SÍNDROME MIELODISPLÁSICO

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ÍNDICE

INTRODUCCIÓN

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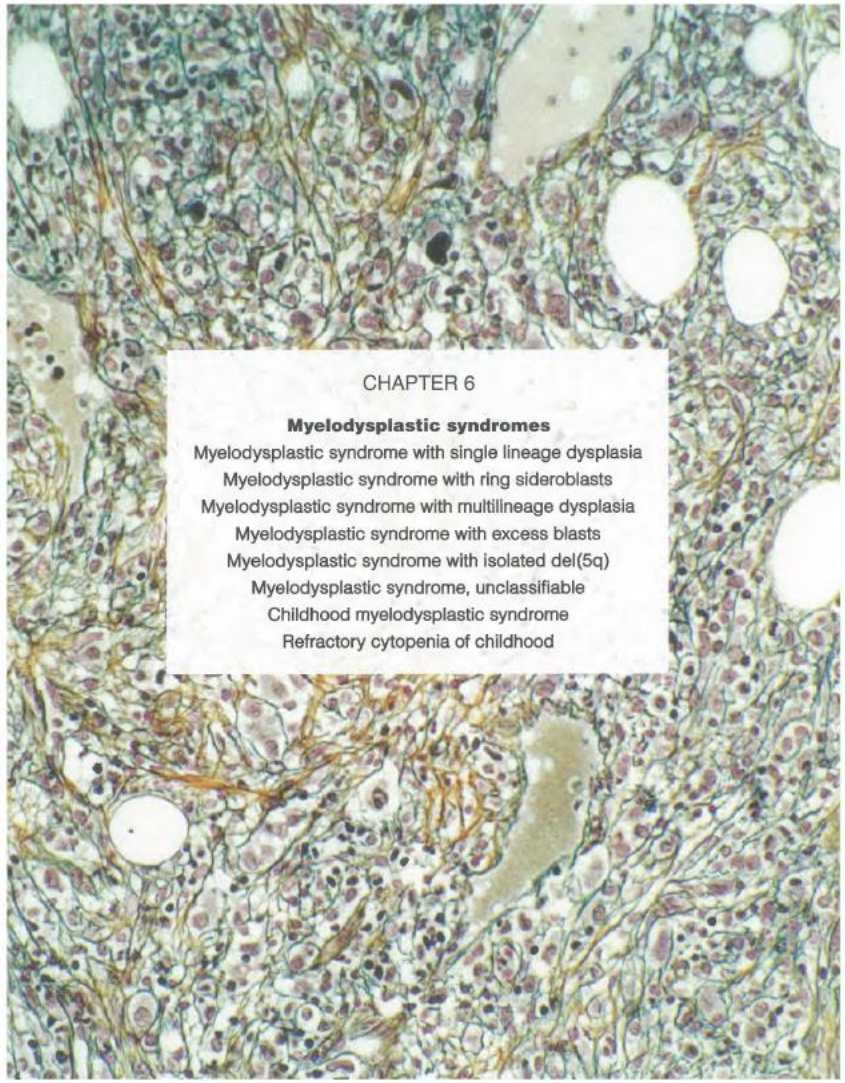
MYELODYSPLASTIC SYNDROMES (D STEENSMA, SECTION EDITOR)

Is There a Role for Flow Cytometry in the Evaluation of Patients With Myelodysplastic Syndromes?

Anna Porwit[1]

INTRODUCCIÓN



- Los hallazgos por CMF no son suficientes para establecer el diagnóstico primario de SMD en ausencia de hallazgos definitivos morfológicos y/o citogenéticos.

INTRODUCCIÓN

Guías españolas SMD y LMMC 2020 - Diagnóstico

Diagnóstico de los síndromes mielodisplásicos

Coordinado por Dra. Lourdes Florensa
Lista de autores de este capítulo en páginas finales

En el presente documento se especifican las recomendaciones que el Comité de Expertos del Grupo Español de SMD (GESMD) considera necesarias para llevar a cabo el diagnóstico de SMD.

Tabla 2. Estudios medulares en los SMD.

Estudios imprescindibles	Aspirado medular	Estudio morfológico (MGG y Perls). Enumeración de blastos* y de dismorfias. Estudio citogenético en al menos 20 metafases. Estudio mutaciones en *SF3B1* cuando se observan 5-14% sideroblastos en anillo siempre que no se cumplan los criterios de SMD con exceso de blastos o de SMD con deleción 5(q) aislada.
	Biopsia medular	En aspirado medular hipoplásico, sospecha de mielofibrosis y en ICUS.
Estudios recomendables en situaciones especiales		FISH (sondas 5q, 7q, CEP8, 20q y cromosoma Y) y/o SNP/CGH arrays. Citometría de flujo (el porcentaje de células CD34+ obtenido por citometría de flujo no debe sustituir al recuento de blastos por morfología). Tinción de PAS. Estudios moleculares en todos los pacientes con SMD especialmente en los SMD sin exceso de blastos. Mutaciones de *JAK2* en pacientes con trombocitosis y/o fibrosis, alteraciones de *PDGFRA, PDGFRB, FGFR1* y *PCM1-JAK2* en casos con eosinofilia, KIT en los SMD asociados a mastocitosis sistémica y *TP53* en SMD con del (5q).

* Se valoran de la totalidad celular. En el caso que se quiera aplicar la clasificación OMS 2008, si existe una cifra de eritroblastos ≥50% de la totalidad celular la valoración de los blastos se realizará en base a la celularidad no eritroide, y si detectan ≥20% blastos se establece el diagnóstico de eritroleucemia. En la clasificación 2017 desaparece esta entidad.
FISH: Hibridación in situ fluorescente; ICUS: citopenia idiopática de significado incierto; SNP/CGH: *single nucleotide polymorphism/comparative genomic hybridization.*

INTRODUCCIÓN

- Enfermedad heterogénea y compleja.
- La utilización generalizada de la CMF para el pronóstico de SMD se ve obstaculizada por la complejidad del análisis.
- Gran variedad de parámetros y clasificaciones para el diagnóstico.
- No existen algoritmos validados que incluyan la CMF para pacientes con citopenias a estudio.
- Valor clínico no se refleja plenamente en el grado de implementación en la práctica habitual.

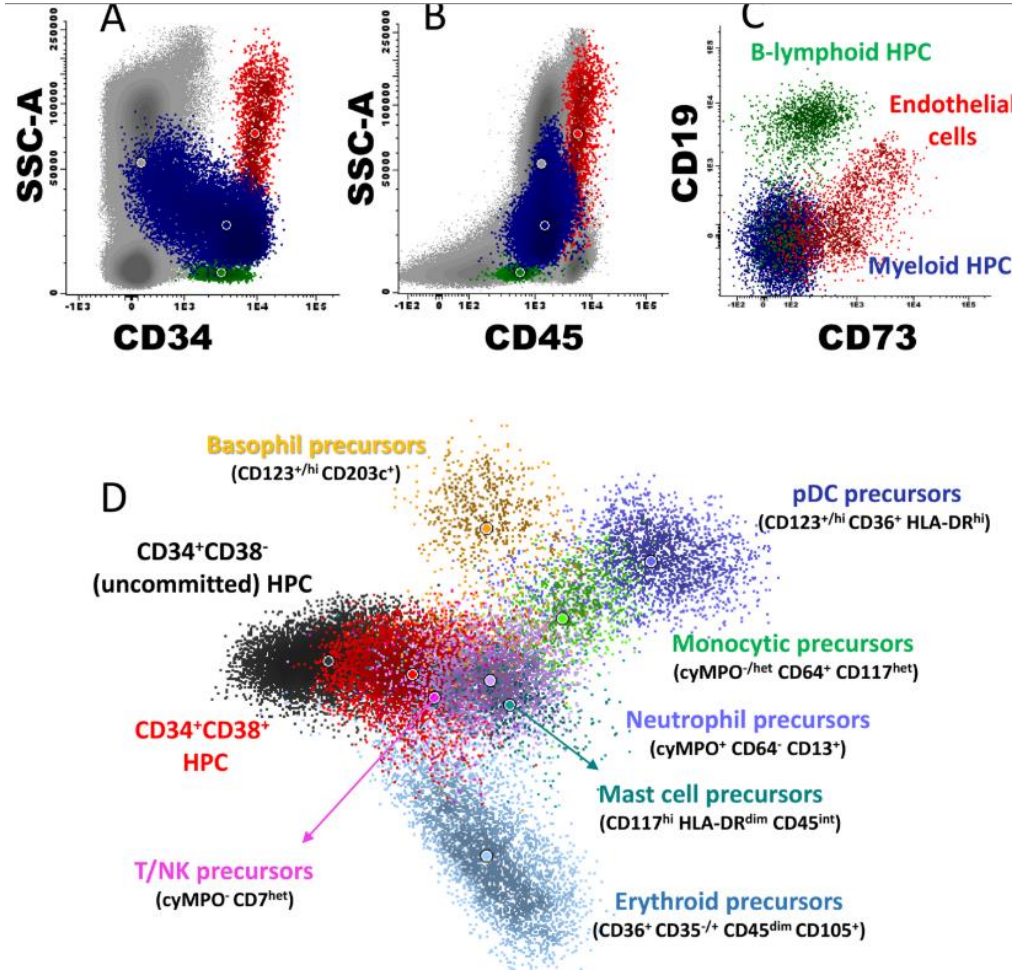
Alhan, C., Westers, T. M., Cremers, E. M. P., Cali, C., Witte, B. I., Ossenkoppele, G. J., & Van De Loosdrecht, A. A. (2016). The myelodysplastic syndromes flow cytometric score: A three-parameter prognostic flow cytometric scoring system. *Leukemia*, *30*(3), 658–665. https://doi.org/10.1038/leu.2015.295

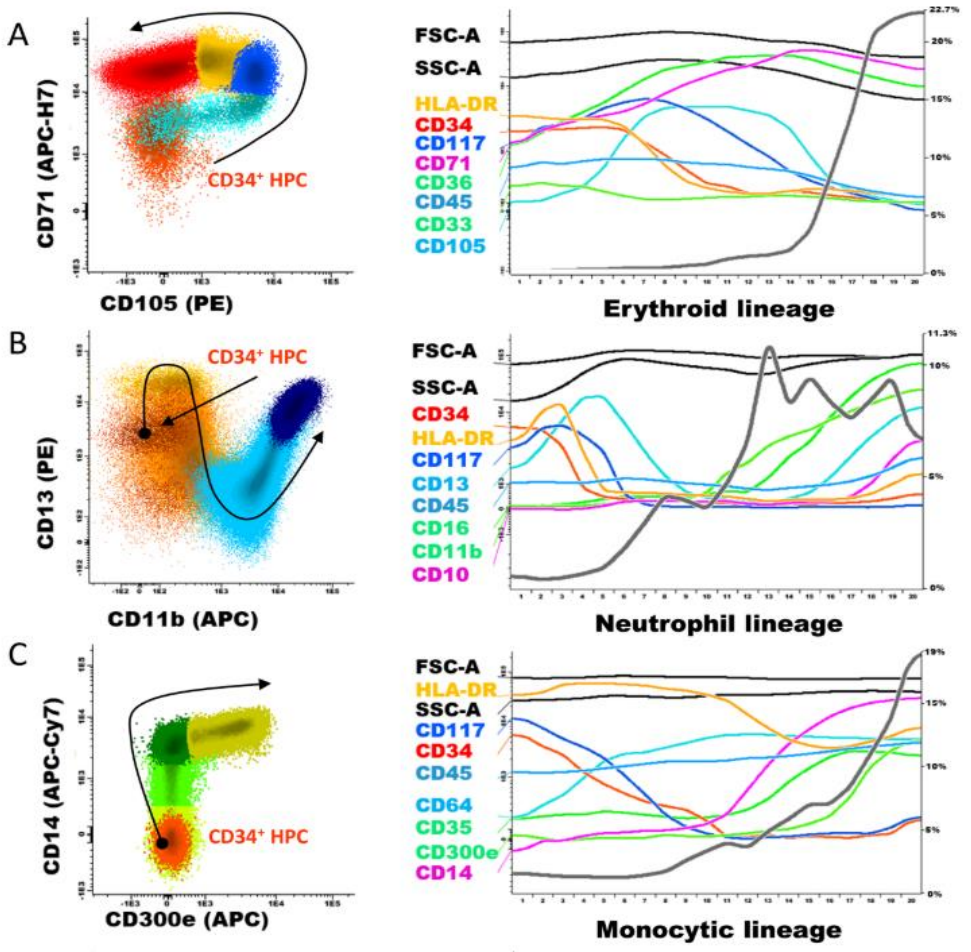
INTRODUCCIÓN

- La aplicación de la CMF en los SMD se basa en el concepto de que la alteración de la hematopoyesis puede estudiarse mediante la expresión de antígenos durante la diferenciación.

Alhan, C., Westers, T. M., Cremers, E. M. P., Cali, C., Ossenkoppele, G. J., & van de Loosdrecht, A. A. (2016). Application of flow cytometry for myelodysplastic syndromes: Pitfalls and technical considerations. *Cytometry Part B - Clinical Cytometry*, *90*(4), 358–367. https://doi.org/10.1002/cyto.b.21333

ESTUDIO DE SMD POR CMF





Orfao, A., Matarraz, S., Pérez-Andrés, M., Almeida, J., Teodosio, C., Berkowska, M. A., & van Dongen, J. J. M. (2019, December 1). Immunophenotypic dissection of normal hematopoiesis. *Journal of Immunological Methods*. Elsevier B.V. https://doi.org/10.1016/j.jim.2019.112684

ESTUDIO DE SMD POR CMF

CD34$^+$ cell compartment	Phenotype	Distribution (%)
% BM CD34$^+$ precursor cells		0.9% (0.2-1.6)
Myeloid precursors	CD38$^+$ CD45^{lo} CD117$^+$ HLA-DR^{het}	77% (57-85%)
Neutrophil lineage	cyMPO$^+$ CD64$^-$ CD13$^+$	33% (26-38%)
Erythroid lineage	CD36$^+$ CD35$^{-/+}$ CD45^{lo} CD105$^+$	35% (24-37%)
Monocytic lineage	cyMPO$^-$ CD64$^+$ CD117^{het}	22% (16-28%)
pDC lineage	CD123$^{+/hi}$ CD36$^+$ CD45^{lo} HLA-DR^{hi}	6% (1-9%)
Basophil lineage	CD123$^{+/hi}$ CD45$^+$ CD117^{lo} HLA-DR^{lo} CD203c$^+$	<1% (0-3%)
Megakaryocytic lineage	CD61$^+$ CD45^{lo} CD203c^{lo}	<1%
Eosinophil lineage	cyMPO$^-$ CD15/CD65$^+$ cyEPO$^+$	<1%
Mast cell lineage	CD117^{hi} HLA-DR^{lo} CD45^{int}	<1%
B-lymphoid precursors	nuTdT$^+$ cyCD79a$^+$ CD19$^+$	23% (<1-45%)
T/NK/DC precursors	cyMPO$^-$ CD7^{het}	12% (10-15%)
% BM CD34$^-$CD117$^+$ myeloid precursors		2.3% (0.8-2.7%)
Neutrophil precursors	cyMPO^{hi} HLA-DR^{het} CD13^{hi}	54.6% (53-69%)
Erythroid precursors	cyMPO$^-$ HLA-DR^{het} CD105$^+$CD36^{hi}	30% (21-40%)
Monocytic precursors	cyMPO$^+$ HLA-DR^{hi} CD13^{int} CD64$^{+/hi}$ CD14$^-$	10% (5-16%)
CD34$^-$CD117$^-$ maturing myeloid cells		
Neutrophil lineage	cyMPO^{lo} HLA-DR$^-$ CD13^{het}	59% (46-74%)
Erythroid lineage	cyMPO$^-$ HLA-DR$^-$ CD105$^-$CD36^{hi}	15% (2-29%)
Monocytic lineage	cyMPO$^+$ HLA-DR^{hi} CD64^{hi} CD14^{het}	4% (2-6%)
pDC lineage	CD123^{hi} CD36$^+$ CD45^{lo} HLA-DR^{hi}	0.2% (0-0.6%)
Basophil lineage	CD123^{hi} CD45$^+$ HLA-DR$^-$ CD203c$^+$	0.4% (0.05-3%)
Eosinophil lineage	cyMPO$^-$ CD15/CD65$^+$ cyEPO$^+$	2.3% (0-4%)
Mast cell lineage	CD117^{hi} HLA-DR$^-$ CD45^{int}	0.005% (0-0.02%)

INTRODUCCIÓN

- En qué casos ayuda la CMF al diagnóstico de SMD:
  - No hay aumento en el número de blastos.
  - No hay ≥ 15% de sideroblastos en anillo.
  - Cariotipo sin alteración citogenética asociada a SMD (50%) o sin metafases analizables.
  - Displasia ≈ 10%.
  - Muestra de poca calidad.

- No permite hallar alteraciones específicas de mielodisplasia. Debe incluirse dentro de un informe de diagnóstico integrado junto con la citología, la citogenética y los estudios moleculares.

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CARACTERÍSTICAS TÉCNICAS

- Recogida de MO en tubo de heparina. EDTA influye en el marcaje de CD11b.
- Conservación a temperatura ambiente y menos de 24 horas. Si marcaje y análisis >24h aumento en el SSC de neutrófilos y disminución de CD14.
- No hay evidencia de cuál es el mejor procedimiento de lisis para la preparación de una MO en pacientes con SMD. Se recomienda realizar el procedimiento de lisar-marcar- lavar.

Duetz, C., Westers, T. M., & Van De Loosdrecht, A. A. (2019, January 1). Clinical Implication of Multi-Parameter Flow Cytometry in Myelodysplastic Syndromes. *Pathobiology*. S. Karger AG. https://doi.org/10.1159/000490727

Alhan, C., Westers, T. M., Cremers, E. M. P., Cali, C., Ossenkoppele, G. J., & van de Loosdrecht, A. A. (2016). Application of flow cytometry for myelodysplastic syndromes: Pitfalls and technical considerations. *Cytometry Part B - Clinical Cytometry*, *90*(4), 358–367. https://doi.org/10.1002/cyto.b.21333

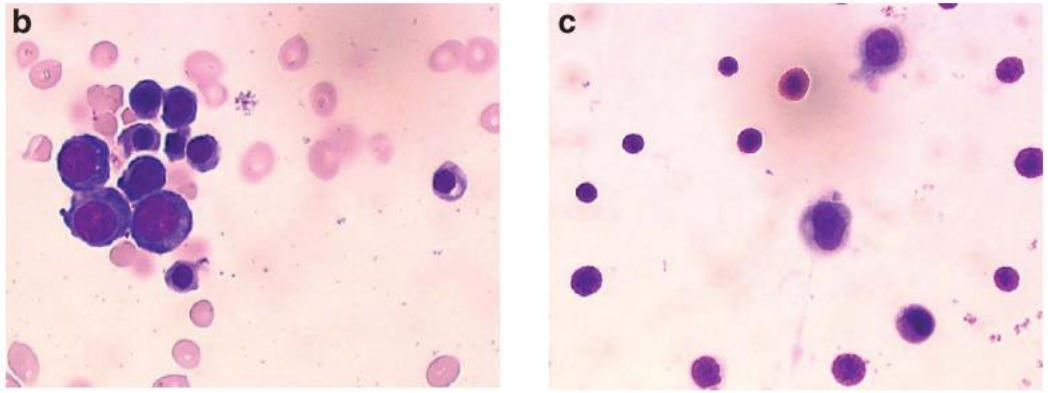
ESTUDIO DE SMD POR CMF

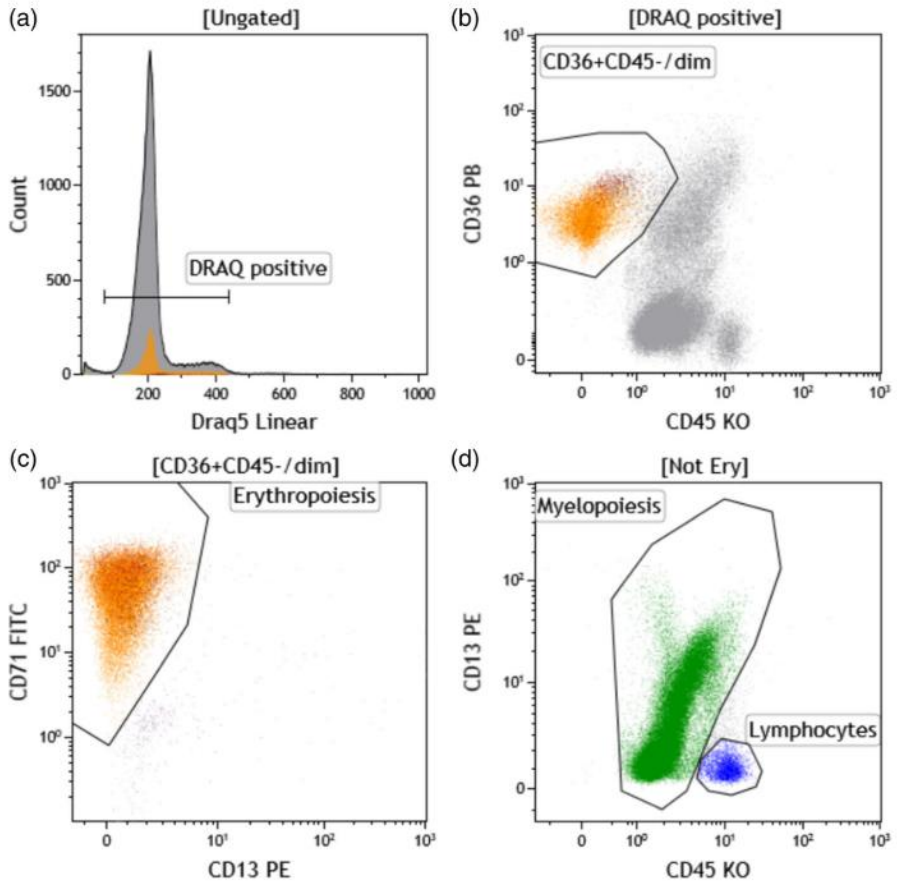
ORIGINAL ARTICLE

CLINICAL CYTOMETRY WILEY

Analysis of erythroid maturation in the nonlysed bone marrow with help of radar plots facilitates detection of flow cytometric aberrations in myelodysplastic syndromes

Despoina Violidaki[1,2] | Olof Axler[1] | Katayoon Jafari[3] | Filippa Bild[1] | Lars Nilsson[4] | Joanna Mazur[5] | Mats Ehinger[1,2] | Anna Porwit[1,2]





Violidaki, D., Axler, O., Jafari, K., Bild, F., Nilsson, L., Mazur, J., … Porwit, A. (2020). Analysis of erythroid maturation in the nonlysed bone marrow with help of radar plots facilitates detection of flow cytometric aberrations in myelodysplastic syndromes. *Cytometry Part B - Clinical Cytometry*, *98*(5), 399–411. https://doi.org/10.1002/cyto.b.21931

Mathis, S., Chapuis, N., Debord, C., Rouquette, A., Radford-Weiss, I., Park, S., … Bardet, V. (2013). Flow cytometric detection of dyserythropoiesis: A sensitive and powerful diagnostic tool for myelodysplastic syndromes. *Leukemia*, *27*(10), 1981–1987. https://doi.org/10.1038/leu.2013.178

ESTUDIO DE SMD POR CMF

CARACTERÍSTICAS TÉCNICAS

- Recogida de MO en tubo de heparina. EDTA influye en el marcaje de CD11b.
- Conservación a temperatura ambiente y menos de 24 horas. Si marcaje y análisis >24h aumento en el SSC de neutrófilos y disminución de CD14.
- No hay evidencia de cuál es el mejor procedimiento de lisis para la preparación de una MO en pacientes con SMD. Se recomienda realizar el procedimiento de lisar-marcar-lavar.
- Eventos: 500,000 por tubo. >250 eventos por población.
- Calidad de la muestra: presencia de precursores, mastocitos, células plasmáticas. PMN <50% de las células de la línea granulocítica.
- **Procedimientos estandarizados; cada laboratorio debe tener sus valores de referencia.**

Duetz, C., Westers, T. M., & Van De Loosdrecht, A. A. (2019, January 1). Clinical Implication of Multi-Parameter Flow Cytometry in Myelodysplastic Syndromes. *Pathobiology*. S. Karger AG. https://doi.org/10.1159/000490727

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ESTUDIO DE SMD POR CMF

¿Qué necesitamos?

- Profesionales entrenados con conocimiento de la médula ósea normal.
- Procesado de la muestra uniforme.
- Estandarización de la estrategia de análisis.

ESTUDIO DE SMD POR CMF

¿Qué buscamos?

- Alteraciones numéricas de los % de las poblaciones celulares.
- Cambios en la complejidad celular.
- Alteraciones de la expresión antigénica.
- Infidelidad de línea.
- Asincronismo madurativo.

ESTUDIO DE SMD POR CMF

Table 1. MFC parameters of interest for MDS

Progenitors		Neutrophils		Monocytes		Erythroid lineage	
CD34	Increase in % or abnormal expression	SSC	Decreased	CD13	Lack of or abnormal expression	CD71	Decreased or heterogeneous expression
CD34+/CD10+ or CD34+ and CD19+	Decrease in %	CD11b	Lack of or abnormal expression	CD14	Lack of or abnormal expression	CD36	Decreased or heterogeneous expression
CD45	Abnormal expression	CD13	Lack of or abnormal expression	CD16	Lack of or abnormal expression	CD117	Abnormal frequency
CD117	Abnormal expression	CD33	Lack of or abnormal expression	CD33	Lack of or abnormal expression	CD105	Abnormal frequency
SSC	Abnormal granularity	CD16	Delayed expression	CD11b	Abnormal expression		
CD13	Overexpression or lack of expression	CD10	Lack of expression	HLA-DR	Abnormal expression		
CD33	Overexpression or lack of expression	CD56	Expression	CD56	Overexpression		
HLA-DR	Overexpression or lack of expression	Subsets	% abnormal distribution of mature and immature subsets	SSC	Decreased or increased		
CD5	Expression on myeloid progenitors	%	As a ratio to lymphocytes	Subsets		% abnormal distribution of mature and immature subsets	
CD7	Expression on myeloid progenitors	CD13/CD11b	Altered pattern	HLA-DR/11b	Altered pattern		
CD19	Expression on myeloid progenitors	CD13/CD16	Altered pattern	CD36/CD14	Altered pattern		
CD56	Expression on myeloid progenitors	CD15/CD10	Altered pattern				
CD11b	Expression on myeloid progenitors						
CD15	Overexpression						

MFC, multi-parameter flow cytometry; MDS, myelodysplastic syndrome; SSC, side scatter.

Duetz, C., Westers, T. M., & Van De Loosdrecht, A. A. (2, 019 January 1). Clinical Implication of Multi-Parameter Flow Cytometry in Myelodysplastic Syndromes. *Pathobiology*. S. Karger AG. https://doi.org/10.1159/000490727

ESTUDIO DE SMD POR CMF

Cellular Compartment	Common aberrancies	
Myelomonocytic progenitors 	↑ Percentage as a fraction of all nucleated cells (>2%), ↑ fraction of CD34+/CD38 dim/-	↑ side scatter
	↓ CD45 intensity	Altered pattern of CD13 and CD33 espression when plotted agains each other (loss of the so-called "boomerang" pattern)
	↑ ↓ CD34 intnsity	Asynchronous expression of of CD11b, CD15 (normally present on mature cells)
	↑ ↓ CD117 intensity	Lineage infidelity: + expression of CD5, CD7, CD19, CD56
	↑ fraction of CD34+ HLA-DR dim/- cells	
Monocytes 	↑ ↓ Percentage of cells	Homogenous ↑ or ↓ of CD13 and/or CD33
	Phenotypic shift towards immaturity ↑ CD15, ↓ CD13, HLA-DR and CD14	+ expression of CD56* or CD2
	Altered pattern of HLA-DR and CD11b when plotted agains each other	
	Altered pattern of CD36 and CD14 when plotted agains each other	
Maturing myeloid cells 	↓ Percentage of cells as ratio to lymphocytes	Altered pattern of CD13 and CD11b when plotted against each other
	↓ SSC as ratio vs SSC of lymphocytes	Altered pattern of CD13 and CD16 when plotted against each other
		Altered expression CD15 and CD10 for stage of maturation; i.e. ↓ CD10 or CD15 expression on mature neutrophils
Hematogones 	↓ pecentage as a fraction of the total CD34+ cellular compartment (≤5%)	
Erythroid compartment 	↑ precentage of nucleated erythroid cells	↓ expression of CD36
	Altered pattern of CD71 and CD235a when plotted against each other	↑ percentage of CD117-positive erythroid precursors
	↓ Expression of CD71	

ESTUDIO DE SMD POR CMF

Table 14. The EuroFlow AML/MDS antibody panel[a,b]

Tube	*PacB*	*PacO*	*FITC*	*PE*	*PerCPCy5.5*	*PECy7*	*APC*	*APCH7*	*Aim*
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	NuTdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation
5	HLADR	CD45	CD15	NG2	CD34	CD117	CD22	CD38	Aberrant marker expression, stem cells
6	HLADR	CD45	CD42a and CD61	CD203c	CD34	CD117	CD123	CD4	Diagnosis and classification of AML Megakaryocytic, basophilic, and plasmacytoid dendritic cell lineages
7	HLADR	CD45	CD41	CD25	CD34	CD117	CD42b	CD9	Characterization of megakaryoblastic leukemia, and systemic mastocytosis

Abbreviations: AML, acute myeloid leukemia; APC, allophycocyanin; BB, backbone; BM, bone marrow; Cy7, cyanin7; FITC, fluorescein isothiocyanate; H7, hilite7; MDS, myelodysplastic syndrome; Nu, nuclear; PacB, pacific blue; PacO, pacific orange; PE, phycoerythrin; PerCPCy5.5, peridinin–chlorophyll–protein–cyanin5.5; PNH, paroxysmal nocturnal hemoglobinuria. [a]Further information about markers and hybridomas is provided in the Appendix. [b]A total of 96 BM samples were evaluated for selection of the BB markers. An additional 84 BM AML samples were evaluated with this version (final) of the panel.

Van Dongen, J. J. M., Lhermitte, L., Böttcher, S., Almeida, J., Van Der Velden, V. H. J., Flores-Montero, J., … Orfao, A. (2012). EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia*, *26*(9), 1908–1975. https://doi.org/10.1038/leu.2012.120

ESTUDIO DE SMD POR CMF

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CD15	Overexpression						

MFC, multi-parameter flow cytometry; MDS, myelodysplastic syndrome; SSC, side scatter.

ESTUDIO DE SMD POR CMF

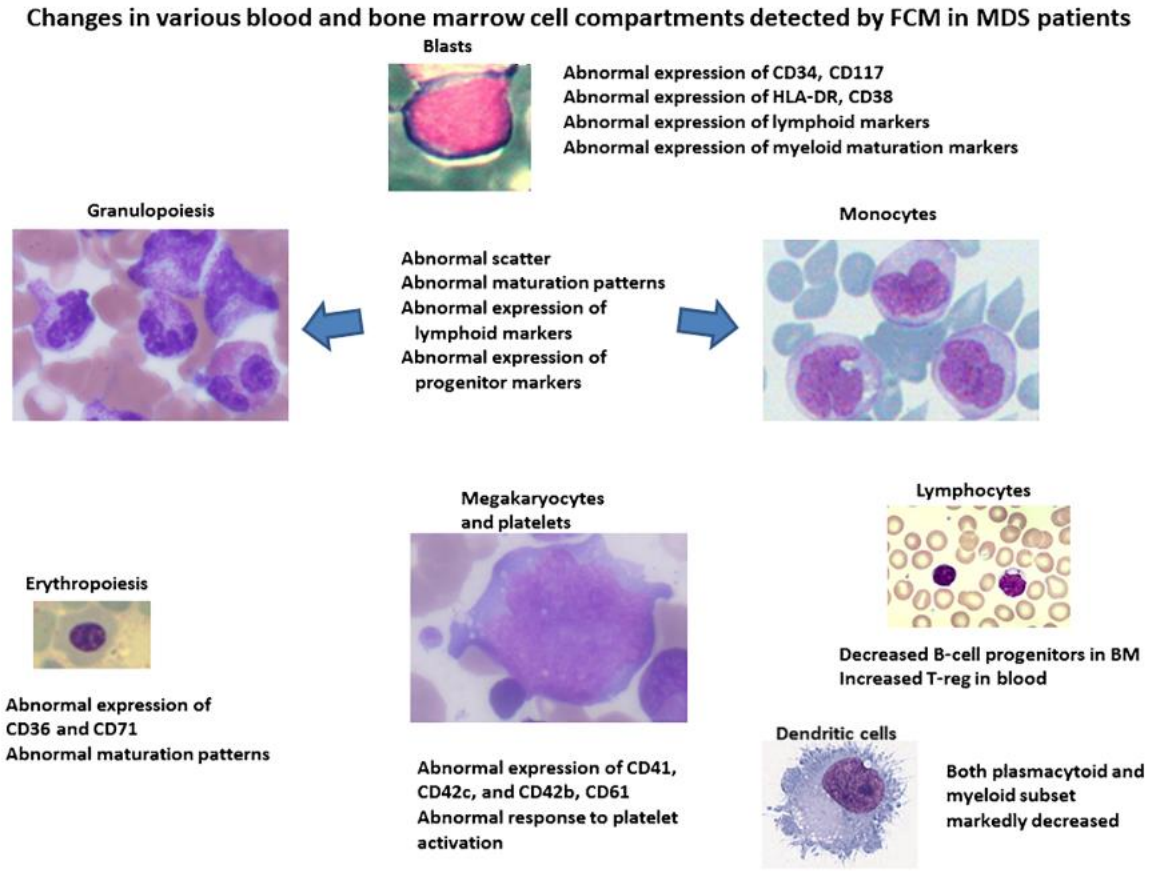
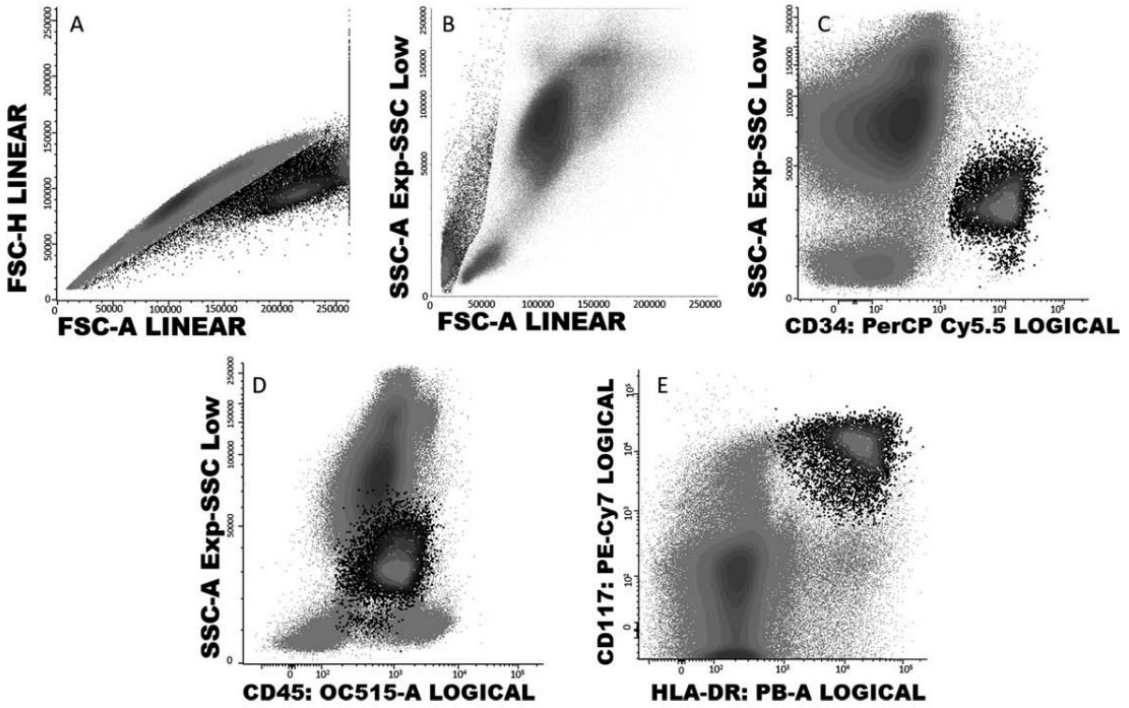


Fig. 1 Changes in various blood and bone marrow cell compartments detected by FCM in MDS patients

Porwit, A. (2015, September 1). Is There a Role for Flow Cytometry in the Evaluation of Patients With Myelodysplastic Syndromes? *Current Hematologic Malignancy Reports*. Current Science Inc. https://doi.org/10.1007/s11899-015-0272-3

Estudio de los progenitores CD34+

- **Aumento del % CD34+/CD19-**
- **Aumento del % CD38-/CD34+**
- SSC y expresión de CD45 alterada.
- Expresión alterada de CD34 y CD117
- Expresión alterada de HLA-DR, CD11b, CD15
- Aumento de CD33+/CD13- o CD33-/CD13+
- Marcadores de infidelidad de linaje.

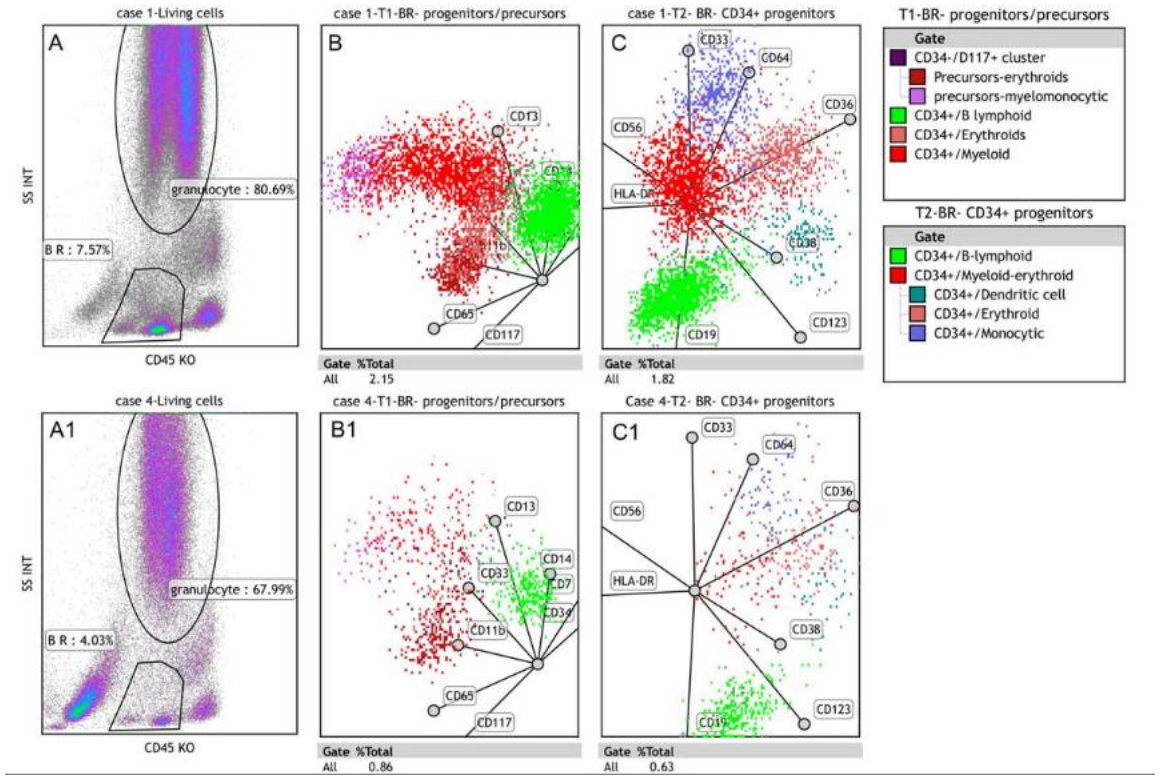


Porwit, A., Van De Loosdrecht, A. A., Bettelheim, P., Eidenschink Brodersen, L., Burbury, K., Cremers, E., … Béné, M. C. (2014). Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes - Proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. *Leukemia*. Nature Publishing Group. https://doi.org/10.1038/leu.2014.191

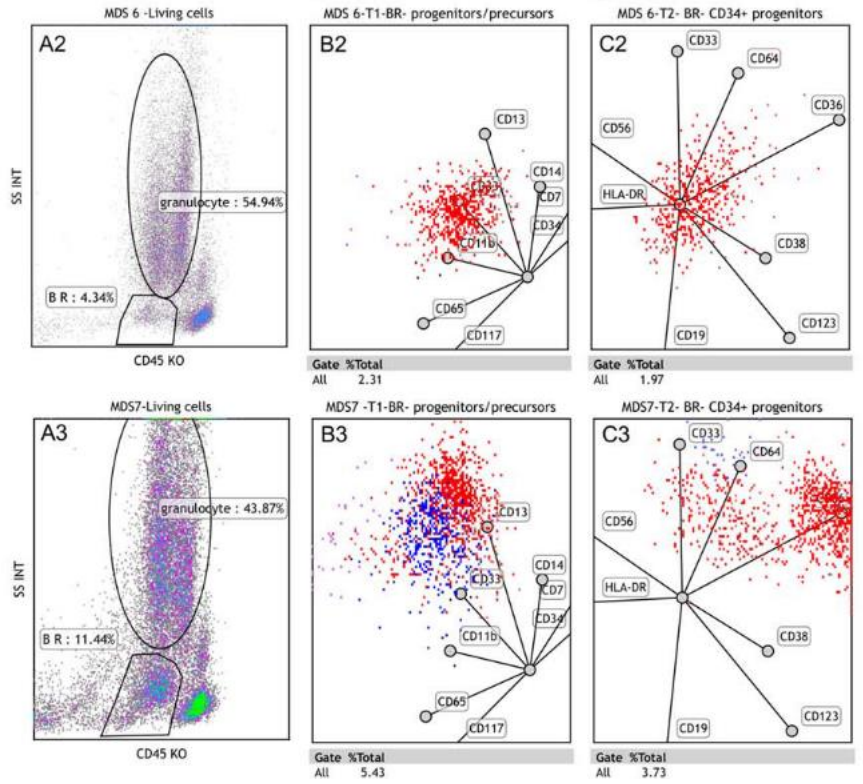
Font, P., Subirá, D., Matarraz, S., Benavente, C., Cedena, M. T., Morado, M., … Díez-Martín, J. L. (2018). Multicenter comparison of CD34+ myeloid cell count by flow cytometry in low-risk myelodysplastic syndrome. Is it feasible? *Cytometry Part B - Clinical Cytometry*, *94*(3), 527–535. https://doi.org/10.1002/cyto.b.21538

Estudio de los progenitores CD34+

MO NORMAL



SMD



Jafari, K., Tierens, A., Rajab, A., Musani, R., Schuh, A., & Porwit, A. (2018). Visualization of Cell Composition and Maturation in the Bone Marrow Using 10-Color Flow Cytometry and Radar Plots. *Cytometry Part B - Clinical Cytometry*, *94*(2), 219–229. https://doi.org/10.1002/cyto.b.21519

Estudio de los progenitores CD34-

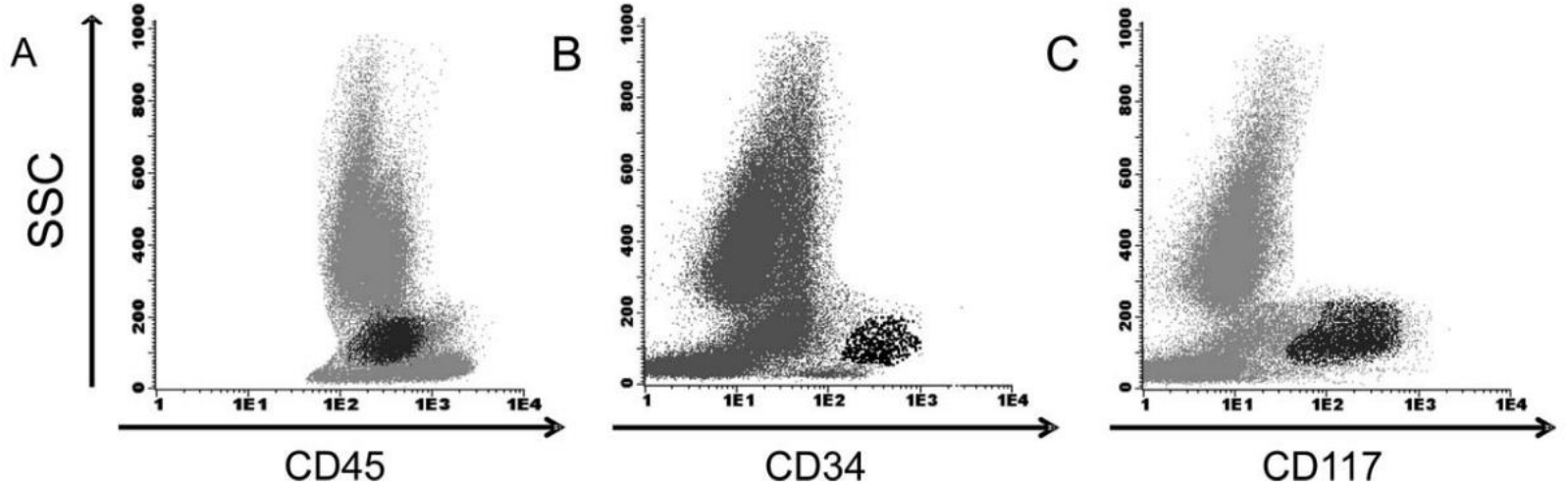
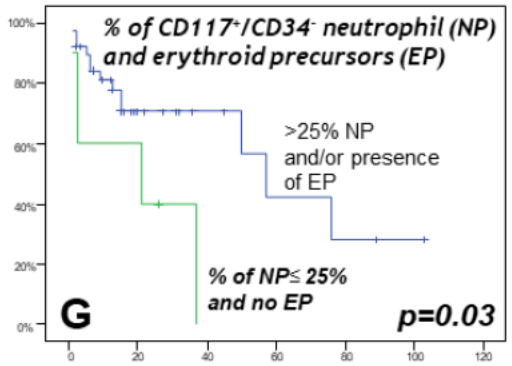


FIG. 5. Abnormal $CD34^{neg}$ myeloid progenitors in a patient with MDS. (**A**) Flow cytometric analysis of a BM sample of a patient with MDS refractory anemia with excess of blasts (5–10%). Myeloid progenitors are defined as $SSC^{intermediate}$ and $CD45^{dim}$ (black dots). (**B**) The myeloid progenitors were back gated in the SSC vs. CD45 plot by gating $CD34^{pos}$ cells. Notably, there is a large population of cells with $SSC^{intermediate}$ and $CD45^{dim}$ properties that are not $CD34^{pos}$. (**C**) The use of CD117 aids in identifying the aberrantly CD34neg myeloid progenitors. The myeloid progenitors are $CD117^{pos}$, $SSC^{intermediate}$ and $CD45^{dim}$ (black dots) but $CD34^{neg}$ as described above.

Alhan, C., Westers, T. M., Cremers, E. M. P., Cali, C., Ossenkoppele, G. J., & van de Loosdrecht, A. A. (2016). Application of flow cytometry for myelodysplastic syndromes: Pitfalls and technical considerations. *Cytometry Part B - Clinical Cytometry*, *90*(4), 358–367. https://doi.org/10.1002/cyto.b.21333

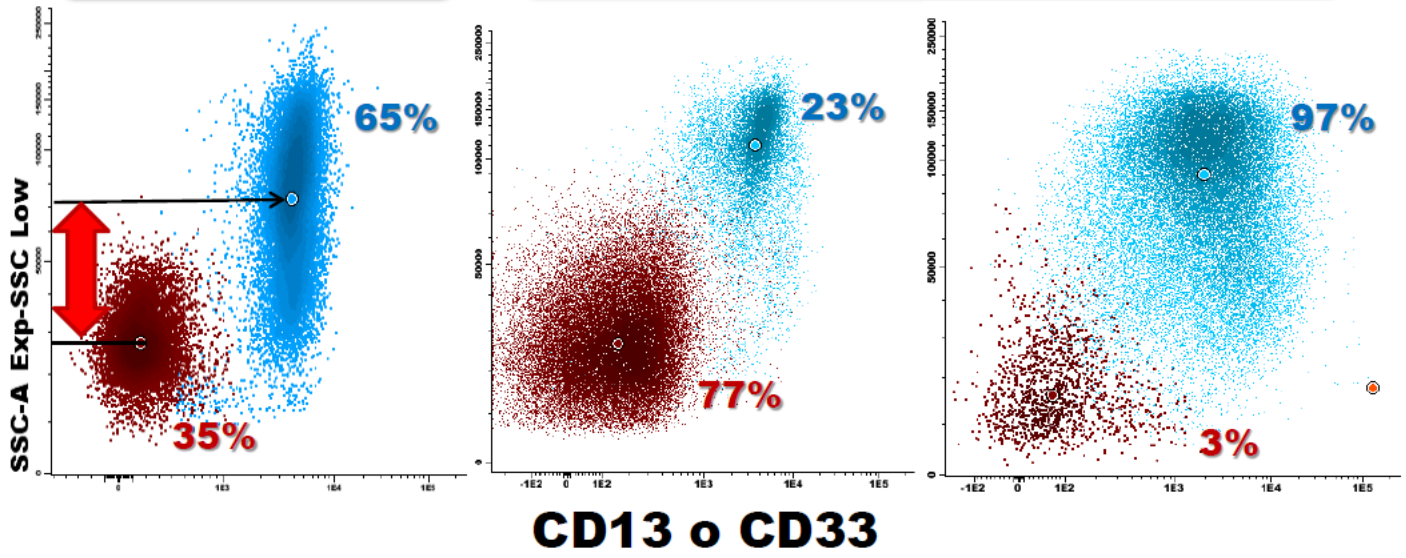
Estudio de los progenitores CD34-

Neutrophil L. in MDS



Gated CD34-CD117+

Normal | MDS

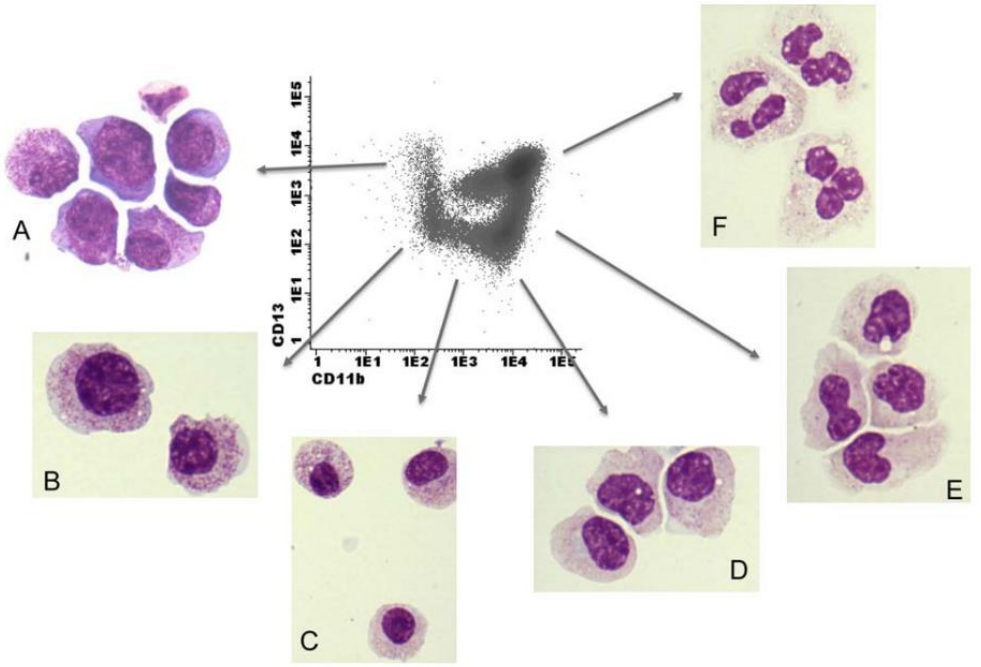


- Imbalance of CD34-CD117+ NP/EP (51% of MDS) is related to adverse outcome

Matarraz S, Cytometry B 2010

Estudio de la serie mieloide granulocítica

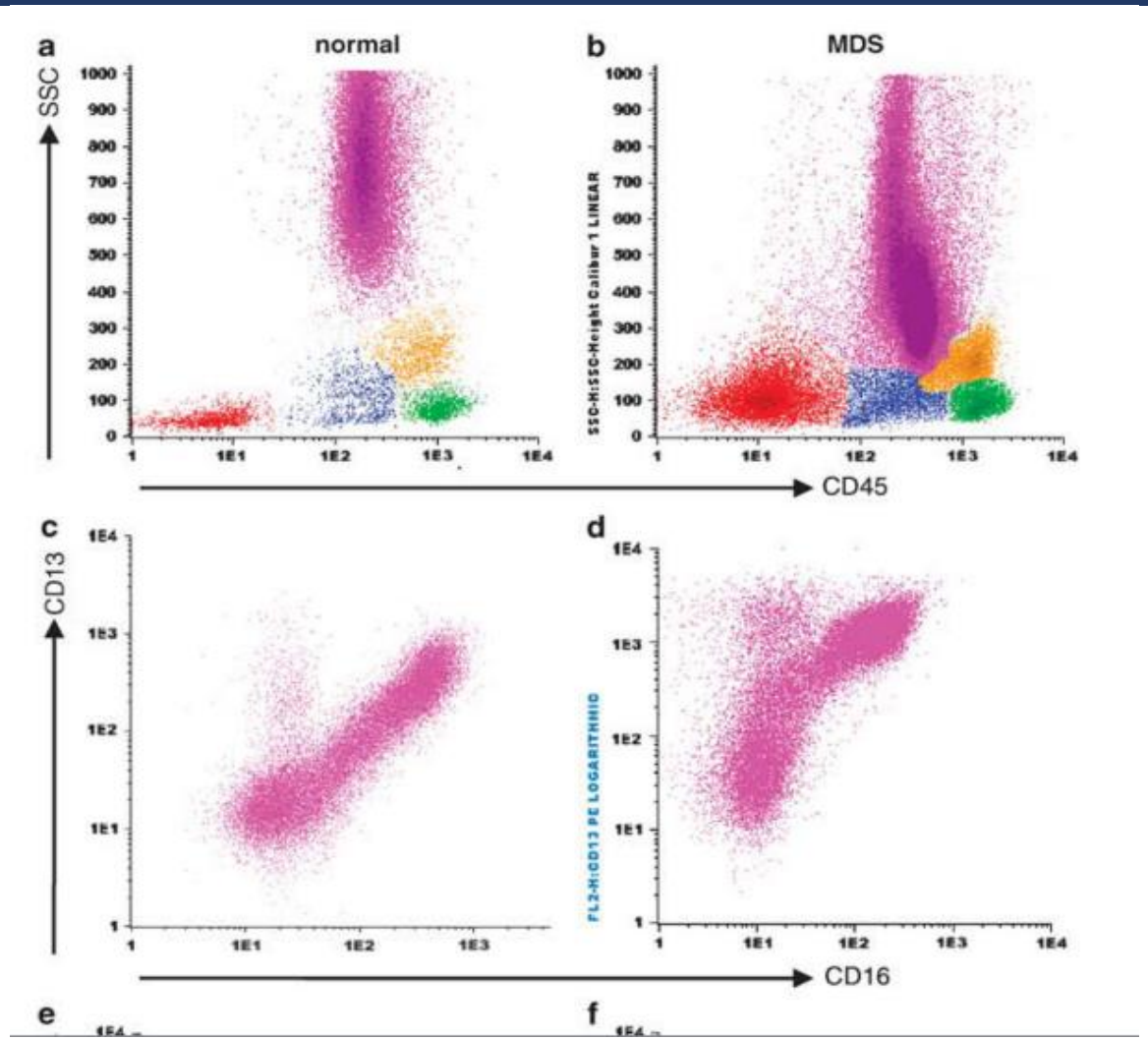
- **SSC Gran/Lym disminuido.**
- Expresión disminuida de CD45
- Aumento de la expresión de CD117
- Expresión asincrónica de CD34
- Aumento de la expresión de HLA-DR
- **Patrón aberrante CD11b/CD16**
- **Patrón aberrante CD13/CD16**
- Aumento de CD33+/CD13- o CD33-/CD13+
- Pérdida de CD10 en los neutrófilos maduros.
- Aumento de la expresión de CD36.
- **Marcadores de infidelidad de linaje.**



Porwit, A., Van De Loosdrecht, A. A., Bettelheim, P., Eidenschink Brodersen, L., Burbury, K., Cremers, E., … Béné, M. C. (2014). Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes - Proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. *Leukemia*. Nature Publishing Group. https://doi.org/10.1038/leu.2014.191

Alhan, C., Westers, T. M., Cremers, E. M. P., Cali, C., Ossenkoppele, G. J., & van de Loosdrecht, A. A. (2016). Application of flow cytometry for myelodysplastic syndromes: Pitfalls and technical considerations. *Cytometry Part B - Clinical Cytometry*, *90*(4), 358–367. https://doi.org/10.1002/cyto.b.21333

Estudio de la serie mieloide granulocítica



Westers, T. M., Ireland, R., Kern, W., Alhan, C., Balleisen, J. S., Bettelheim, P., … Van De Loosdrecht, A. A. (2012). Standardization of flow cytometry in myelodysplastic syndromes: A report from an international consortium and the European LeukemiaNet Working Group. *Leukemia*, *26*(7), 1730–1741. https://doi.org/10.1038/leu.2012.30

Estudio de la serie monocítica

- SSC disminuido
- Expresión disminuida de CD45
- Expresión asincrónica de CD34
- Disminución de la expresión de HLA-DR
- Disminución de CD11b
- **Patrón aberrante CD11b/CD16**
- Patrón aberrante CD11b/HLA-DR
- Aumento de CD33+/CD13- o CD33-/CD13-
- Disminución de CD15
- Aumento de la expresión de CD36.
- **Marcadores de infidelidad de linaje.**

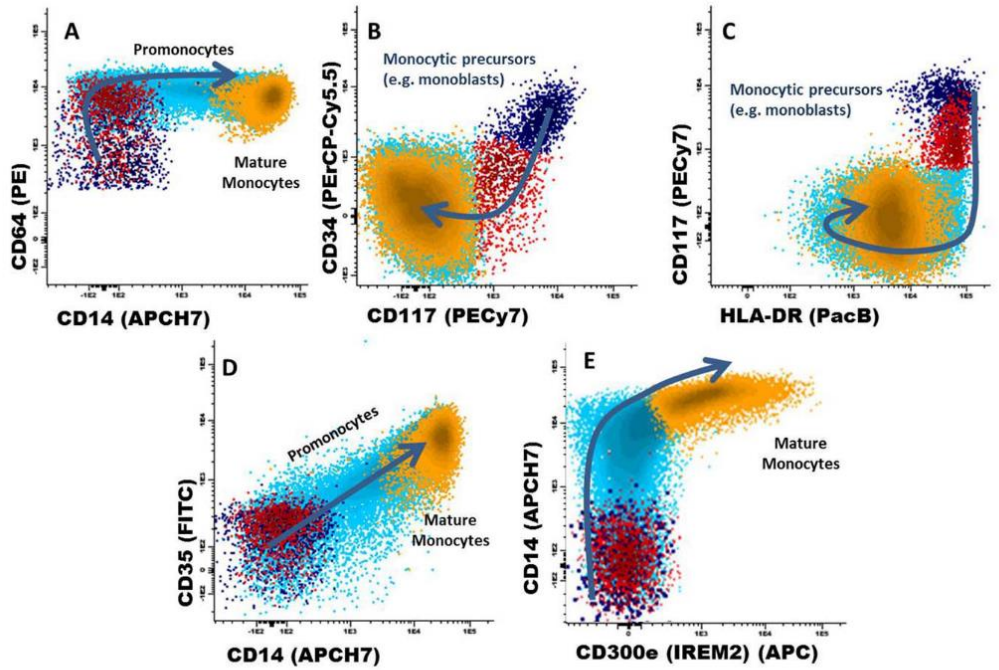
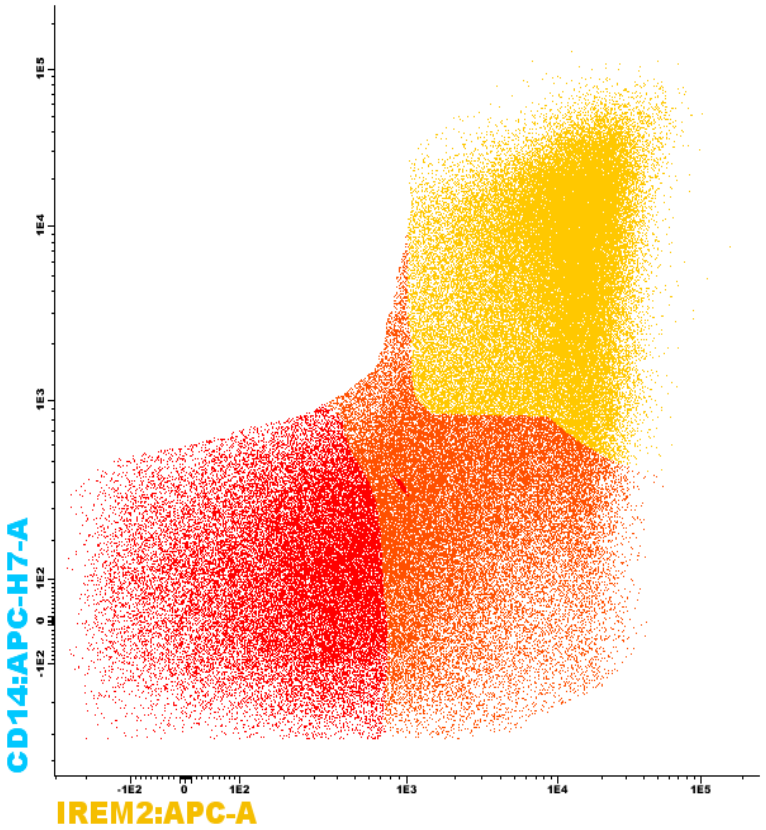


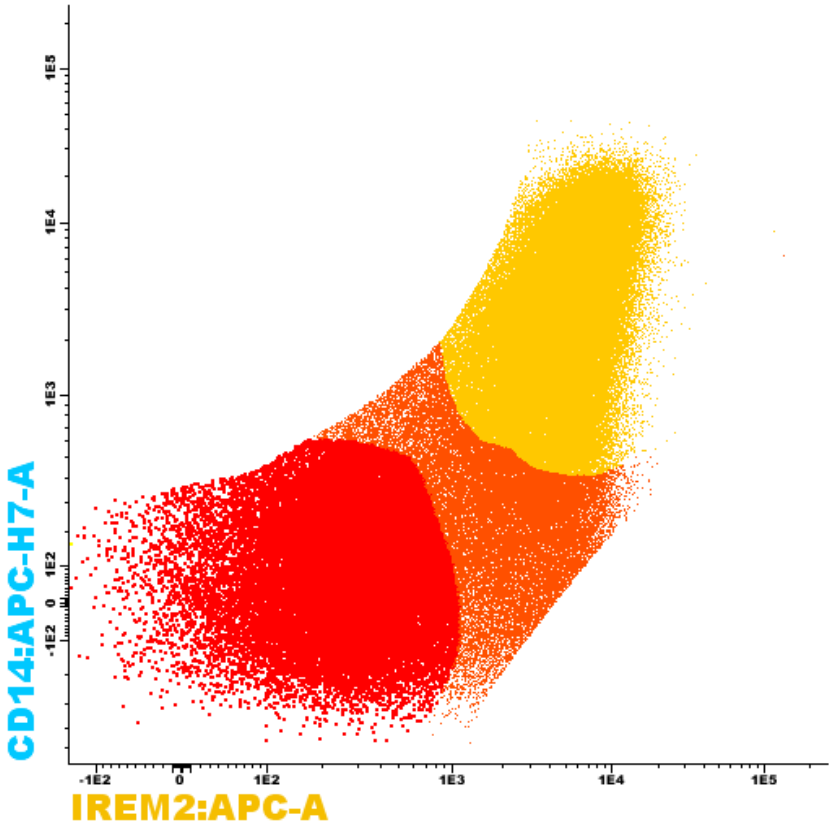
FIG. 1. Phenotypic patterns of monocytic maturation in normal bone marrow. Abbreviations: PerCPCy5.5, peridinin-chlorophyll-protein-cyanin5.5; PacB, pacific blue; PacO, pacific orange; PE, phycoerythrin; Cy7, cyanin7; APC, allophycocyanin; H7, hilite7; FIFC, fluorescein isothiocyanate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Porwit, A., Van De Loosdrecht, A. A., Bettelheim, P., Eidenschink Brodersen, L., Burbury, K., Cremers, E., … Béné, M. C. (2014). Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes - Proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. *Leukemia*. Nature Publishing Group. https://doi.org/10.1038/leu.2014.191

Matarraz, S., Almeida, J., Flores-Montero, J., Lécrevisse, Q., Guerri, V., López, A., … Orfao, A. (2017, May 1). Introduction to the diagnosis and classification of monocytic-lineage leukemias by flow cytometry. *Cytometry Part B - Clinical Cytometry*. John Wiley and Sons Inc. https://doi.org/10.1002/cyto.b.21219

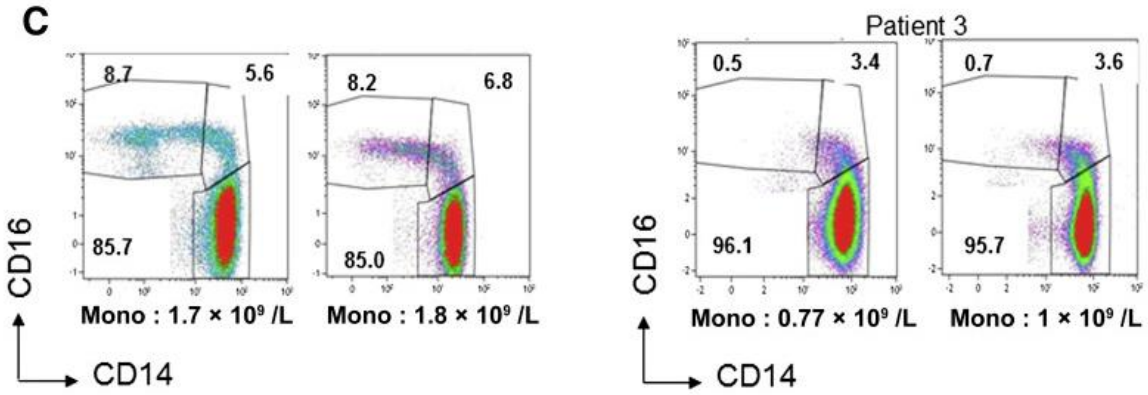
Estudio de la serie monocítica



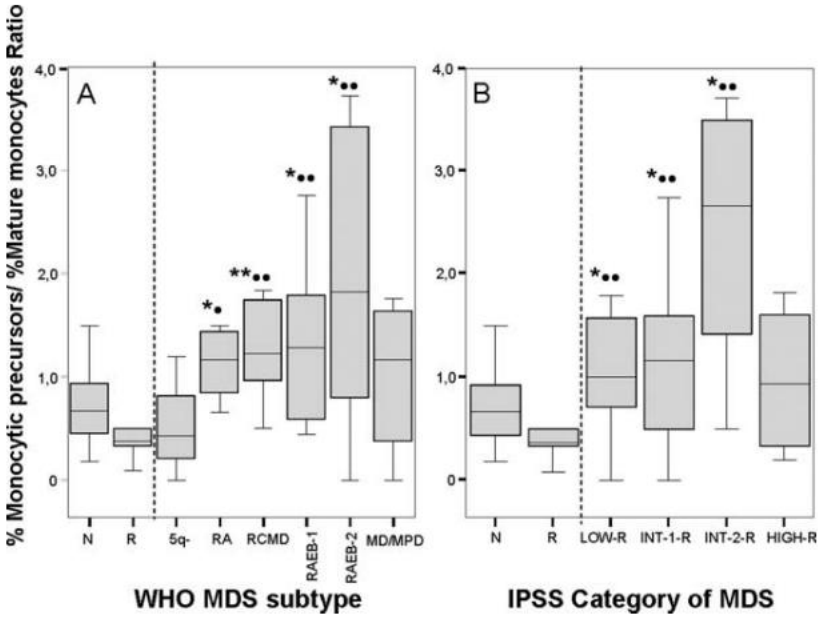


Dra. Margarita Trejos

Estudio de la serie monocítica



Dorothée Selimoglu-Buet, Orianne Wagner-Ballon, Véronique Saada, Valérie Bardet, Raphaël Itzykson, Laura Bencheikh, Margot Morabito, Elisabeth Met, Camille Debord, Emmanuel Benayoun, Anne-Marie Nloga, Pierre Fenaux, Thorsten Braun, Christophe Willekens, Bruno Quesnel, Lionel Adès, Michaela Fontenay, Philippe Rameau, Nathalie Droin, Serge Koscielny, Eric Solary; on behalf of the Groupe Francophone des Myélodysplasies, Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. *Blood* 2015; 125 (23): 3618–3626. doi: https://doi.org/10.1182/blood-2015-01-620781



Matarraz S, López A, Barrena S, Fernandez C, Jensen E, Flores-Montero J, Rasillo A, Sayagues JM, Sa´nchez ML, Ba´rcena P, Hernandez-Rivas JM, Salvador C, Fernandez-Mosteirı´n N, Giralt M, Perdiguer L, Laranjeira P, Paiva A, Orfao A. Bone marrow cells from myelodysplastic syndromes show altered immunophe- notypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. Cytometry Part B 2010; 78B: 154–168.

Estudio de la serie eritroide

ANÁLISIS DE LA SERIE ERITROIDE: PROBLEMAS

- Pocos estudios y falta de estandarización.
- Calidad de las muestras.
- Preparación de las muestras y método de lisis.
- Estrategia de análisis complicada.

Sin embargo, la diseritropoyesis es una de las características más frecuentes observadas en los SMD.

Westers, T. M., Cremers, E. M. P., Oelschlaegel, U., Johansson, U., Bettelheim, P., Matarraz, S., … van de Loosdrecht, A. A. (2017). Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group. *Haematologica*, *102*(2), 308–319. https://doi.org/10.3324/haematol.2016.147835

Estudio de la serie eritroide

EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group

Theresia M. Westers,[1] Eline M.P. Cremers,[1] Uta Oelschlaegel,[2] Ulrika Johansson,[3] Peter Bettelheim,[4] Sergio Matarraz,[5] Alberto Orfao,[5] Bijan Moshaver,[6] Lisa Eidenschink Brodersen,[7] Michael R. Loken,[7] Denise A. Wells,[7] Dolores Subirá,[8] Matthew Cullen,[9] Jeroen G. te Marvelde,[10] Vincent H.J. van der Velden,[10] Frank W.M.B. Preijers,[11] Sung-Chao Chu,[12] Jean Feuillard,[13] Estelle Guérin,[13] Katherina Psarra,[14] Anna Porwit,[15,16] Leonie Saft,[16] Robin Ireland,[17] Timothy Milne,[17] Marie C. Béné,[18] Birgit I. Witte,[19] Matteo G. Della Porta,[20] Wolfgang Kern[21] and Arjan A. van de Loosdrecht,[1] on behalf of the IMDSFlow Working Group

Haematologica 2017
Volume 102(2):308-319

- Cohorte de aprendizaje: 245 SMD, 290 patológico, 142 controles normales.
- Cohorte de validación: 129 SMD, 153 patológico, 49 controles normales.

ANÁLISIS:

- CV CD36: 4 PUNTOS
- CV CD71: 3 PUNTOS
- MFI CD71: 2 PUNTOS (si disminuido)
- % progenitores eritroides CD117+: 2 PUNTOS (si disminuido o aumentado)

≥ 5 PUNTOS: alteraciones eritroides asociadas A SMD

E: 90%, S: 33%

	10th percentile	90th percentile	# of PC cases*	# of NBM cases
relative %NEC	-	**268%**	238	139
%CD71^{dim}	-	**17%**	250	129
relative MFI of CD71	**45%**	-	250	126
relative CV of CD71	-	**133%**	165	88
relative MFI of CD36	**53%**	-	203	124
relative CV of CD36	-	**145%**	177	92
relative %CD117 progenitors	**37%**	**222%**	180	122
relative %CD105 progenitors	**50%**	**184%**	52	59
relative MFI of CD105	**52%**	**113%**	70	47

Note: : Cut-off values represent the 10th and 90th percentiles of results for erythroid markers among pathological controls in the learning cohort. The number of pathological control (PC) cases that were available to calculate cut-off values are displayed (*). Most values (except for %CD71^{dim}) are expressed as ratio to the median value determined in the set of normal bone marrow samples. The utmost right column displays the number of normal bone marrow (NBM) cases that were available to calculate these median values. Abbreviations: CV: coefficient of variation; dim: diminished; MFI: mean fluorescence intensity; NEC: nucleated erythroid cells.

Example on how to translate these reference ranges for application in a single center:
In case a sufficient amount of data is present regarding a large variation of pathological controls, 10th and 90th percentiles calculated from a center's own cohort may be applied when comparable to the herein described reference values.
Otherwise, collect data on an appropriate amount of normal bone marrow samples (min. 10) and determine median values to calculate the cut-offs for the parameters in the erythroid score.
For instance, the median CV value of CD71 in your set of normal bone marrow samples is 67 and the median percentage of CD117^{+} erythroid progenitors in the erythroid compartment is 8%. Then the reference values for CD71 CV is: 133/100*67=89; a CD71CV>89 should be considered increased. "133" is the 90th percentile for CD71 CV from supplementary table 2A.
Similarly for %CD117^{+}: lower cut-off 37/100*8=3.0 and highest cut-off 222/100*8=17.8; i.e. a CD117+ percentage (erythroid compartment) below 3.0% or above 17.8% should be considered aberrant.

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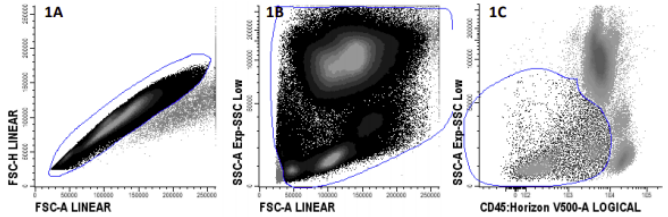
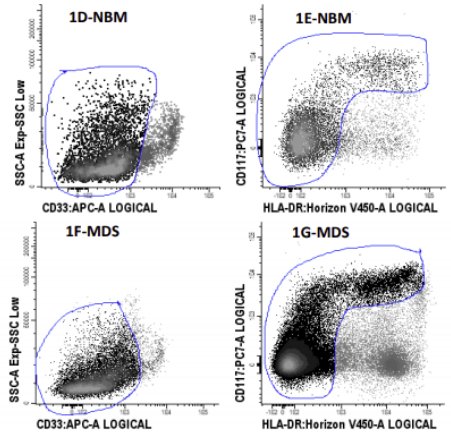
Estudio de la serie eritroide

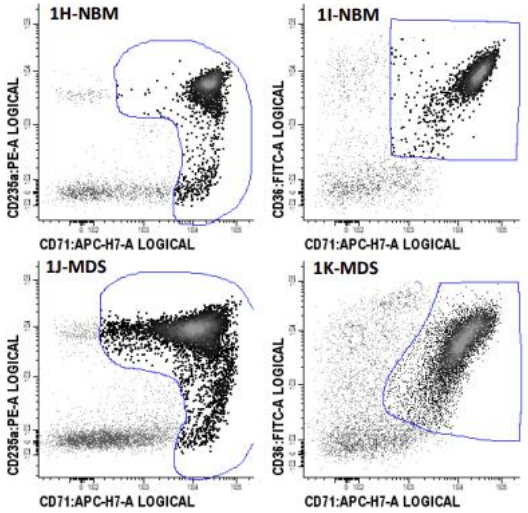
1. **Percentage of nucleated erythroid cells of total nucleated cells**
- Select singlets in FSC-A vs. FSC-H plot (when possible); figure 1A.
- Exclude debris in FSC-SSC plot (when available a nuclear dye can be helpful); figure 1B.
- Select CD45$^{negative\text{-}to\text{-}dim}$ with SSC$^{low\text{-}to\text{-}int}$ in CD45 vs. SSC plot; figure 1C.

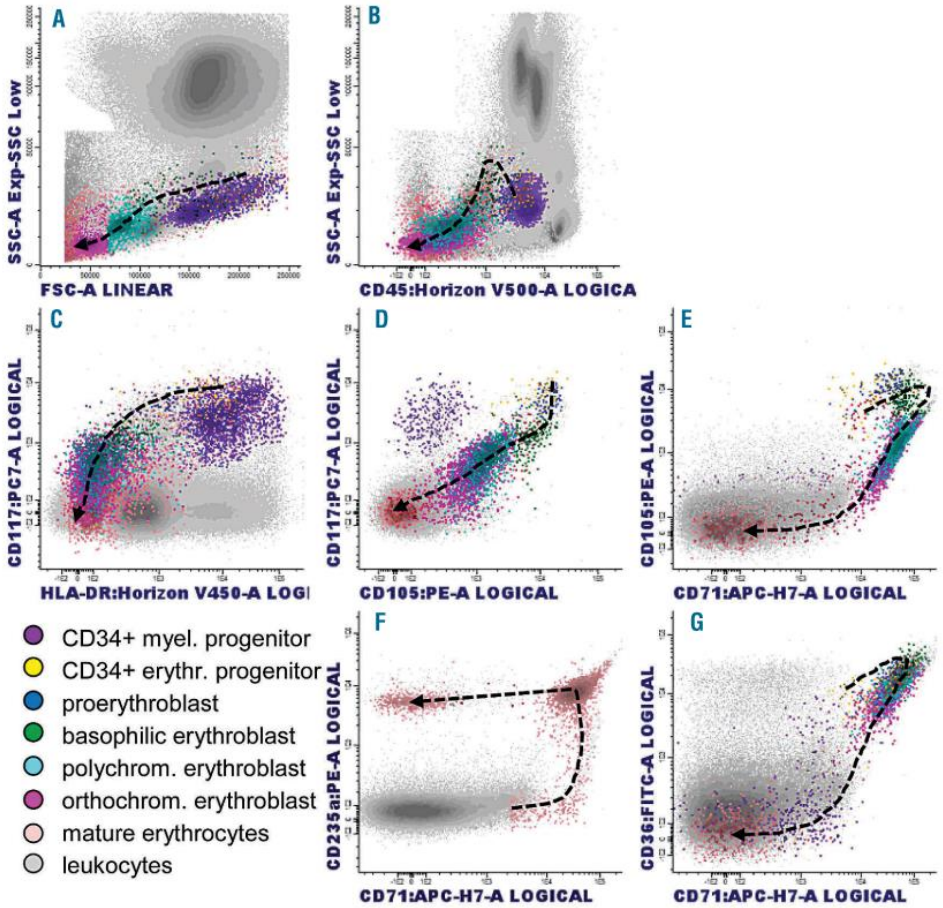


- CD45dim myeloid and B cell precursors are present in the current selected population, therefore … Exclude remaining myeloid cells by e.g. the selection of CD33 negative cells or CD13 negative cells (not shown). An example is shown for a normal bone marrow (NBM) sample and a MDS sample (figures 1D and F). B cell progenitors and other contaminating non erythroid cells may be excluded by their CD117^{-}HLA-DR^{+} phenotype (figures 1E and G).



- Next, exclude remaining mature erythrocytes (CD235a^{+}CD71^{-} or CD36^{-}CD71^{-}) and platelets (CD36+CD71-); figures 1H-K





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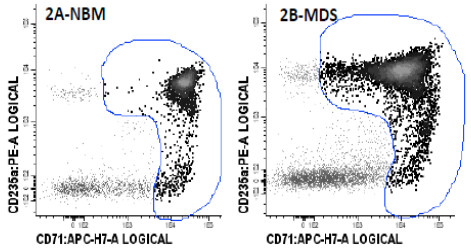
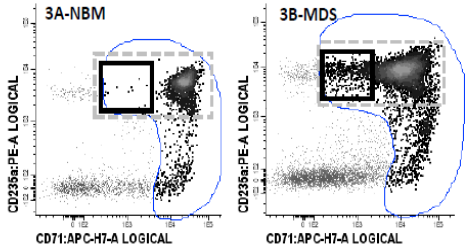
Estudio de la serie eritroide

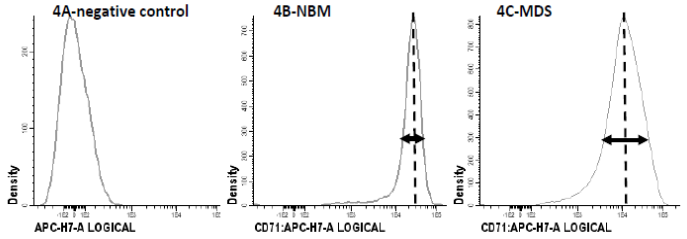
2. Interpret the CD71 vs. CD235a erythroid differentiation pattern as normal or aberrant according to your own center's reference profiles; Figures 2A and 2B
This can be performed by "eye balling", by automated reference plots, by occurrence of a CD71^{dim} population, by increase in CV of CD71 expression or altered mean fluorescence intensity, by altered percentage of precursors (CD71^{+}CD235a^{-}). Some of these features are described below.



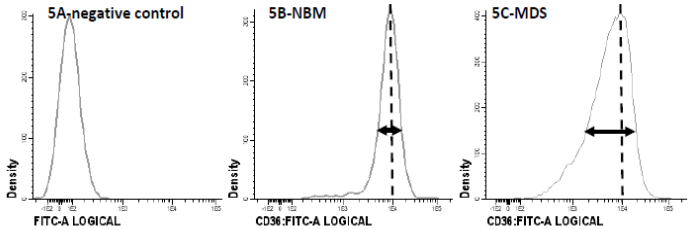
3. CD71^{dim} fraction as percentage of the CD235a^{+} CD71^{+} erythroid cells. The CD71^{dim} fraction is mostly absent in NBM and may be present in MDS (boxes in figures 3A and B). The CD71^{dim} fraction may also be evaluated in the CD71 vs. CD36 plot (Figures 1I and 1K)



4. CD71 expression. CD71 expression level can be analyzed as fluorescence intensity or coefficient of variation (CV). The CV represents a homogenous or aberrantly heterogeneous expression profile (arrows). With regard to expression level, preferably use geo mean of fluorescence intensity as compared to unstained cells (or an irrelevant antibody as back ground/auto fluorescence; figure 4A); use median if geo mean is not available. Examples in figures 4B and 4C. Erythroid cells in MDS-example in figure 4C display lower expression of CD71 and increased CV (more heterogeneous) as compared to NBM in figure 4B.

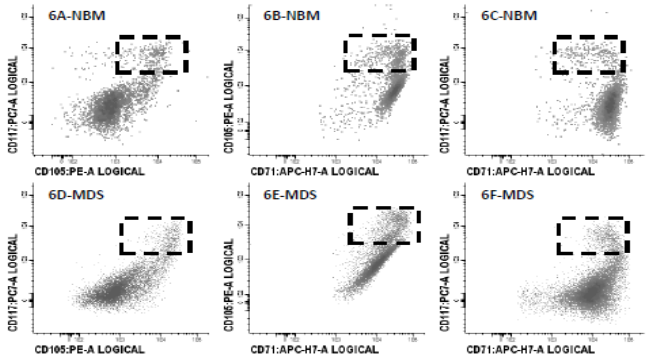


5. CD36 expression. CD36 expression level can be analyzed as fluorescence intensity or coefficient of variation (CV). With regard to expression level, preferably use geo mean of fluorescence intensity as compared to unstained cells (or an irrelevant antibody as back ground/auto fluorescence; figure 5A); use median if geo mean is not available. Examples in figures 6B and 6C. Erythroid cells in MDS-example in figure 5C display increased CV (more heterogeneous) as compared to NBM in figure 5B.

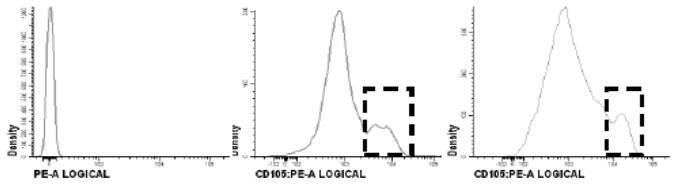


6. **Percentage CD117+ immature erythroid cells within erythroid fraction** defined as CD45$^{negative\text{-}to\text{-}dim}$ with SSC$^{low\text{-}to\text{-}int}$ CD117^{+} and CD71^{+}; figures 6A-F. Beware to exclude myeloid precursors. Since CD117^{+} erythroid cells may be hard to distinguish from myeloid progenitors an alternative might be to enumerate the CD105$^{bright+}$ immature erythroid cells within the erythroid fraction.
An alternative strategy for enumerating CD117^{+} progenitors has been previously described by Matarraz et al. (Cytometry Part B: Clinical Cytometry 2010, 78B, pages 154–168).

7. **Percentage CD105^{+} immature erythroid cells within erythroid fraction** defined as: CD45$^{negative\text{-}to\text{-}dim}$ with SSC$^{low\text{-}to\text{-}int}$ CD105bright^{+} and CD71^{+} (beware not to include myeloid precursors (CD105^{-}CD117^{+}); figures 6A-F.



8. **CD105 expression.** CD105 expression level can be analyzed on immature CD105^{bright} cells. Examples in figures 7A, 7B and 7C. Immature CD105^{bright} erythroid cells in the MDS-example in figure 7C display increased expression as compared to NBM in figure 7B.



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CMF Y CITOGENÉTICA

Four-Color Flow Cytometry Shows Strong Concordance With Bone Marrow Morphology and Cytogenetics in the Evaluation for Myelodysplasia

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Key Words: Myelodysplasia; Myelodysplastic syndromes; Flow cytometry; Antigen expression; Cytogenetics; Bone marrow

DOI: 10.1309/6PBP78G4FBA1FDG6

Alteraciones CMF estudiadas (4 colores):

- Desviaciones en la intensidad de los antígenos mieloides.
- Expresión anormal homogénea de antígeno, alteración de la maduración.
- Expresión asincrónica de 2 antígenos mieloides.
- Expresión aberrante de antígenos no mieloides.
- Disminución de SSC en los granulocitos.

- 124 MO con citopenia y/o monocitosis.
- 46,7% CMF alterada, 15,3% alteraciones leves con significado indeterminado, 37.9% CMF normal.
- **CMF alterado en el 89% de los casos alterados morfológicamente.**
- **CMF alterado 94% de los casos con citogenética alterada.**
- Concordancia de CMF con morfología y citogenética.
- Sensibilidad 89%, especificidad 88%.

Table 1
Flow Cytometrically Defined Patient Groups*

	Flow Cytometric Results			
	Normal (n = 47)	Indeterminate (n = 19)	Abnormal (All Cases) (n = 58)	Abnormal (<5% Blasts) (n = 32)
Mean age (y)	58.2	63.3	65.9†	64.5
No. (%) males	23 (49)	11 (58)	31 (53)	19 (59)
Hemoglobin, g/dL (g/L)	11.8 (118)	11.2 (112)	9.8 (98)‡	9.8 (98)‡
MCV, μm^3 (fL)	91.7 (91.7)	92.1 (92.1)	96.6 (96.6)†	97.4 (97.4)†
Neutrophil count, /μL (× 10^9/L)	3,100 (3.1)	4,000 (4.0)	4,300 (4.3)	4,200 (4.2)
Platelet count, × 10^3/μL (× 10^9/L)	190 (190)	163 (163)	103 (103)§	130 (130)
Mean myeloid blast %‖	1.3	1.3	6.1‡	2.1†
Mean abnormal myeloid antigens	0	1.9	5.7‡	5.1‡
Mean nonmyeloid antigens	0	0.1	0.6‡	0.6‡
No. (%) of cases with				
Abnormal morphologic features	3 (6)	3 (16)	50 (86)¶	26 (81)¶
Abnormal cytogenetics	1 (2)	1 (5)	31 (53)¶	16 (50)¶
Mean No. of cytogenetic abnormalities#	0.02 (1)	0.05 (1)	1.6 (2.9)‡	1.3 (2.6)†
No. (%) of cases meeting "gold standard" criteria**	4 (9)	4 (21)	51 (88)¶	27 (84)¶

Kussick, S. J., Fromm, J. R., Rossini, A., Li, Y., Chang, A., Norwood, T. H., & Wood, B. L. (2005). Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. *American Journal of Clinical Pathology*, *124*(2), 170–181. https://doi.org/10.1309/6PBP78G4FBA1FDG6

CMF Y CITOGENÉTICA

Original Article

Clinical Utility of Multiparameter Flow Cytometry in the Diagnosis of 1013 Patients With Suspected Myelodysplastic Syndrome

Correlation to Cytomorphology, Cytogenetics, and Clinical Data

Wolfgang Kern, MD; Claudia Haferlach, MD; Susanne Schnittger, PhD; and Torsten Haferlach, MD

- 1013 MO con sospecha de SMD.
- **Concordancia CM y CMF: 82%.**
- Porcentaje de blastos por CM correlación fuerte con la CMF.
- **La especificidad CMF aumenta cuando hay mayor número de marcadores aberrantes detectados. Se correlaciona con IPSS.**

**12 pacientes citomorfología normal presentaban alteraciones citogenéticas de SMD; en el 50% la CMF demostró alteraciones de SMD.

Table 3. Diagnostic Results of Multiparameter Flow Cytometry in Cytogenetically Defined Myelodysplastic Syndrome Subgroups

Cytogenetic Result	Total No. of Patients	No. of Patients With Results in Agreement With MDS by MFC Independent of CM Result (%)
Normal karyotype	768	257 (33.5)
Del(5q)	43	33 (76.7)
Aberrations of chromosome 7	14	14 (100)
Trisomy 8	30	25 (83.3)
Del(20q)	21	18 (85.7)
Complex karyotype	23	19 (82.6)
Loss of Y-chromosome	43	22 (51.2)
Other aberrations	71	58 (81.7)

MFC indicates multiparameter flow cytometry; MDS, myelodysplastic syndrome; CM, cytomorphology; Del, deletion.

Kern, W., Haferlach, C., Schnittger, S., & Haferlach, T. (2010). Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: Correlation to cytomorphology, cytogenetics, and clinical data. *Cancer*, *116*(19), 4549–4563. https://doi.org/10.1002/cncr.25353

CMF Y CITOGENÉTICA

Original Article

Clinical Utility of Multiparameter Flow Cytometry in the Diagnosis of 1013 Patients With Suspected Myelodysplastic Syndrome

Correlation to Cytomorphology, Cytogenetics, and Clinical Data

Wolfgang Kern, MD; Claudia Haferlach, MD; Susanne Schnittger, PhD; and Torsten Haferlach, MD

Progenitores:

- CD11b, CD56, CD7
- **Progenitores >5%:**
  - **Alteraciones en cromosoma 7, cariotipo complejo.**
- **Del(20q) o del(5q) expresión anormal de CD11b.**

Granulocitos:

- Alteración de CD13/CD16; CD56, CD33-.
- Disminución del ratio SSC granulocitos: linfocitos.
- **Alteraciones cromosoma 7 y cariotipo complejo: CD13/CD16**.
- **5q- : CD56+.**
- **Trisomía cromosoma 8: CD13/CD16, CD11b/CD16, CD33-.**

Monocitos:

- Expresión de CD56, CD16, CD2, falta de expresión de CD13.
- **Alteraciones cromosoma 7: CD56+**
- **Cariotipo complejo: HLA-DR-.**
- **Trisomía cromosoma 8: CD13-, CD56+**

Eritrocitos:

- Alteración en la expresión de CD71.
- **5q-: CD71-**

Kern, W., Haferlach, C., Schnittger, S., & Haferlach, T. (2010). Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: Correlation to cytomorphology, cytogenetics, and clinical data. *Cancer*, *116*(19), 4549–4563. https://doi.org/10.1002/cncr.25353

CMF Y CITOGENÉTICA

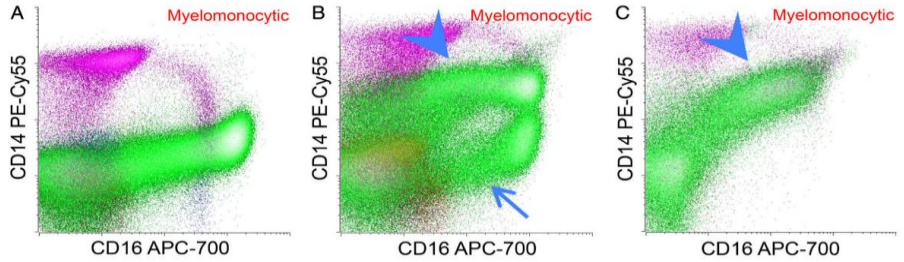
Original Article

Immunophenotypic Features of Myeloid Neoplasms Associated with Chromosome 7 Abnormalities

Xueyan Chen,[1*] Brent L. Wood,[1,2] and Sindhu Cherian[1]
[1]Department of Laboratory Medicine, University of Washington, Seattle, Washington
[2]Seattle Cancer Care Alliance, Seattle, Washington

Table 2
Immunophenotypic Abnormalities on Maturing Granulocytic and Monocytic Cells in Myeloid Neoplasms with Monosomy 7 and del(7q)

Abnormalities	Monosomy 7 (*N* = 98)	del(7q) (*N* = 61)	*P* value (Fishers exact test)
Increased CD14 on granulocytic cells, No. (%)	91 (92.9%)	5 (8.2%)	<0.0001
Increased CD13 on granulocytic cells, No. (%)	85 (86.7%)	48 (78.7%)	0.193
Abnormal CD13 vs. CD16 pattern, No. (%)	55 (56.1%)	42 (68.9%)	0.133
CD56 on granulcoytic cells, No. (%)	38 (38.8%)	22 (36.1%)	0.867
CD56 on monocytic cells, No. (%)	41 (41.8%)	19 (31.1%)	0.184
CD64 retention on granulocytic cells, No. (%)	57 (58.2%)	17 (27.9%)	0.0003
Expanded immature monocytic population, No. (%)	11 (11.2%)	4 (6.6%)	0.411
Immature monocytic cells % (of white cells), median (range)	12.0% (3.9–53.8%)	9.1% (5.5–18.5%)	
Total numbers of aberrancies, median (range)	4 (0–6)	3 (0–5)	



Chen, X., Wood, B. L., & Cherian, S. (2019). Immunophenotypic Features of Myeloid Neoplasms Associated with Chromosome 7 Abnormalities. *Cytometry Part B - Clinical Cytometry*, *96*(4), 300–309. https://doi.org/10.1002/cyto.b.21775

Myelodysplastic syndromes with a deletion 5q display a characteristic immunophenotypic profile suitable for diagnostics and response monitoring

Feature			Score
CD45-MFI-ratio	(lympho *vs.* myPC)	≤7.0	**10**
myPC		>2.0%	**3**
SSC-ratio	(granulo *vs.* lympho)	<6.0	**2**
CD71	(granulo)	≤20%	**1.5**
Sex		female	**1.5**

It should be mentioned that MDS with del(5q) cases showed relatively high Wells and iFC scores. In MDS with del(5q), the common MFC aberrations were a low number of B-cell precursors, low CD45 ratio of $CD34^+$ blasts, low granularity of granulocytes, and high level of $CD7^+CD34^+$ cells. MDS with del(5q) had low frequencies of

Sullivan, R. O., & Walsh, M. (2007). L ETTERS TO THE To the Editor :, *27*(8), 970–972.

Davydova, Y. O., Parovichnikova, E. N., Galtseva, I. V., Kokhno, A. V., Dvirnyk, V. N., Kovrigina, A. M., … Savchenko, V. G. (2020). Diagnostic significance of flow cytometry scales in diagnostics of myelodysplastic syndromes. *Cytometry Part B - Clinical Cytometry*. https://doi.org/10.1002/cyto.b.21965

SISTEMAS DE PUNTUACIÓN

AL MENOS 3 ALTERACIONES EN DOS COMPARTIMENTOS DIFERENTES.

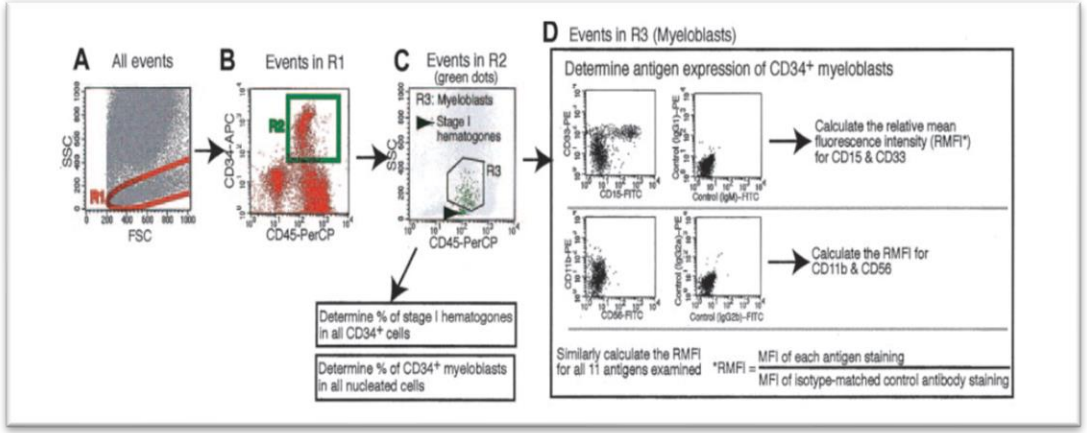
SIEMPRE INTERPRETADO CON CITOLOGÍA Y CITOGENÉTICA

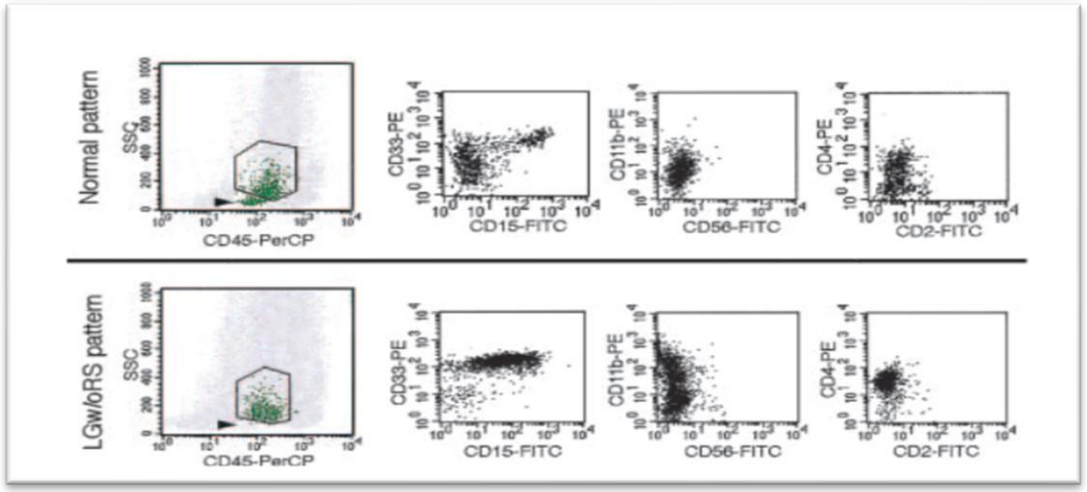
SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS

NEOPLASIA

Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes

Kiyoyuki Ogata, Yoshifumi Kishikawa, Chikako Satoh, Hideto Tamura, Kazuo Dan, and Akio Hayashi





- 90 MO controles vs 27 MO SMD BR sin SA.
- Expresión de antígenos (RMFI)en CD34+: CD2, CD4, CD10, CD11b, CD13, CD15, CD33, CD56, CD117, CD123, and CD133
- Porcentaje hematogonias estadio I
- Porcentaje de mieloblastos Cd34+ en todas las células nucleadas.

Table 3. FCM scores of controls and LGw/oRS patients

	First cohort		Second cohort		Both cohorts	
	Controls, no.	LGw/oRS patients, no.	Controls, no.	LGw/oRS patients, no.	Controls, no.	LGw/oRS patients, no.
Total	50	12	40	15	90	27
Score						
0	40	1	31	2	71	3
1	4	1	9	1	13	2
2	6	3	0	3	6	6
3	0	2	0	4	0	6
4	0	1	0	3	0	4
5	0	3	0	1	0	4
6	0	1	0	0	0	1
7	0	0	0	1	0	1
3 or higher	0	7	0	9	0	16

*One point was given for each FCM parameter selected from Table 2 (ie, stage I hematogones, CD34+ myeloblasts, CD4, CD56, CD11b, CD13, CD15, CD33, CD117, and CD133) if the data on each parameter were outside the RR.

OGATA: CD34+
CD4+; CD56+
CD11b+; CD15+
Aumento de expresión: CD117; CD133; CD13; CD133
Aumento de mieloblastos CD34+
Disminución de hematogonias EI

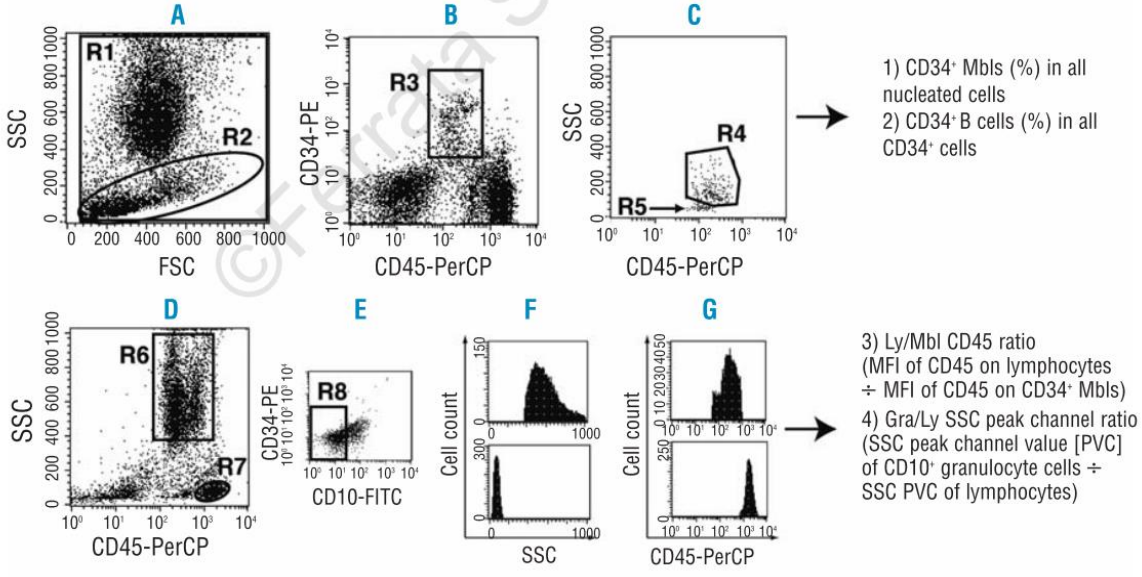
OGATA
S: 59%
E: 100%

Ogata, K., Kishikawa, Y., Satoh, C., Tamura, H., Dan, K., & Hayashi, A. (2006). Diagnostic application of flow cytometric characteristics of CD34 + cells in low-grade myelodysplastic syndromes. *Blood*, *108*(3), 1037–1044. https://doi.org/10.1182/blood-2005-12-4916

SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS

Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study

Kiyoyuki Ogata,[1] Matteo G. Della Porta,[2] Luca Malcovati,[2] Cristina Picone,[2] Norio Yokose,[3] Akira Matsuda,[4] Taishi Yamashita,[1,5] Hideto Tamura,[1] Junichi Tsukada,[6] and Kazuo Dan[1]



- 106 MO controles, 134 SMD BR (84 sin SA ni aberrancias citogenéticas)
- CD34+ myeloblasts <2.4%, CD34+ B cells >5%, 4<Ly/Mbl CD45 ratio <7.8, and Gra/Ly SSC peak ratio >6
- CD11b expression on CD34+ myeloblasts <10%, CD15 expression on CD34+ myeloblasts <20%, and CD56 expression on CD34+ myeloblasts <10%

Table 2. Flow scores of patients in the prospective cohorts.

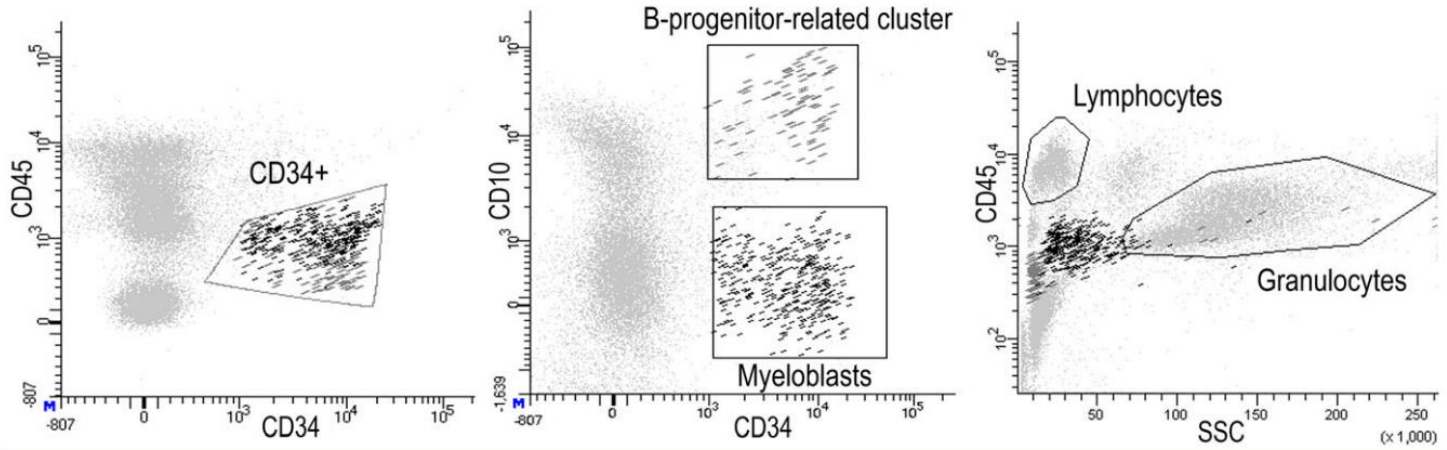
	Flow score using 4 parameters									Flow score using 7 parameters									
	0	1	2	3	4	Cases positive/ cases examined	Sensitivity (%) 2 or more[1]	Specificity (%) 2 or more[1]	Likelihood ratio 2 or more[1]	0	1	2	3	4	5	Cases positive/ cases examined	Sensitivity (%) 2 or more[1]	Specificity (%) 2 or more[1]	Likelihood ratio 2 or more[1]
Japanese cohort																			
Non-clonal cytopenia	35	7	1	0	0	1/43				35	7	1	0	0	0	1/43	72	98	30.9
All low-grade MDS patients	12	14	7	11	2	20/46	44 (29-59)	98 (88-100)	18.7 (3.6-108.8)	2	11	15	12	5	1	33/46	(57-84)	(88-100)	(6.5-176.1)
Patients with conventional markers	4	4	5	6	1	12/20	60 (36-81)	98 (88-100)	25.8 (5.2-151.5)	0	4	7	7	1	1	16/20	80 (56-94)	98 (88-100)	34.4 (7.7-195.0)
Patients without conventional markers	8	10	2	5	1	8/26	31 (14-52)	98 (88-100)	13.2 (2.4-80.7)	2	7	8	5	4	0	17/26	65 (44-83)	98 (88-100)	28.1 (5.8-162.8)
Italian cohort																			
Non-clonal cytopenia	38	20	5	0	0	5/63				22	12	4	0	0	0	4/38			
All low-grade MDS patients	8	18	37	20	5	62/88	71 (60-80)	92 (82-97)	8.9 (4.2-20.4)	3	6	26	25	6	0	57/66	86 (76-94)	90 (75-97)	8.2 (3.9-19.4)
Patients with conventional markers	1	7	13	9	3	25/33	76 (58-89)	92 (82-97)	9.5 (4.6-20.7)	1	3	5	5	5	0	15/19	79 (63-89)	90 (75-97)	7.5 (3.3-16.2)
Patients without conventional markers	7	11	24	11	2	37/55	67 (53-79)	92 (82-97)	8.5 (4.0-19.5)	2	3	21	20	1	0	42/47	89 (77-97)	90 (75-97)	8.5 (4.1-18.4)

[1]*Data are the diagnostic power of the "flow score 2 or more." Data in parentheses are 95% CI.*

OGATA
S: 65%; 89%
E: 98%, 90%

Ogata, K., Della Porta, M. G., Malcovati, L., Picone, C., Yokose, N., Matsuda, A., … Dan, K. (2009). Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: A prospective validation study. *Haematologica*, *94*(8), 1066–1074. https://doi.org/10.3324/haematol.2009.008532

SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS



Parameter	Cut-off values	Score*
Myeloblast (% of CD45+ cells)	> 2 %	1
B-progenitor-related cluster size (% of CD34+)	< 5 %	1
Lymphocyte to myeloblast CD45 ratio	≤ 4 or ≥ 7.5	1
Granulocyte to lymphocyte SSC ratio	≤ 6	1

*MDS is indicated for samples obtaining ≥ 2 points

OGATA
S: 65%; 89%
E: 98%, 90%

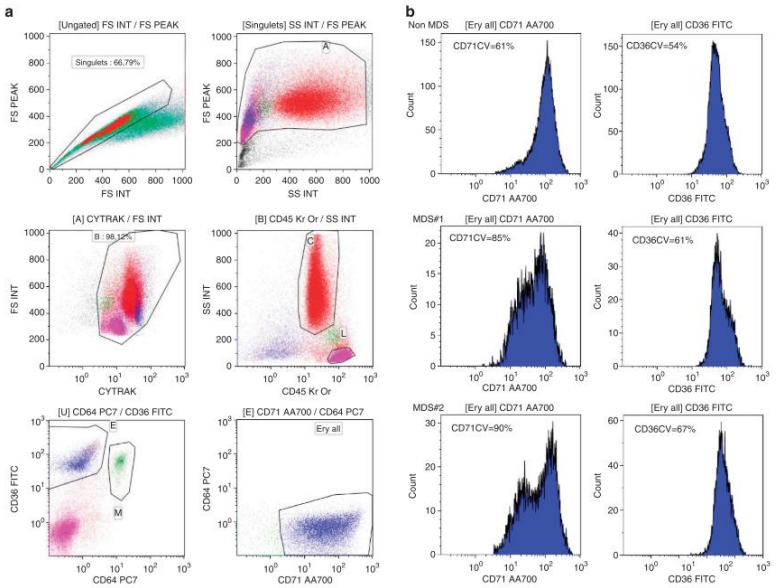
SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS

ORIGINAL ARTICLE

Flow cytometric detection of dyserythropoiesis: a sensitive and powerful diagnostic tool for myelodysplastic syndromes

S Mathis[1,2,7], N Chapuis[1,2,7], C Debord[1], A Rouquette[3], I Radford-Weiss[4], S Park[2,5], F Dreyfus[2,5], C Lacombe[1,2], MC Bèné[6], O Kosmider[1,2], M Fontenay[1,2] and V Bardet[1,2]

- 163 pacientes
  - 126 con citopenia; 46 controles.
  - No lisis. CyTrak orange



a

RED-score parameter	Threshold	Points
CD71CV (%)	<80	0
	≥80	3
CD36CV (%)	<65	0
	≥65	2
Hb level (g/dL)	>10.5 (F) or >11.5 (M)	0
	≤10.5 (F) or ≤11.5 (M)	2

- Altamente sugestivo de SMD si ≥3
- S: 80%, E: 90%, VPP: 97%
- Si lo combinas con Ogata S: 88%

Mathis, S., Chapuis, N., Debord, C., Rouquette, A., Radford-Weiss, I., Park, S., … Bardet, V. (2013). Flow cytometric detection of dyserythropoiesis: A sensitive and powerful diagnostic tool for myelodysplastic syndromes. *Leukemia*, *27*(10), 1981–1987. https://doi.org/10.1038/leu.2013.178

SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS

EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group

Theresia M. Westers,[1] Eline M.P. Cremers,[1] Uta Oelschlaegel,[2] Ulrika Johansson,[3] Peter Bettelheim,[4] Sergio Matarraz,[5] Alberto Orfao,[5] Bijan Moshaver,[6] Lisa Eidenschink Brodersen,[7] Michael R. Loken,[7] Denise A. Wells,[7] Dolores Subirá,[8] Matthew Cullen,[9] Jeroen G. te Marvelde,[10] Vincent H.J. van der Velden,[10] Frank W.M.B. Preijers,[11] Sung-Chao Chu,[12] Jean Feuillard,[13] Estelle Guérin,[13] Katherina Psarra,[14] Anna Porwit,[15,16] Leonie Saft,[16] Robin Ireland,[17] Timothy Milne,[17] Marie C. Béné,[18] Birgit I. Witte,[19] Matteo G. Della Porta,[20] Wolfgang Kern[21] and Arjan A. van de Loosdrecht,[1] on behalf of the IMDSFlow Working Group

Haematologica 2017
Volume 102(2):308-319

- Cohorte de aprendizaje: 245 SMD, 290 patológico, 142 controles normales.
- Cohorte de validación: 129 SMD, 153 patológico, 49 controles normales.

ANÁLISIS:

- CV CD36: 4 PUNTOS
- CV CD71: 3 PUNTOS
- MFI CD71: 2 PUNTOS (si disminuido)
- % progenitores eritroides CD117+: 2 PUNTOS (si disminuido o aumentado)

≥ 5 PUNTOS: alteraciones eritroides asociadas A SMD

E: 90%, S: 33%

	10th percentile	90th percentile	# of PC cases*	# of NBM cases
relative %NEC	-	**268%**	238	139
%CD71^{dim}	-	**17%**	250	129
relative MFI of CD71	**45%**	-	250	126
relative CV of CD71	-	**133%**	165	88
relative MFI of CD36	**53%**	-	203	124
relative CV of CD36	-	**145%**	177	92
relative %CD117 progenitors	**37%**	**222%**	180	122
relative %CD105 progenitors	**50%**	**184%**	52	59
relative MFI of CD105	**52%**	**113%**	70	47

Note: : Cut-off values represent the 10th and 90th percentiles of results for erythroid markers among pathological controls in the learning cohort. The number of pathological control (PC) cases that were available to calculate cut-off values are displayed (*). Most values (except for %CD71^{dim}) are expressed as ratio to the median value determined in the set of normal bone marrow samples. The utmost right column displays the number of normal bone marrow (NBM) cases that were available to calculate these median values. Abbreviations: CV: coefficient of variation; dim: diminished; MFI: mean fluorescence intensity; NEC: nucleated erythroid cells.

Example on how to translate these reference ranges for application in a single center:
In case a sufficient amount of data is present regarding a large variation of pathological controls, 10th and 90th percentiles calculated from a center's own cohort may be applied when comparable to the herein described reference values.
Otherwise, collect data on an appropriate amount of normal bone marrow samples (min. 10) and determine median values to calculate the cut-offs for the parameters in the erythroid score.
For instance, the median CV value of CD71 in your set of normal bone marrow samples is 67 and the median percentage of CD117^{+} erythroid progenitors in the erythroid compartment is 8%. Then the reference values for CD71 CV is: 133/100*67=89; a CD71CV>89 should be considered increased. "133" is the 90th percentile for CD71 CV from supplementary table 2A.
Similarly for %CD117^{+}: lower cut-off 37/100*8=3.0 and highest cut-off 222/100*8=17.8; i.e. a CD117+ percentage (erythroid compartment) below 3.0% or above 17.8% should be considered aberrant.

Westers, T. M., Cremers, E. M. P., Oelschlaegel, U., Johansson, U., Bettelheim, P., Matarraz, S., … van de Loosdrecht, A. A. (2017). Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group. *Haematologica*, *102*(2), 308–319. https://doi.org/10.3324/haematol.2016.147835

SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS / PRONÓSTICOS

Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation

Denise A. Wells, Martin Benesch, Michael R. Loken, Carlos Vallejo, David Myerson, Wendy M. Leisenring, and H. Joachim Deeg

- 115 SMD, 104 patológicos, 25 sanos.

Table 3. Components of flow scoring system

Points	Myeloid abnormailities	Monocytic abnormalities
0	Appropriate CD45/SSC	Appropriate CD45/SSC
	Heterogeneous CD11b$^+$, HLA-DR$^-$	CD11b$^+$, heterogeneous HLA-DR
	Normal relationships of CD13 and CD16	CD13/CD16 coexpression
	CD33$^+$	CD33/CD14 coexpression
	CD19/CD5/CD34/CD56/CD7$^-$	CD19/CD5/CD34/CD56/CD7$^-$
	Synchronous shift to the left	
1	One of the following is present:	One of the following is present:
	Abnormal granularity	Abnormal granularity
	Abnormal decrease in CD45 expression	Abnormal CD11b or HLA-DR expression
	Presence of HLA-DR or lack of CD11b	Loss of CD13 or CD16
	Convex or abnormal relationship between CD13 and CD16	Presence of CD56
	Expression of CD56 on subpopulation of myeloid cells	Lack of CD33 or CD14
	Lack of CD33 expression (may be a normal variant)	
	Asynchronous shift to the left	
2	2-3 of the above abnormalities or presence of CD34 on myeloid cells or presence of lymphoid antigens on myeloid cells	2-3 of the above abnormalities or presence of CD34 on monocytes or presence of lymphoid antigens on monocytes (exception is CD4)
3	4 or more of the above abnormalities or 1 or more of the abnormalities plus presence of CD34 or lymphoid antigen expression on myeloid cells	4 or more of the above abnormalities or 1 or more of the abnormalities plus presence of CD34 or lymphoid antigen expression on monocytes

- Grupos: bajo (0-1), moderado (2-3) y alto (4 o más alteraciones).
- Puntuación ≥2.
- S:70 %; E:93
- Correlación con IPSS y con el riesgo citogenético según el IPSS.
- Correlación con evolución post alo-TPH.

Wells, D. A., Benesch, M., Loken, M. R., Vallejo, C., Myerson, D., Leisenring, W. M., & Deeg, H. J. (2003). Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood*, *102*(1), 394–403. https://doi.org/10.1182/blood-2002-09-2768

SISTEMAS DE PUNTUACIÓN DIAGNÓSTICOS

How to report FCM findings?

Guidelines of the IMDSflow WG on FCM in MDS 2015

- A:
  FCM analysis: *NO* MDS-related features
- B:
  FCM analysis: some changes often seen in MDS
- C:
  FCM analysis: consistent with MDS

Loosdrecht AA van de, Westers TM. J Nat Compr Cancer Netw 2013;11:892-902
Westers TM, et al., Leukemia 2012;26:1730-41; Porwit A, et al., Leukemia 2014;28:1793-98

Integrated Flow Cytometric diagnostic approach
Scoring system

Diagnostic flow score (Ogata et al.)	<2	<2	<2	<2		≥2	≥2	≥2	≥2
Dysplasia by FC myeloid progenitors	-	-	+	+		-	-	+	+
Dysplasia by FC - Neutrophils (SSC or two or more other aberrancies) - Monocytes (CD56 or two or more other aberrancies) - Erythroid precursors (CD36 and/or CD71)	-	+	-	+		-	+	-	+
Conclusion	A	A/B	A/B	C		A/B	B/C	B/C	C

Loosdrecht AA van de, Westers TM. J Natl Comp Canc Netw 2013;11:892-902;
Porwit A, Loosdrecht AA van de, et al., Leukemia 2014;28:1793-98

S:80%
E: 95%

Porwit, A. (2015, September 1). Is There a Role for Flow Cytometry in the Evaluation of Patients With Myelodysplastic Syndromes? *Current Hematologic Malignancy Reports*. Current Science Inc. https://doi.org/10.1007/s11899-015-0272-3

SISTEMAS DE PUNTUACIÓN PRONÓSTICOS

ORIGINAL ARTICLE

The myelodysplastic syndromes flow cytometric score: a three-parameter prognostic flow cytometric scoring system

C Alhan[1], TM Westers[1], EMP Cremers[1], C Cali[1], BI Witte[2], GJ Ossenkoppele[1] and AA van de Loosdrecht[1]

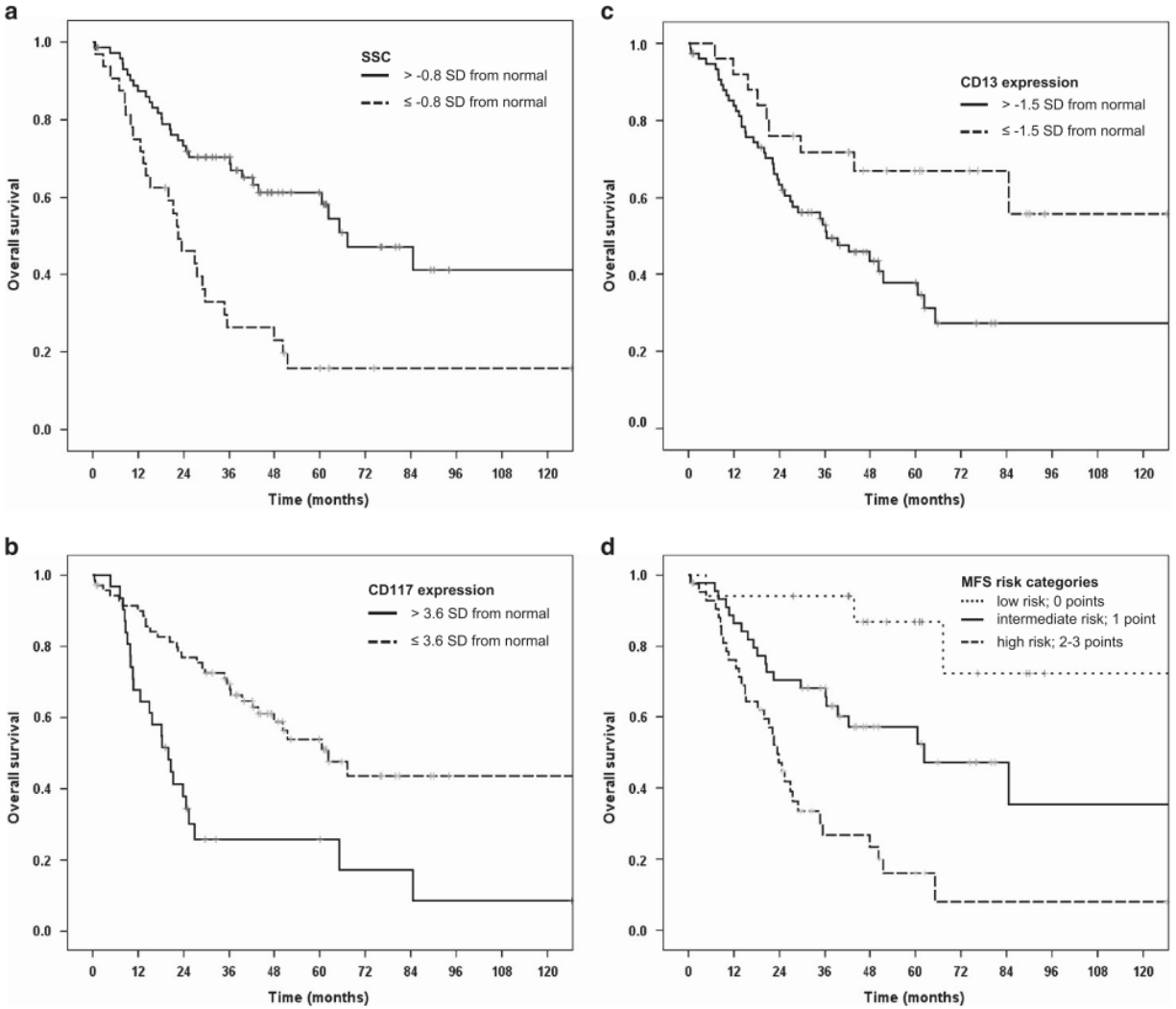
The prognosis of myelodysplastic syndromes (MDS) is currently estimated by using the revised International Prognostic Scoring System (IPSS-R). Several studies have shown that further refinement of prognostication for MDS can be achieved by adding flow cytometric parameters. However, widespread implementation of flow cytometry for the prognosis of MDS is hampered by complexity of the analysis. Therefore, the aim of this study was to construct a robust and practical flow cytometric score that could be implemented as a routine procedure. To achieve this, bone marrow aspirates of 109 MDS patients were analyzed by flow cytometry. A second cohort consisting of 103 MDS patients was used to validate the MDS flow cytometric score (MFS). The parameters forming the MFS were sideward light scatter and CD117 expression of myeloid progenitor cells and CD13 expression on monocytes. Three MFS risk categories were formed. Patients with MDS and intermediate MFS scores had significantly better overall survival (OS) compared with the patients with high MFS scores. The MFS further refined prognostication within the IPSS-R low-risk category, by identifying patients with worse OS in case of high MFS. In conclusion, a practical three parameter flow cytometric prognostic score was constructed enabling further refinement of prognostication of MDS.

Leukemia (2016) **30,** 658–665; doi:10.1038/leu.2015.295

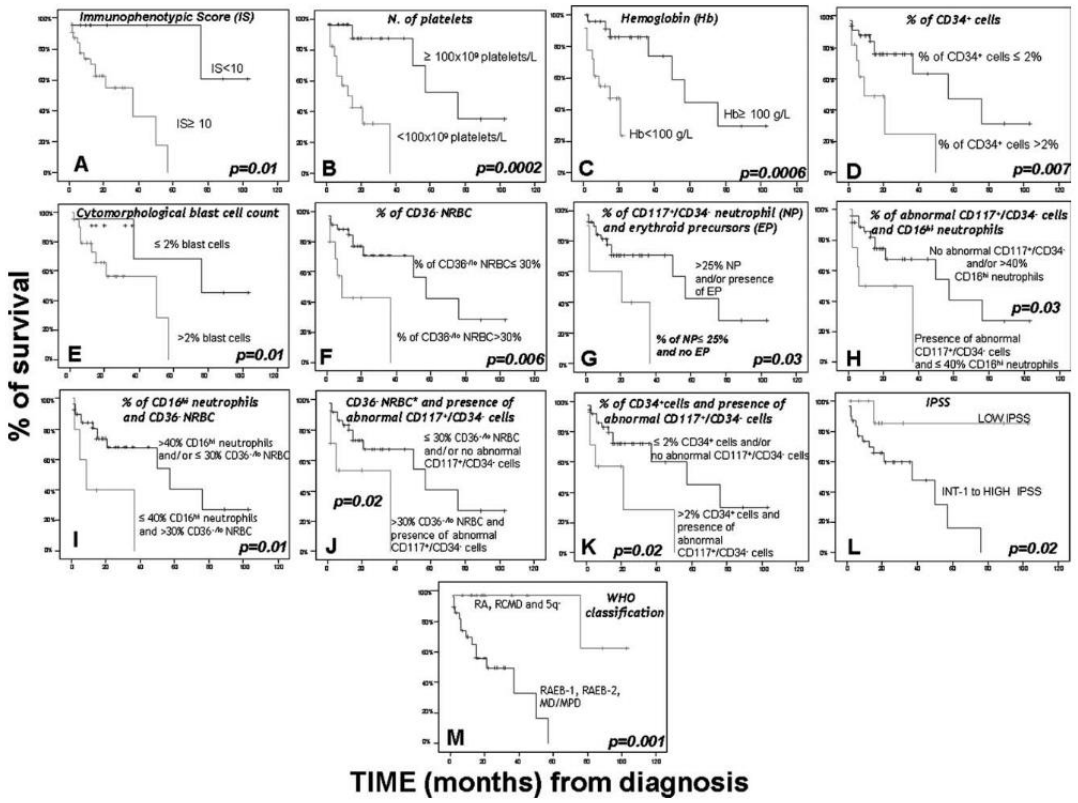
Table 3. Components of the MFS

	Deviation from normal[a]	*Hazard ratio*	*Number of points*
Myeloid progenitors decreased SSC	⩽−0.8 s.d.	2.4	1
Myeloid progenitors increased CD117 expression	>3.6 s.d.	2.7	1
Monocytic cells decreased CD13expression[b]	⩽−1.5 s.d.	2.4	1

Note: The variables are described in such a manner that these are associated with adverse outcome. [a]These cutoff values were acquired by applying recursive partitioning (STREE). [b]Decreased expression of CD13 on monocytes is associated with favorable outcome. Low MFS group: 0 points, intermediate MFS group: 1 point, high MFS group: 2–3 points.



SISTEMAS DE PUNTUACIÓN PRONÓSTICOS



Results: Although MDS-associated phenotypes were detected in reactive BM, the overall immunophenotypic profile of BM cells allowed an efficient discrimination between MDS and both normal and reactive BM, once the number and degree of severity of the abnormalities detected per patient were simultaneously considered in the proposed IS. Interestingly, increasingly higher IS were found among patients with MDS showing adverse prognostic factors and in low- versus high-grade cases. The most informative prognostic factors included the number of $CD34^{+}$ cells, presence of aberrant $CD34^{-}/CD117^{+}$ precursors, decreased mature neutrophils and $CD34^{-}$ erythroid precursors, and increased numbers of $CD36^{-/lo}$ erythroid precursors; in addition, the IS was an independent prognostic factor for overall survival.

Matarraz S, López A, Barrena S, Fernandez C, Jensen E, Flores-Montero J, Rasillo A, Sayagues JM, Sa´nchez ML, Ba´rcena P, Hernandez-Rivas JM, Salvador C, Fernandez-Mosteirı´n N, Giralt M, Perdiguer L, Laranjeira P, Paiva A, Orfao A. Bone marrow cells from myelodysplastic syndromes show altered immunophe- notypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. Cytometry Part B 2010; 78B: 154–168.

CONCLUSIONES

approach to diagnosing MDS, we suggest a combination of CM, CG, and MFC. Further studies should be performed to confirm these results and especially to define standards for the application of MFC in the diagnostic workup of MDS.[34,35]

entification of myeloid or monocytic dyspoiesis and abnormal
mmunophenotypes of myeloblasts in patients with MDS by flow
tometry may add prognostic significance beyond that obtained
morphology and cytopenias. Future studies could conceivably
mbine and integrate multidimensional flow cytometric analyses
ith other parameters of MDS to further prognostic accuracy.

in cases with relatively low blast percentages an
cases with minimal immunophenotypic abnormalitie
myeloid blasts. Future prospective studies will be he
to both confirm these findings and to elucidate the m
anism underlying this reproducible immunophenot
aberrancy.

management of MDS-patients, as it is useful in diagnosis, risk stratification and therapy guidance. Further research is required to compile optimal and simple panels that can be easily implemented in routine laboratory medicine. Additionally, embracing novel techniques such as automated analysis and high-dimensional single-cell analysis will, potentially increase the clinical impact of MFC.

d percentages of erythroid progenitors (CD117
tion of the defined marker set demonstrated hi
ity. Future studies should assess the contribution
cted erythroid markers to the evaluation of t
l progenitors, the maturing myelomonocytic lin

dardization of flow cytometry approaches, improvement of routine laboratory practices is mandatory to reach a good reproducibility of cell counts, and sets the basis for the use of CD34+ cell enumeration as a complimentary test to BM blast count in MDS patients.

CONCLUSIONES

Servicio de hematología y hemoterapia.
Sección Diagnóstico.

GENERALITAT VALENCIANA

CASTELLÓ

INMUNOFENOTIPO SÍNDROMES MIELODISPLÁSICOS

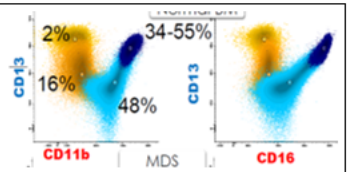
Nombre:
Hemograma:

CD34 %:
- Alteración R M/L:
- Homogéneo:
- Disminución SSC:
- Alteración expresión CD45:
- Disminución u homogeneidad en CD13:
- Disminución u homogeneidad en CD33:
- Expresión anormal CD117:
- Aumento expresión HLA-DR o pérdida de expresión:
- CD11b:
- CD36 homogéneo:
- CD15 -:
- CD7:
- CD19:
- CD56:
- NUtdT:

Cell lineage	Phenotype	Frequency Normal BM (%)
Neutrophil	CyMPO$^+$, CD64$^-$, CD13^{dim}	30 (15-45)
B-Lymphoid	nTdT$^+$, cyCD79a$^+$, CD19$^+$	20 (7-44)
Erythroid	CD36$^+$, CD64$^-$, CD45^{dim}, CD105$^+$	20 (11-34)
pDC	CD123^{hi}, HLA-DR$^+$, CD36$^-$	6 (1-9)
Monocytic	CyMPO^{dim}, CD64$^+$, HLA-DR$^+$, CD117^{dim}	23 (10-40)
Basophil	CD123^{hi}, HLA-DR^{dim}, CD117^{dim}, CD45^{dim}, CD203c$^+$	<1 (<1-3)
Megakaryocytic	CD61$^+$, CD45^{dim}	<1
Eosinophil	CyMPO$^+$, CD15/65$^+$, CyPEo$^+$	<1
Mast cell	CD117^{hi}, HLA-DR^{dim}, CD45^{dim}	<1

% MIELOIDE:
% MONOCITO:
% LINFOIDE:
% ERITROIDE:

NEUTRÓFILOS %:
- Disminución SSC
- CD45 anormal:
- Aumento de células inmaduras:
- Disminución u homogeneidad en CD33:
- Disminución u homogeneidad en CD33:
- Patrón CD11b/CD13 alterado
- Disminución 11B
- Patrón CD16/CD13 alterado
- CD15 - / débil:
- HLA-DR +:
- CD36+
- CD10+/CD16-:
- CD64+ homogéneo:
- CD7:
- CD19:
- CD56:

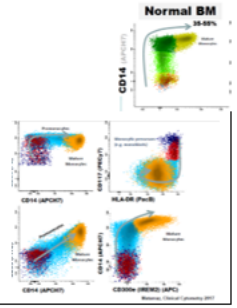


Servicio de hematología y hemoterapia.
Sección Diagnóstico.

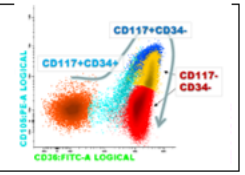
GENERALITAT VALENCIANA

CASTEL

MONOCITOS %:
- Disminución SSC:
- Disminución CD45:
- Aumento células inmaduras.
- Disminución o CD33 heterogéneo
- Patrón CD11b / HLA-DR alterado
- Disminución HLA-DR:
- Disminución o CD13-:
- Disminución o CD36-:
- Disminución o CD14-:
- Disminución o CD64-:
- Disminución o CD11c-:
- Disminución o 300e-:
- CD7:
- CD19:
- CD56:



ERITROIDE %:
- Aumento CD45:
- Disminución CD71 o exp. Heterogénea:
- Patrón CD71/CD235a alterado:
- Disminución o aumento de CD105:
- Disminución o aumento de CD36:
- Disminución o aumento de CD71:
- Expresión CD117



LINFOIDE %:
- Disminución / aumento:
- Disminución SSC:
- CD117

AÑADIR:
- RED-Score
- Ogata

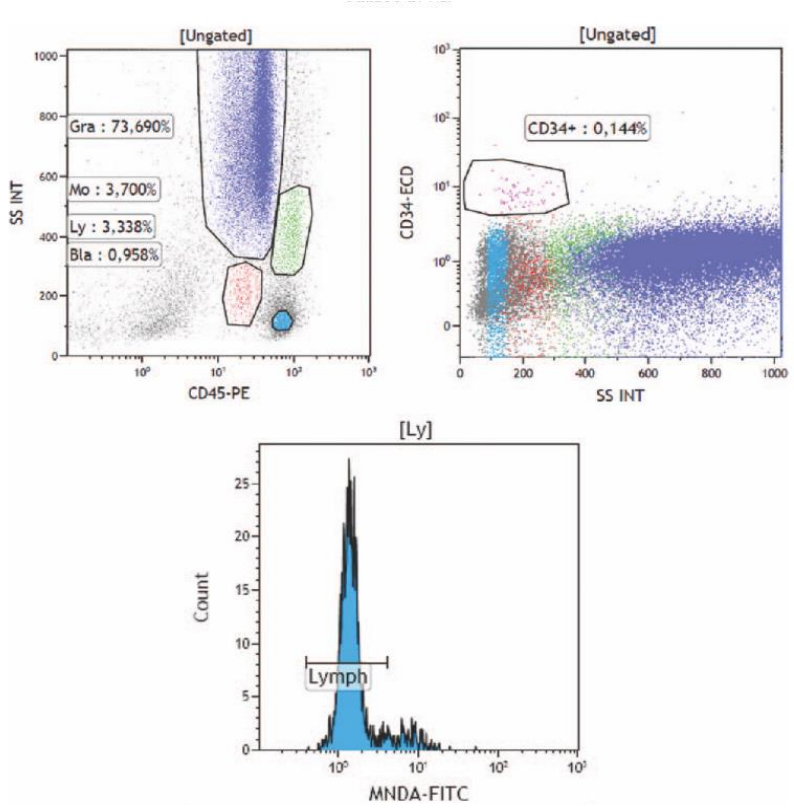
ANALIZAR 10 MO CONTROL

CONCLUSIONES

Original Article

Evaluation of Flow Cytometric Assessment of Myeloid Nuclear Differentiation Antigen Expression as a Diagnostic Marker for Myelodysplastic Syndromes in a Series of 269 Patients

Frauke Bellos, Tamara Alpermann, Elena Gouberman, Claudia Haferlach, Susanne Schnittger, Torsten Haferlach, and Wolfgang Kern*
MLL Munich Leukemia Laboratory, Munich, Germany



ORIGINAL ARTICLE

CLINICAL CYTOMETRY WILEY

Automated leukocyte parameters are useful in the assessment of myelodysplastic syndromes

Anna Shestakova[1,2] | Ali Nael[1,3] | Virgilita Nora[1] | Sherif Rezk[1] | Xiaohui Zhao[1]

Profiling myelodysplastic syndromes by mass cytometry demonstrates abnormal progenitor cell phenotype and differentiation

Gregory K. Behbehani[1,2,3] | Rachel Finck[1] | Nikolay Samusik[1] | Kunju Sridhar[2] | Wendy J. Fantl[4] | Peter L. Greenberg[2,3] | Garry P. Nolan[1,3,4]

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[3]Stanford Cancer Institute, Stanford, California

[4]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Stanford University School of Medicine, Stanford, California

CONCLUSIONES

- La CMF es útil para el diagnóstico y la estratificación de SMD.
- Más investigación para conseguir paneles más sencillos que puedan ser implementados para los estudios de rutina.
- Nuevas técnicas como el análisis automatizado o el análisis masivo que aumentarán el potencial de la CMF en los SMD.
- Análisis siempre integrado con la citomorfología y citogenética.

MUCHAS GRACIAS.