



FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI



Ematologia di Laboratorio:
Percorsi diagnostici e obiettivi Clinici

Il contributo della Citometria Automatizzata nell'inquadramento diagnostico delle Sindromi Mielodisplastiche

Paolo Danise (Nocera Inferiore)

Maria Gioia (Palermo)

Maria Caterina Maconi (Reggio Emilia)

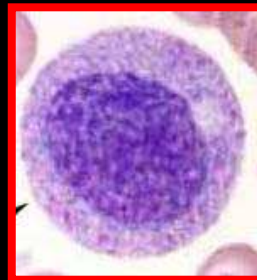
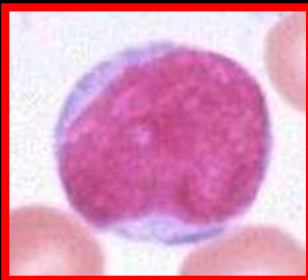
Vincenzo Rocco (Benevento)

Maria Grazia Silvestri (Bologna)

Donatella Tanca (Lavagna)



È utile un nuovo allarme “*displasia*”?



Automated flagging influences the inconsistency and bias of band cell and atypical lymphocyte morphological differentials

Wim van der Meer^{1,*}, Colin Stephen Scott²
and Marinus H. de Keijzer¹

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*These results suggest that morphologists access to an analyzer report and flagging information is unlikely to affect the randomness of band cell determinations but **may induce observer bias in variant lymphocyte estimates.***

Epidemiologia delle MDS

blood

2008-112: 45-52
 Prepublished online Apr 28, 2008;
 doi:10.1182/blood-2008-01-134858

Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs

Dana E. Rollison, Nadia Howlander, Martyn T. Smith, Sara S. Strom, William D. Merritt, Lynn A. Ries, Brenda K. Edwards and Alan F. List

**Registro di Dusseldorf circa
 4/100.000/anno**

**Programma SEER 2001-2003 negli
 USA 3,4/100.000/anno**

**L'incidenza delle MDS è simile a quella
 del MM e della CLL e più elevata
 rispetto alla Leucemia Acuta e alle
 MPD**

	SEER		
	Rate†	Count	%
Total	3.42	7 076	
Year of diagnosis‡			
2001	3.29	2 246	31.7
2002	3.38	2 349	33.2
2003	3.58	2 481	35.1
Sex‡			
Male	4.51	3 803	53.7
Female	2.71	3 273	46.3
Age‡			
Less than 40 y	0.14	186	2.6
40 to 49 y	0.71	246	3.5
50 to 59 y	2.05	524	7.4
60 to 69 y	7.57	1 135	16.0
70 to 79 y	20.94	2 393	33.8
80 y and older	36.41	2 592	36.6
Race			
White	3.48	6 068	86.9
Black	3.02	500	7.2
Asian/Pacific Islander	2.63	400	5.7
AI/AN	1.02	13	0.2
Ethnicity§			
Hispanic	2.79	516	7.3
Non-Hispanic	3.47	6 560	92.7

Aumentato carico di lavoro per gli ematologi di laboratorio per la necessità:

- di proporre una diagnosi;
- di diagnosi differenziale rispetto ad altre patologie con caratteristiche citometriche simili;
- di valutare variazioni del quadro ematologico nei frequenti controlli eseguiti dai pazienti MDS.

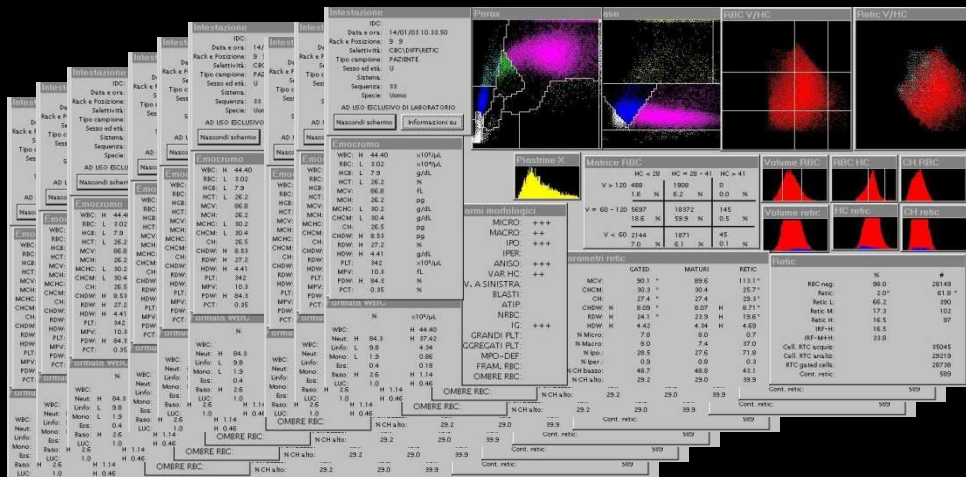


Table 1. Proposal for updated WHO classification of myelodysplastic syndromes 2008

Disease	Blood findings	Bone marrow findings
Refractory cytopenias with unilineage dysplasia (RCUD): Refractory anemia (RA) Refractory neutropenia (RN)	Unicytopenia or bicytopenia* No or rare blasts (<1%)†	Unilineage dysplasia; ≥10% of the cells of the affected lineage are dysplastic < 5% blasts < 15% of the erythroid precursors are ringed

Sangue Periferico

Blasti (<1%; ≤1%; 2-4%)

Corpi di Auer

Midollo

Displasia ≥10% delle cellule di 1 o più linee

Sideroblasti ad anello

Blasti (<5%; 5%-9%; >10)

Corpi di Auer

platelet count
No or rare blasts (< 1%)

< 5% blasts
Isolated del(5q) cytogenetic abnormality
No Auer rods

*Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U

† If the marrow myeloblast percentage is < 5% but there are 2% to 4% myeloblasts in the blood, the diagnostic classification is RAEB-1.

If the marrow myeloblast percentage is < 5% and there are 1% myeloblasts in the blood, the case should be classified as MDS-U.

‡ Cases with Auer rods and < 5% myeloblasts in the blood and < 10% in the marrow should be classified as RAEB-2.

Serie eritroide

- Forma:
 - anisocitosi
 - dimorfismo
 - pochilocitosi: schistociti, dacriociti, ovalociti, stomatociti...
 - emazie a bersaglio
- Colore:
 - anisocromia
 - policromasia
- Inclusioni eritrocitarie:
 - punteggiatura basofila (RNA)
 - corpi di Howell-Jolly (DNA)
 - corpi di Pappenheimer (siderosomi)
- Eritroblasti in circolo (diseritropoietici):
 - bi- o multinuclearità
 - asincronie e megaloblastosi
 - frammentazione e bizzarrie nucleari...

- vacuoli
- aree di basofilia, corpi di Döhle

- Tendenza all'omogeneità morfologica nei singoli casi:
 - displasia tipo I (piccoli, iposegmentati)
 - displasia tipo II (grandi, segmentazione nucleare variabile)

Altre serie

- Eosinofili e basofili:
 - anomalie della morfologia nucleare
 - anomalie delle granulazioni
- Granulociti immaturi (dismorfici) e/o blasti (tipo I, II o III)
- Monociti:
 - promonociti
 - anomalie della morfologia nucleare
 - anomalie delle granulazioni e del citoplasma

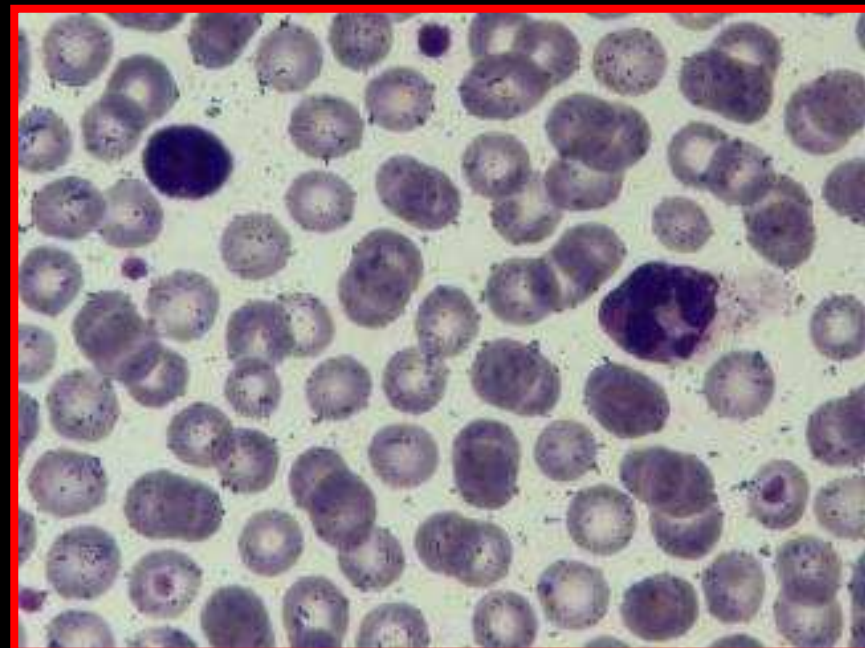
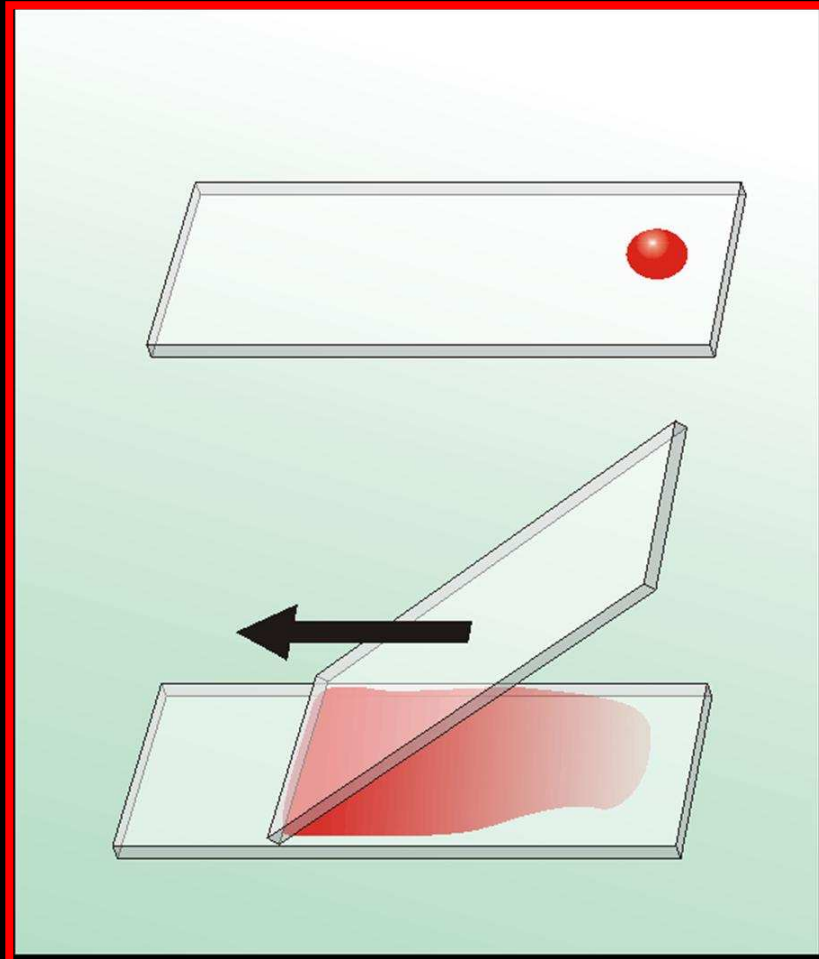
Granulociti neutrofili

- Nucleo:
 - lobularità:
 - iposegmentazione (pseudo-Pelger)
 - ipersegmentazione
 - densità cromatinica:
 - aumentata → picnotica (pseudo-Pelger)
 - ridotta → spugnosa
 - corpi Jolly-like
- Citoplasma:
 - anomalie delle granulazioni:
 - riduzione o assenza
 - aumento
 - distribuzione irregolare
 - deficienza di perossidasi (o altri enzimi)

Serie piastrinica

- Dimensioni:
 - aumento dell'anisocitosi fisiologica
 - macrotrombociti
 - piastrine giganti
 - microtrombociti
- Morfologia:
 - riduzione o assenza delle granulazioni (piastrine grigie)
 - addensamento centrale di granuli ipercromici (linfocito-simili)
- Lembi di citoplasma di megacariociti (piastrine non separate)
- Micromegacariociti
- Nuclei nudi di megacariociti

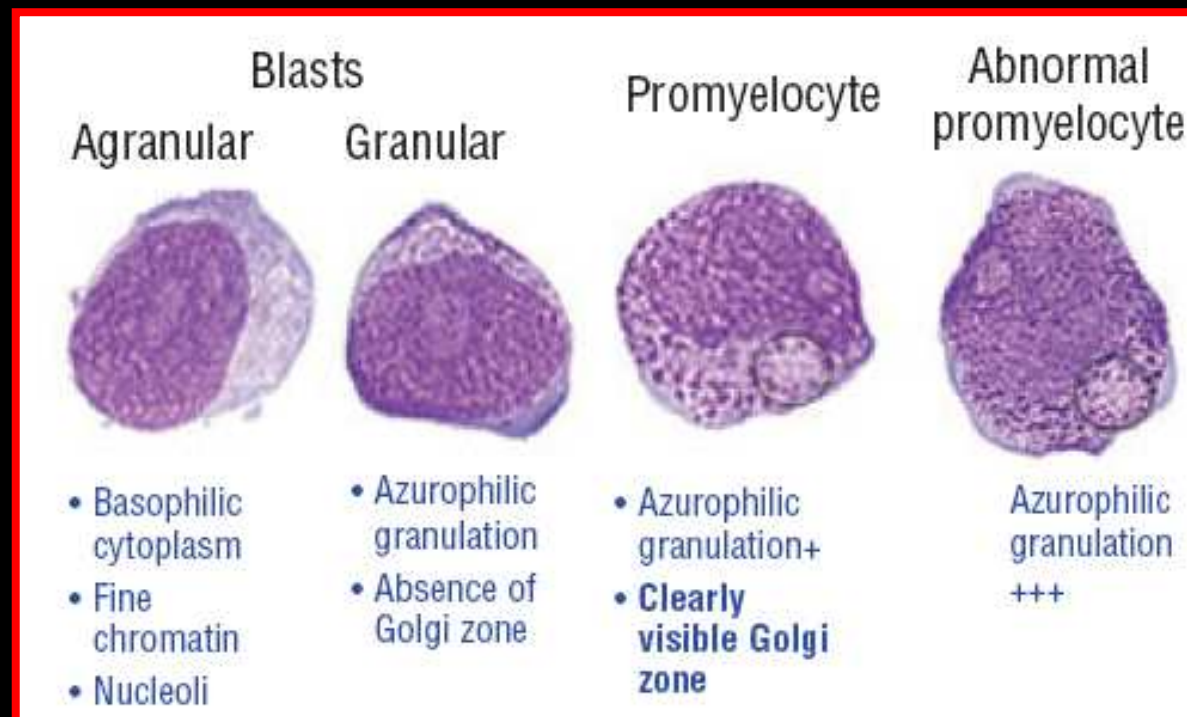
**Difficoltà nella standardizzazione
della preparazione degli strisci di
sangue periferico**



Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts

Haematologica 2009

Ghulam J. Mufti,¹ John M. Bennett,² Jean Goasguen,³ Barbara J. Bain,⁴ Irith Baumann,⁵ Richard Brunning,⁶ Mario Cazzola,⁷ Pierre Fenaux,⁸ Ulrich Germing,⁹ Eva Hellström-Lindberg,¹⁰ Itsuro Jinnai,¹¹ Atsushi Manabe,¹² Akira Matsuda,¹³ Charlotte M. Niemeyer,¹⁴ Guillermo Sanz,¹⁵ Masao Tomonaga,¹⁶ Teresa Vallespi,¹⁷ and Ayami Yoshimi¹⁸

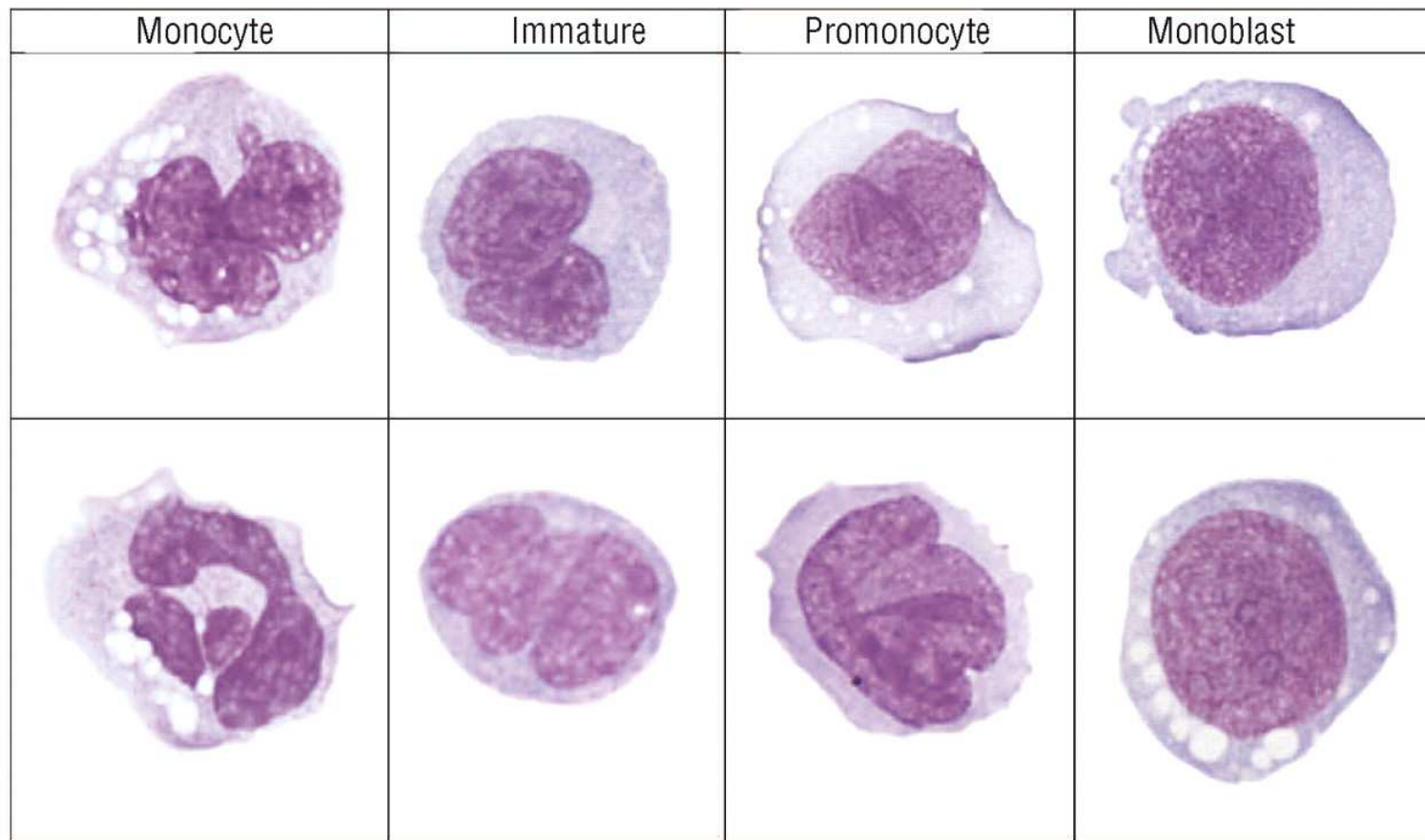


Morphological evaluation of monocytes and their precursors

Haematologica 2009

Jean E. Goasguen,¹ John M. Bennett,² Barbara J. Bain,² Teresa Vallespi,⁴ Richard Brunning,⁵ and Ghulam J. Mufti⁶
for the International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS)

¹University of Rennes, Rennes, France; ²James P. Wilmot Cancer Center, Rochester, New York, USA; ³St Mary's Hospital Campus of Imperial College Faculty of Medicine, London, UK; ⁴Hospital Universitario Vall d'Hebron, Barcelona, Spain; ⁵University of Minnesota, Minneapolis, Minnesota, USA and ⁶King's College London, UK

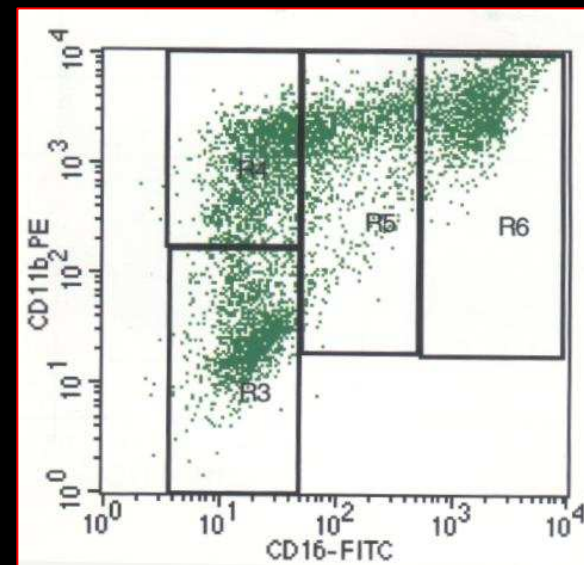
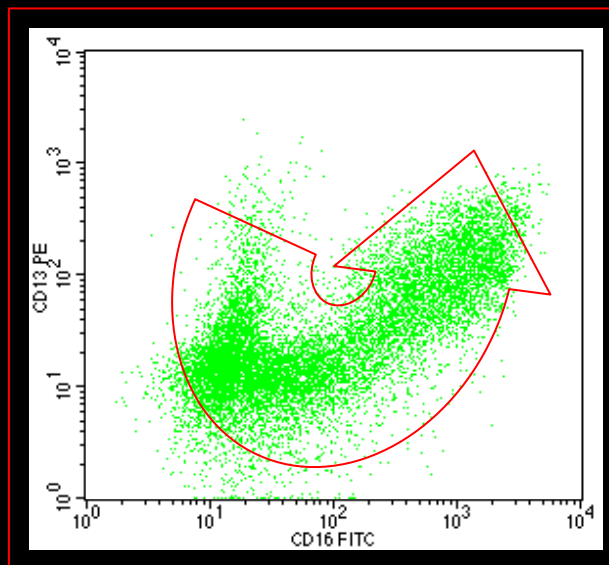


Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes

Haematologica 2009

Arjan A. van de Loosdrecht,¹ Canan Alhan,¹ Marie Christine Béné,² Matteo G. Della Porta,³ Angelika M. Dräger,¹ Jean Feuillard,⁴ Patricia Font,⁵ Ulrich Germing,⁶ Detlef Haase,⁷ Christa H. Homburg,⁸ Robin Ireland,⁹ Joop H. Jansen,¹⁰ Wolfgang Kern,¹¹ Luca Malcovati,³ Jeroen G. te Marvelde,¹² Ghulam J. Mufti,⁹ Kiyoyuki Ogata,¹³ Alberto Orfao,¹⁴ Gert J. Ossenkoppele,¹ Anna Porwit,¹⁵ Frank W. Preijers,¹⁰ Stephen J. Richards,¹⁶ Gerrit Jan Schuurhuis,¹ Dolores Subirá,¹⁷ Peter Valent,¹⁸ Vincent H.J. van der Velden,¹² Paresh Vyas,¹⁹ August H. Westra,¹ Theo M. de Witte,¹⁰ Denise A. Wells,²⁰ Michael R. Loken,²⁰ and Theresia M. Westers¹

Il Gruppo ha concluso che nonostante le forti evidenze di un impatto della FCM nelle MDS, sono necessarie delle ulteriori valutazioni in gruppi di pazienti di controllo per la validazione di marker e pattern immunofenotipici, come pure un'ulteriore standardizzazione in studi multicentrici.



blood

2007 110: 4385-4395
Prepublished online Aug 28, 2007;
doi:10.1182/blood-2007-03-082404

New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients

Detlef Haase, Ulrich Germing, Julie Schanz, Michael Pfeilstöcker, Thomas Nösslinger, Barbara Hildebrandt, Andrea Kundgen, Michael Lübbert, Regina Kunzmann, Aristoteles A. N. Giagounidis, Carlo Aul, Lorenz Trümper, Otto Krieger, Reinhard Stauder, Thomas H. Müller, Friedrich Wimazal, Peter Valent, Christa Fonatsch and Christian Steidl

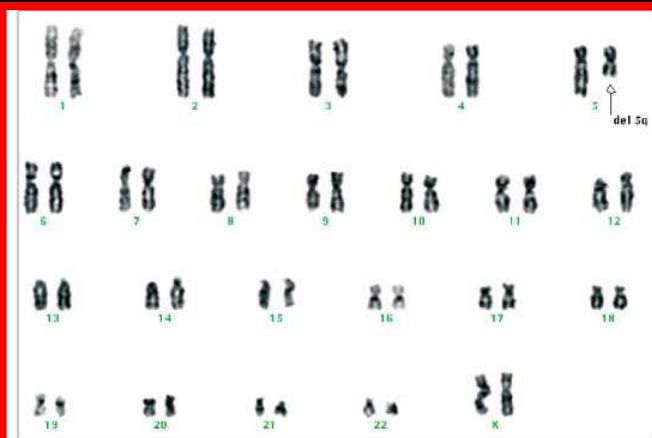
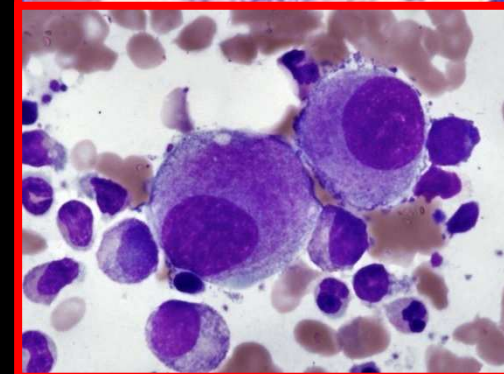
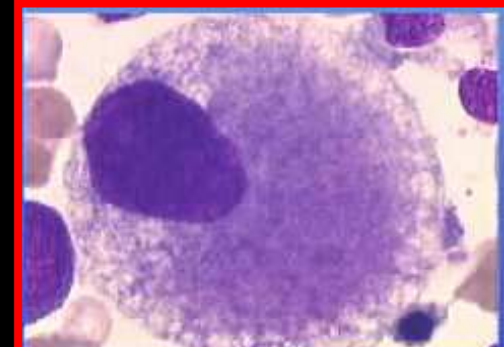
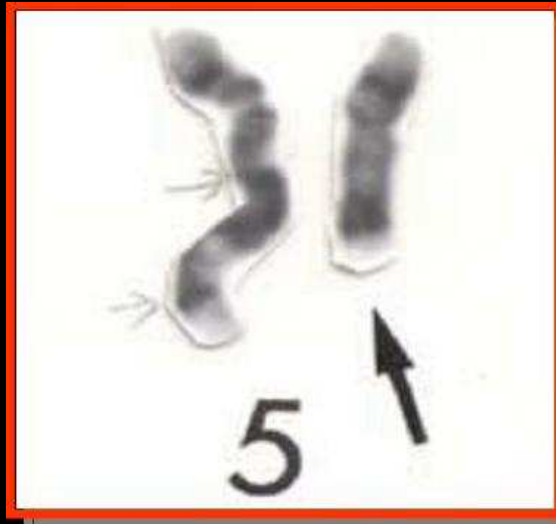


Fig. 2—G-banded karyotype obtained from the myelodysplastic syndrome patient at diagnosis: 46, XX, del(5)(q13;q33). The arrow shows the chromosomal abnormality



International Scoring System for Evaluating Prognosis in Myelodysplastic Syndromes

Peter Greenberg, Christopher Cox, Michelle M. LeBeau, Pierre Fenaux, Pierre Morel, Guillermo Sanz, Miguel Sanz, Teresa Vallespi, Terry Hamblin, David Oscier, Kazuma Ohyashiki, Keisuke Toyama, Carlo Aul, Ghulam Mufti and John Bennett

Table 3. IPSS for MDS: Survival and AML Evolution

Prognostic Variable	Score Value				
	0	0.5	1.0	1.5	2.0
BM blasts (%)	<5	5-10	—	11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

Scores for risk groups are as follows: Low, 0; INT-1, 0.5-1.0; INT-2, 1.5-2.0; and High, ≥ 2.5 .

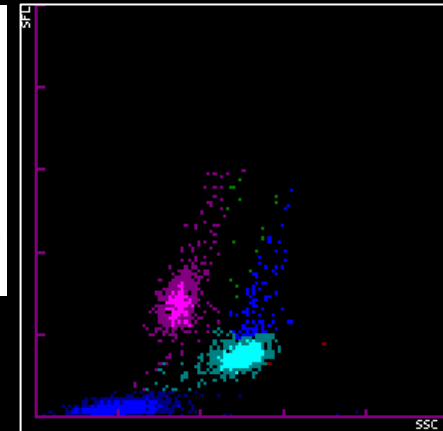
* Good, normal, $-Y$, $\text{del}(5q)$, $\text{del}(20q)$; Poor, complex (≥ 3 abnormalities) or chromosome 7 anomalies; Intermediate, other abnormalities.

Allarmi morfologici

MICRO:	+
MACRO:	+++
IPO:	++
IPER:	
ANISO:	+++
VAR HC:	++
DEV. A SINISTRA:	
BLASTI:	
ATIP:	
NRBC:	
IG:	
GRANDI PLT:	
AGGREGATI PLT:	
MPO-DEF:	
FRAM. RBC:	
OMBRE RBC:	
DISPLASIA	+++

The utility of the Sysmex XE-2100 analyzer's NEUT-X and NEUT-Y parameters for detecting neutrophil dysplasia in myelodysplastic syndromes

J. R. FURUNDARENA, M. ARAIZ, M. URANGA, M. R. SAINZ, A. AGIRRE, M. TRASSORRAS, N. URESANDI, M. C. MONTES, N. ARGOITIA

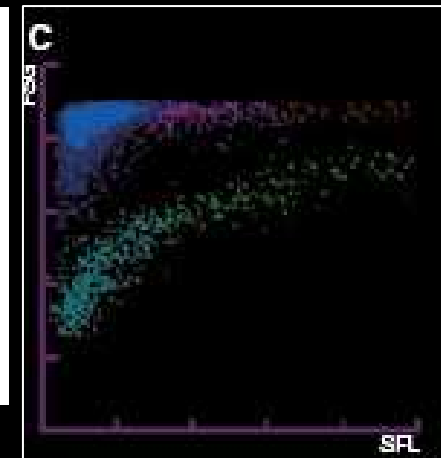


European Journal of Haematology ISSN 0902-4441

ORIGINAL ARTICLE

Aberrant increase in the immature platelet fraction in patients with myelodysplastic syndrome: a marker of karyotypic abnormalities associated with poor prognosis

Naomi Sugimori¹, Yukio Kondo¹, Masami Shibayama², Mika Omote², Akiyoshi Takami³, Chiharu Sugimori¹, Ken Ishiyama¹, Hirohito Yamazaki¹, Shinji Nakao¹



I PARAMETRI POSIZIONALI DI LH 750 NELLA VALUTAZIONE DELLA DISPLASIA GRANULOCITARIA DI VARIA ORIGINE

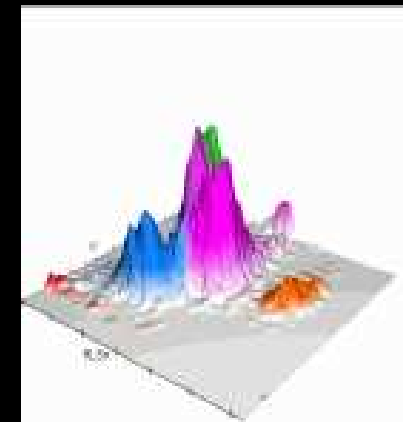
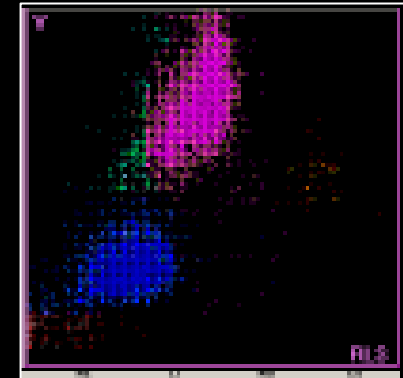


C. Piccinini, B. Biasioli Dipartimento di Medicina di Laboratorio – AOU Trieste

IMPORTANZA DELL'ETEROGENEITA' VOLUMETRICA DEI GRANULOCITI NEUTROFILI PER IL RICONOSCIMENTO DELLA DISPLASIA GRANULOCITARIA UTILIZZANDO, NELLA ROUTINE, I PARAMETRI POSIZIONALI DI COULTER LH 750

C. Piccinini, D. Casotto, M. Poletto, B. Biasioli

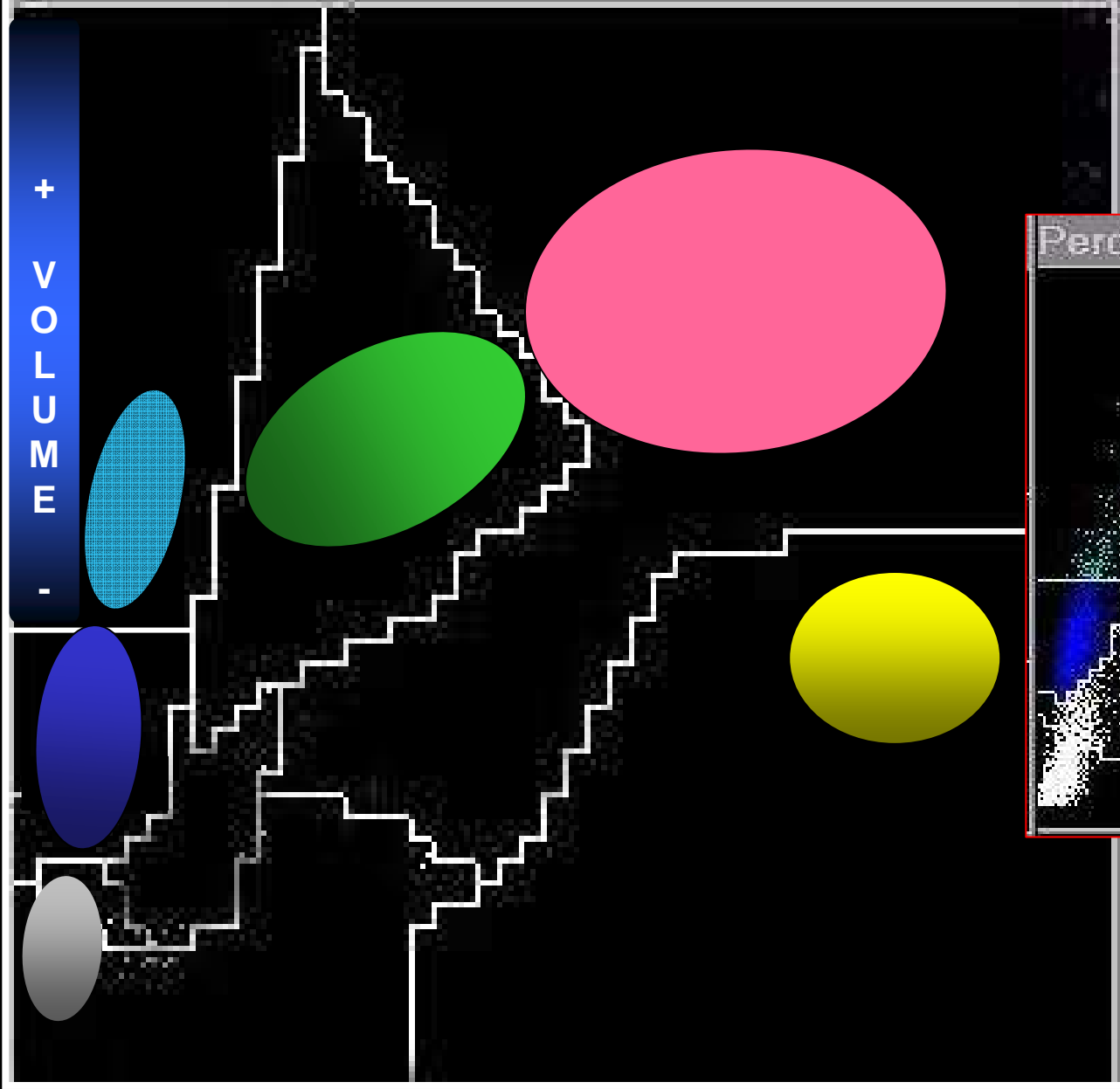
DAI di Medicina di Laboratorio, S.C. di Patologia Clinica, S.S. di Ematologia di Laboratorio AOU Trieste



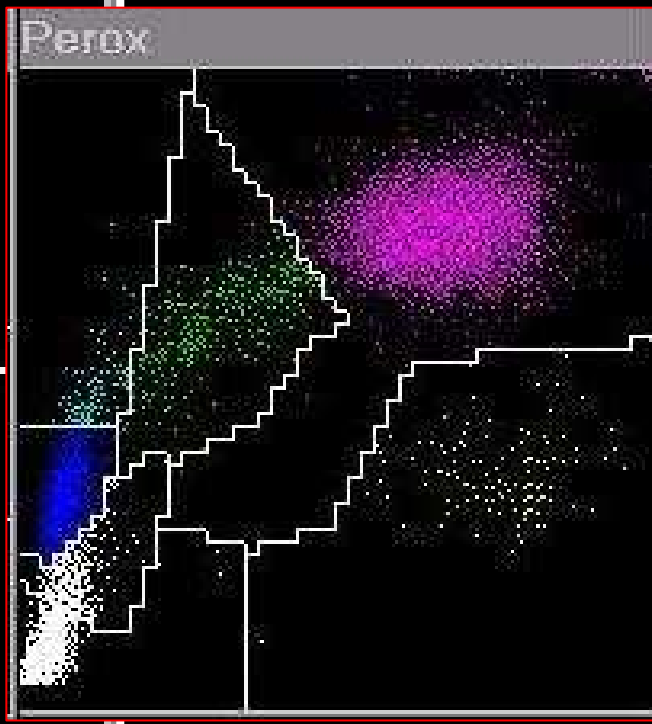
Perox

- Attività perossidasi +

+
V
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L
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-

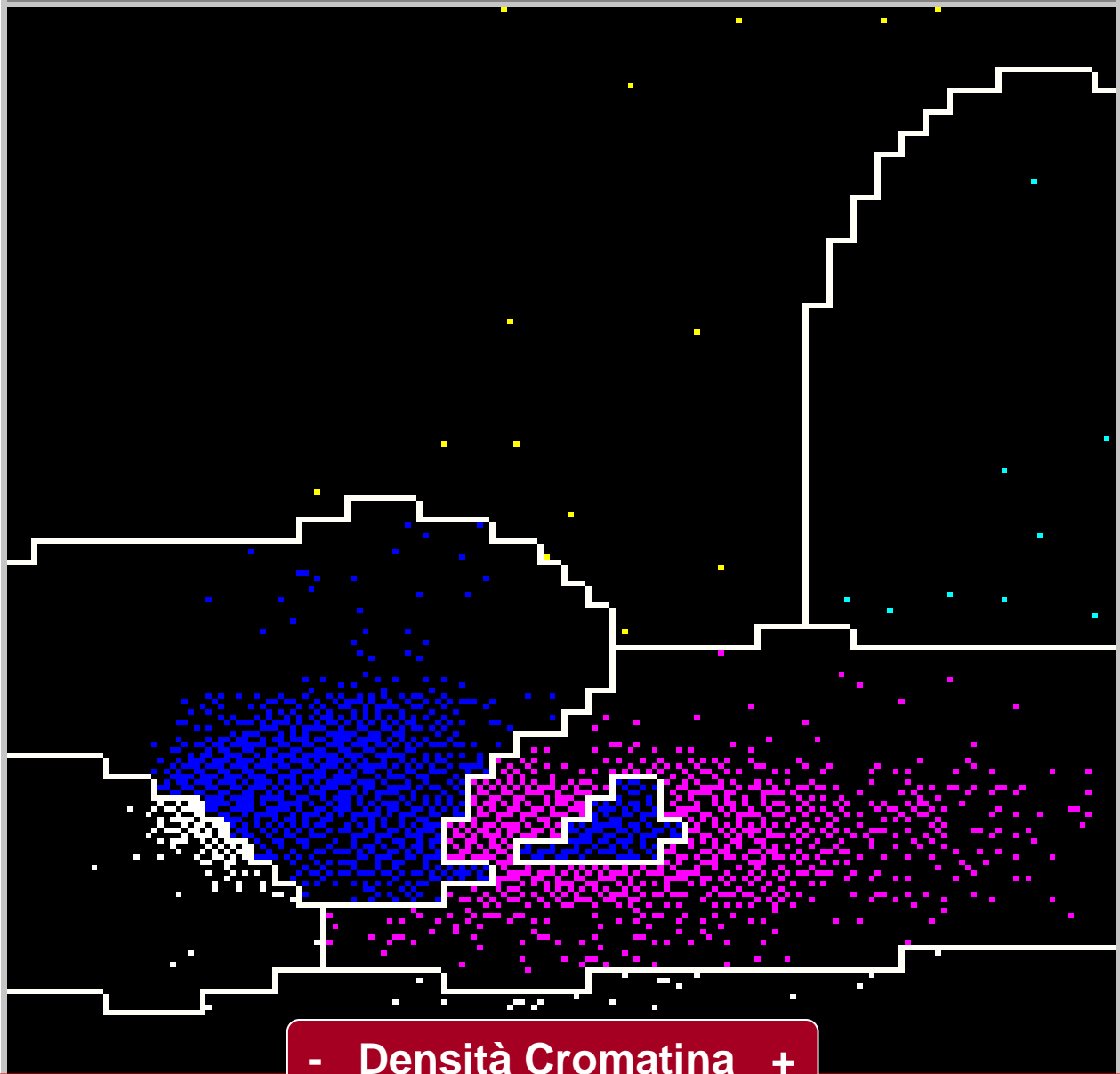


**ADVIA 2120
Siemens**



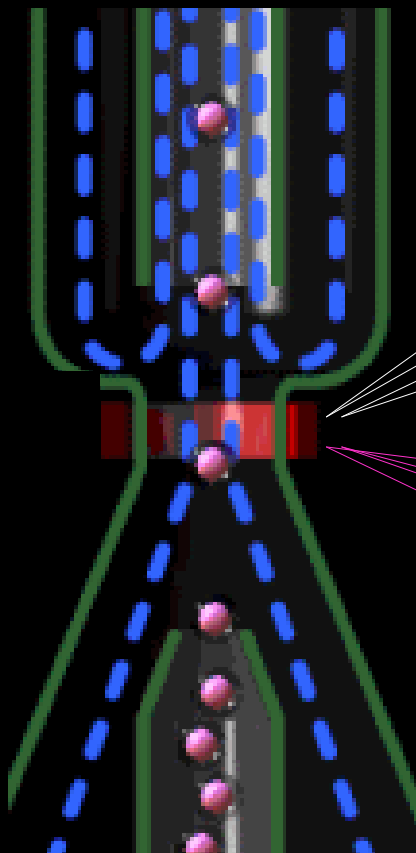
Baso

+
VOLUME
-



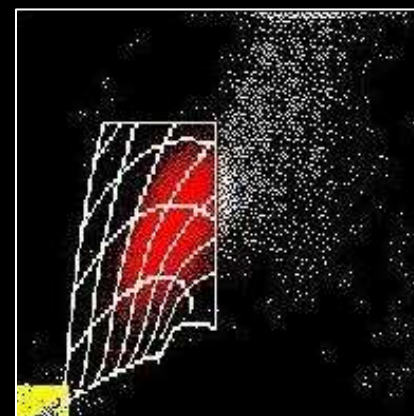
- Densità Cromatina +

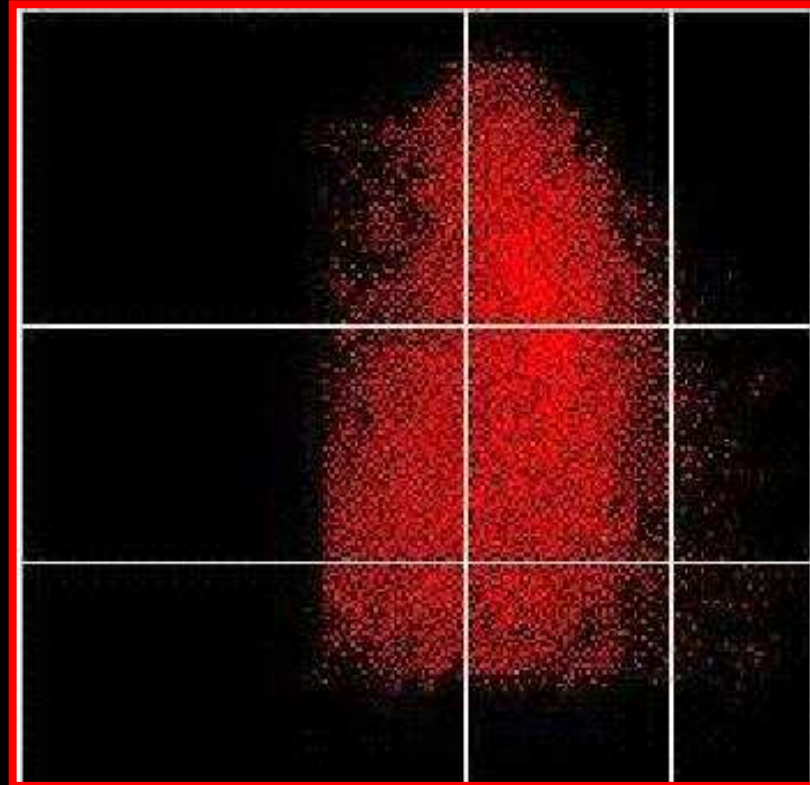
Siemens – Advia e GBR



High angle detector
 5° - 15°
LUCE RIFRATTA
Concentrazione Hb

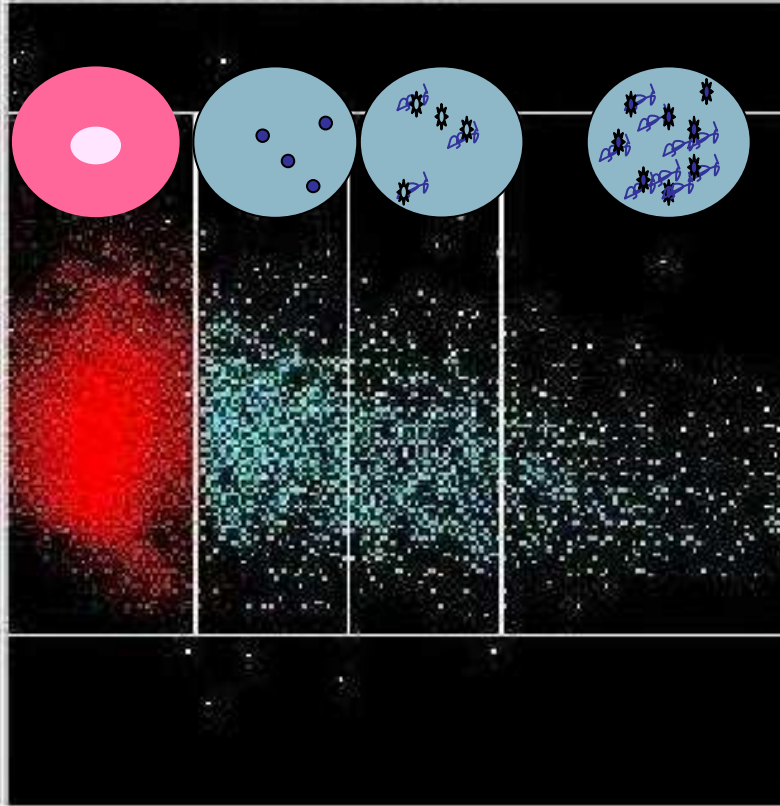
Low angle detector
 2° - 3°
SCATTER
Volume RBC





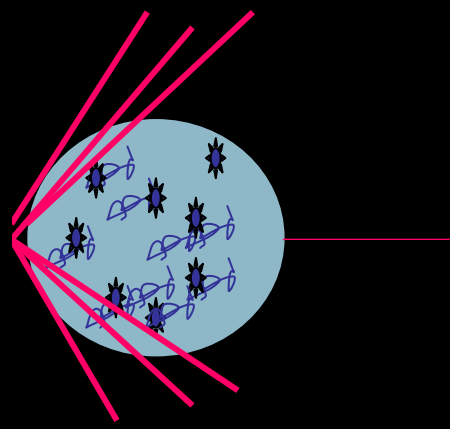
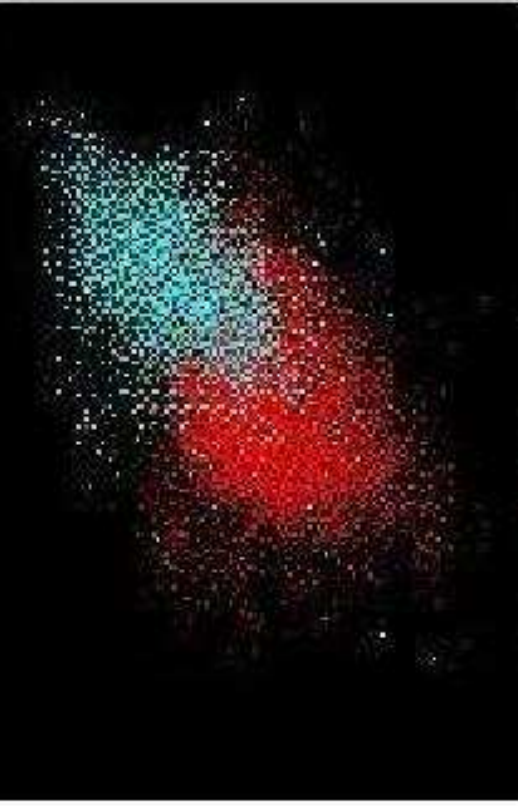
	HC <28	HC 28-41	HC >41
V > 120	946 (4.7%)	6256 (31.1%)	9 (0.0%)
V 60-120	3082 (15.3%)	7553 (37.6%)	64 (0.3%)
V < 60	981 (4.6%)	1221 (6.1%)	40 (0.2%)

Ass scatter retic



Retic V/HC

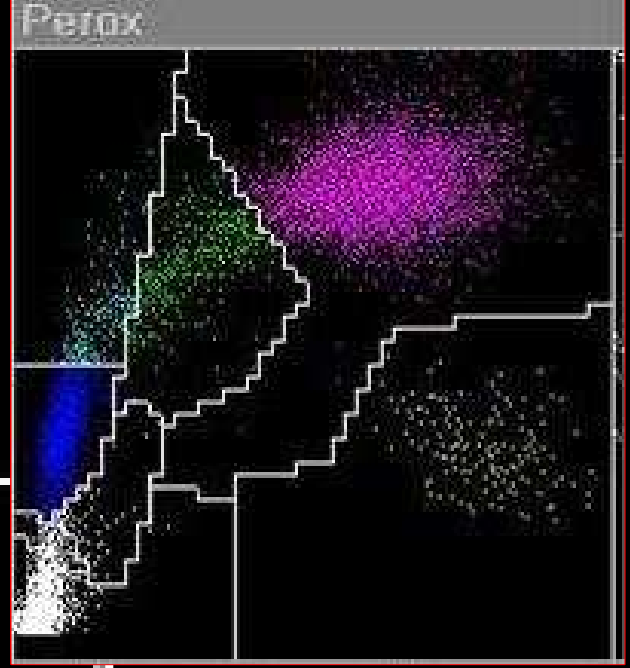
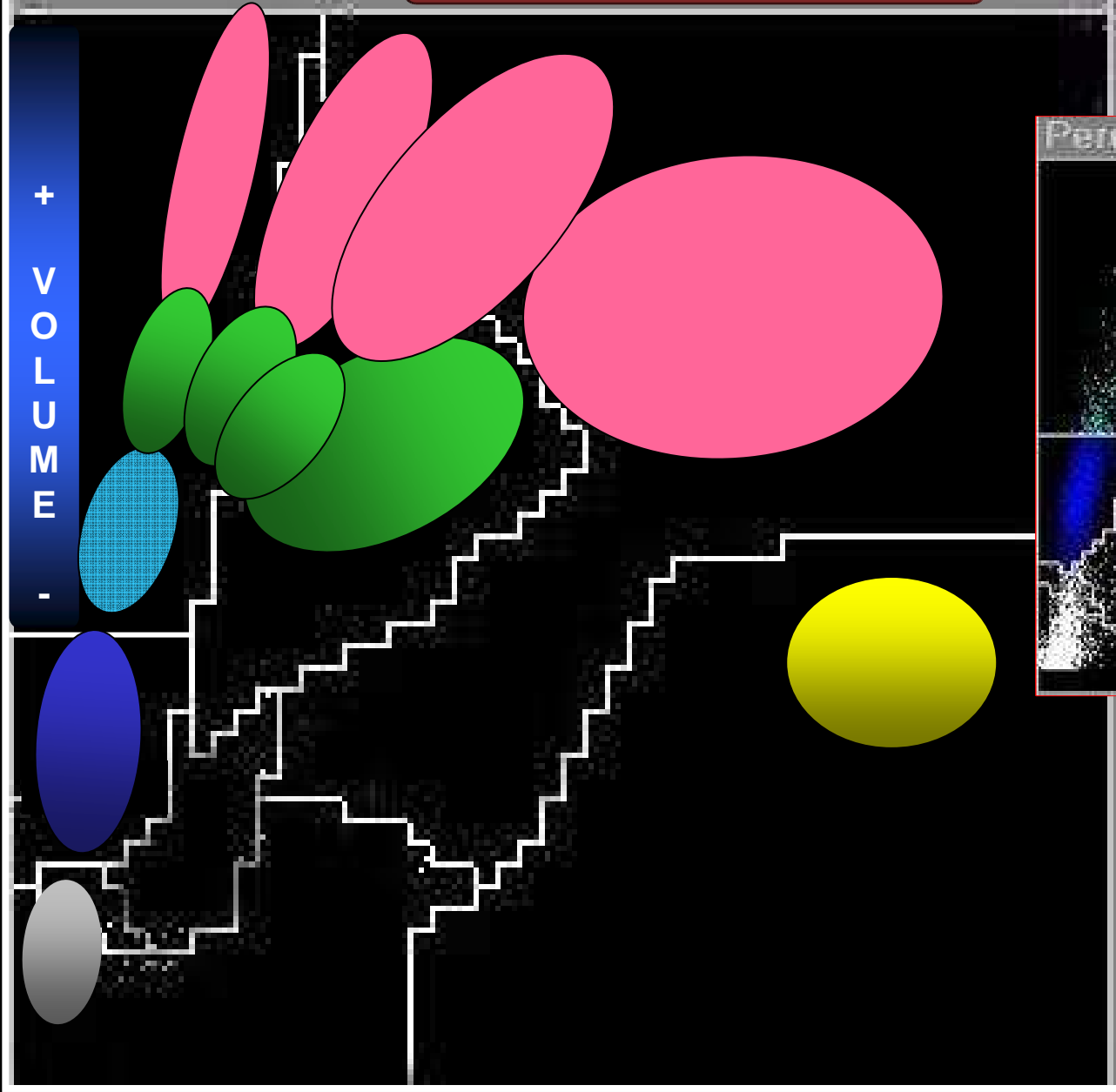
- MCVr
- CHCMr
- CHr
- RDWr
- HDWr
- CHDWr

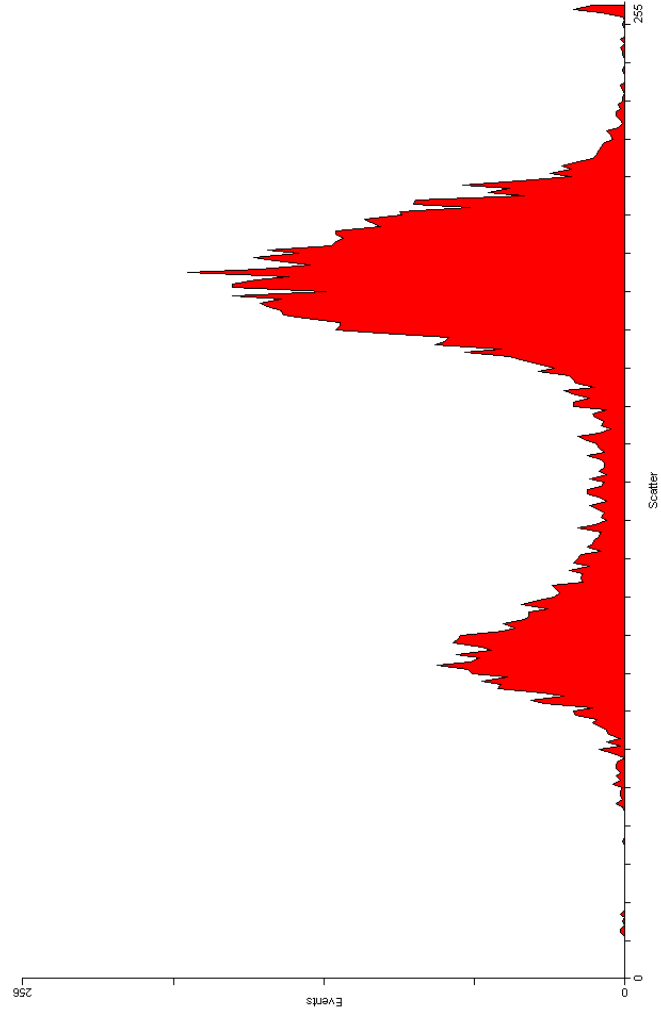
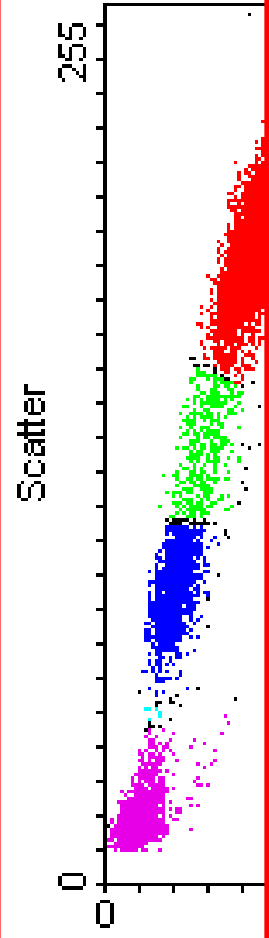


Perox

- Attività perossidasi +

+
VOLUME
-





Scatter

255

0

Events

255

Scatter

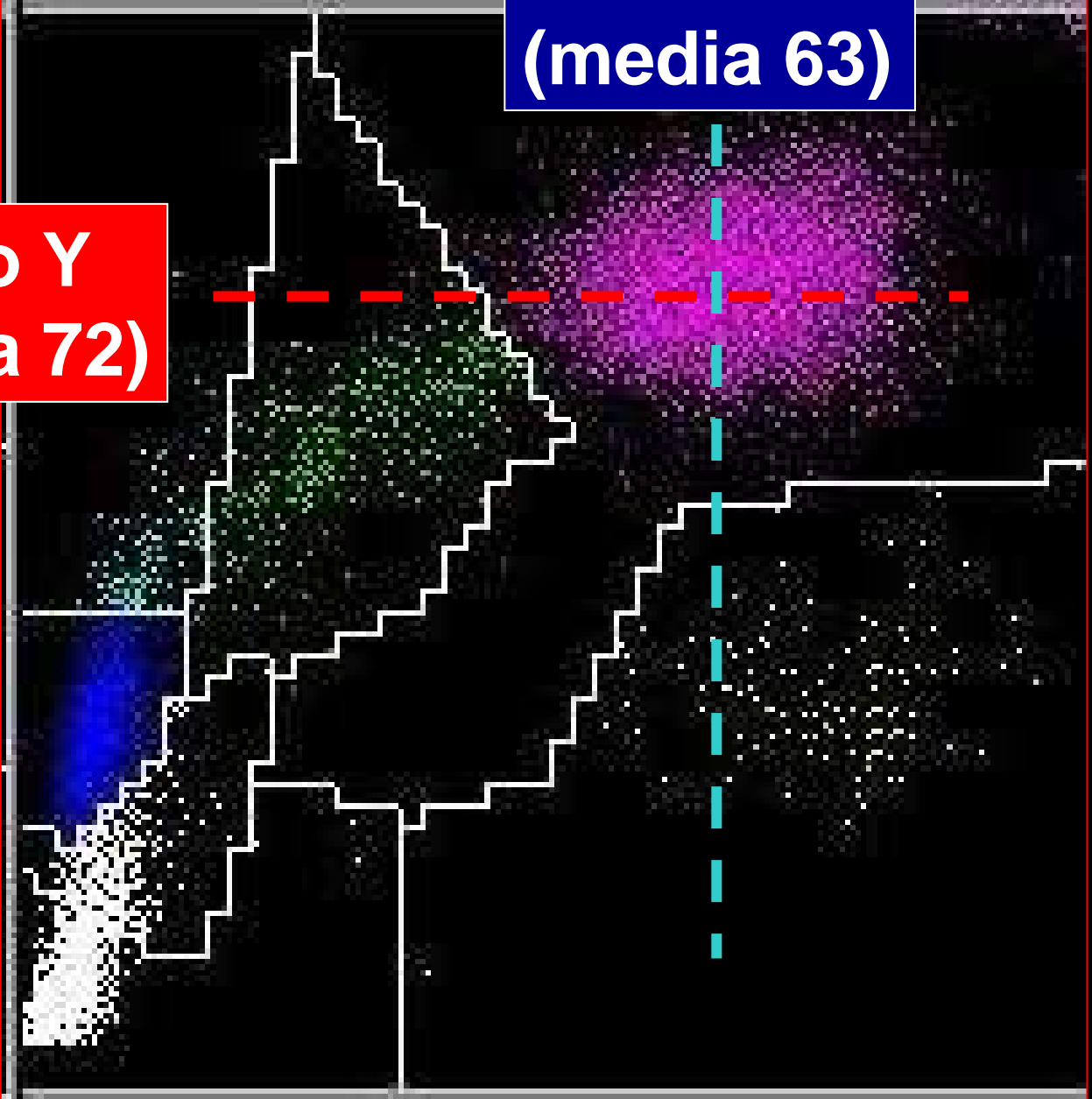
255

0

Perox

Neutro X
(media 63)

Neutro Y
(media 72)



Test	Med.	Limiti	Da 30/12/08 17.29		A 03/02/09 11.49	
CHCM	33,52	36,00 33,00 30,00				
WBCB	7,435	11,00 7,000 3,000				
WBCP	7,447	11,00 7,000 3,000				
NEUTx	62,43	68,00 63,00 58,00				
NEUTy	74,55	77,00 72,00 67,00				

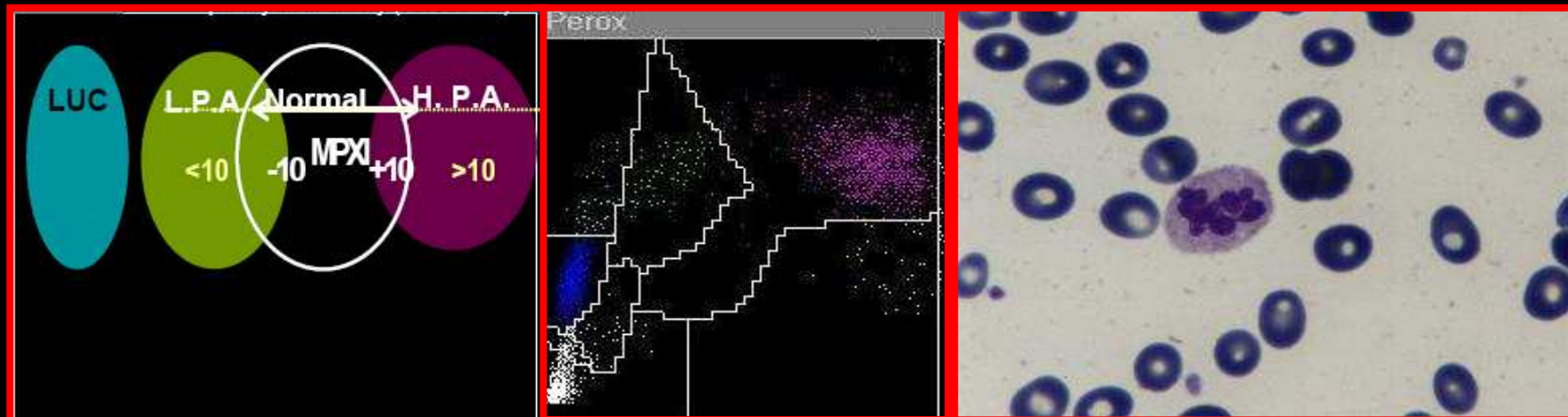
blood

1990 76: 1004-1007

Neutrophil myeloperoxidase measurement uncovers masked megaloblastic anemia [see comments]

ML Gulley, SA Bentley and DW Ross

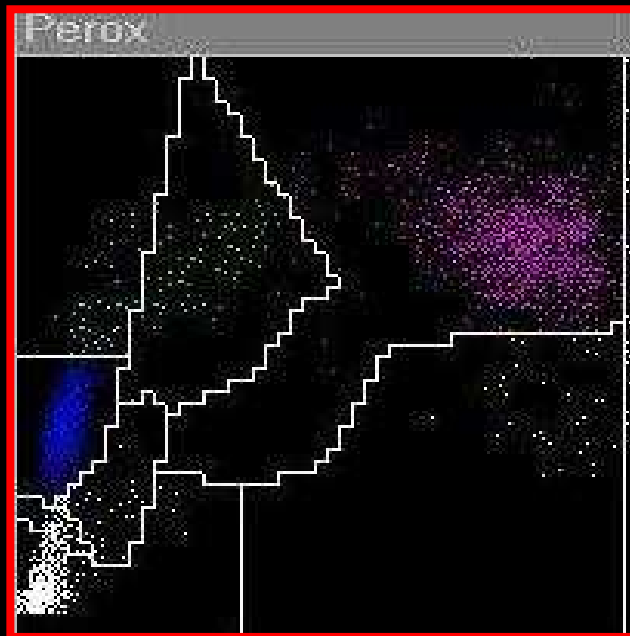
Gli AA concludevano che l'elevato MPXI non era specifico per magaloblastosi essendo ritrovato anche in alcune **MDS**, in alcune malattie mieloproliferative e nella AML FAB-M3 (dove l'elevato MPXI è dovuto alla presenza di granulociti immaturi nel sangue periferico).



Role of myeloperoxidase index in differentiation of megaloblastic and aplastic anemia

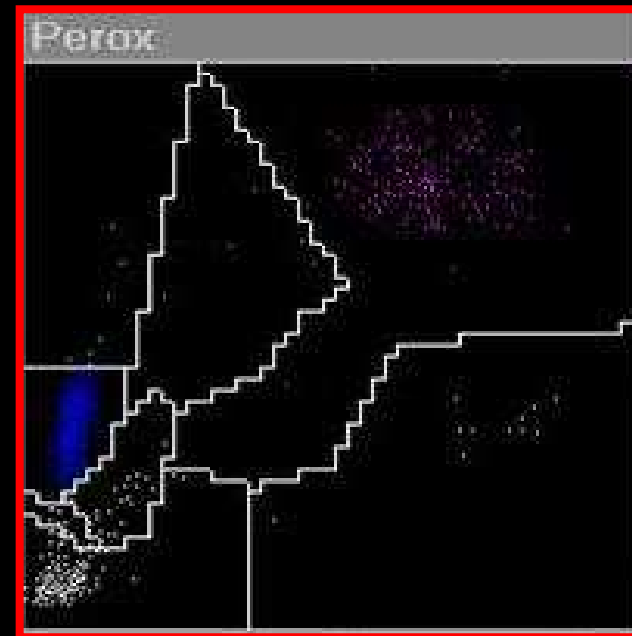
Jamal Eivazi Ziaei, Saeed Dastgiri

Hematology and Oncology Department, Imam Hospital, Tabriz, Iran



Anemia Megaloblastica

MPXI medio = 18,3



Anemia Aplastica

MPXI medio = 1,8

REVIEW

Francesco Lanza

Clinical manifestation of myeloperoxidase deficiency

Secondary MPO deficiency occurs in the following clinical situations:

- Hematological neoplasms (especially those involving the maturation processes of the granulocytic series): chronic myeloid leukemia, acute myeloid leukemia, polycythemia vera, myelofibrosis with myeloid metaplasia, myelodysplastic syndromes
- Disseminated cancers (apparently related to cytostatic drug administration)
- Drugs (cytotoxic agents, some anti-inflammatory compounds)
- Iron deficiency
- Lead intoxication
- Thrombotic diseases
- Renal transplantation
- Severe infectious diseases
- Diabetes mellitus
- Neuronal lipofuscinosis
- Pregnancy

Oltre a ribadire la possibile Carenza di Mieloperossidasi acquisita nella carenza di Ferro, è da notare il possibile deficit di perossidasi nelle MDS

Pseudo-eosinofilia da mielodisplasia

U. Bolzern,
H.R. Recalde,
I. Chiesa, F. Motta
Laboratorio di Ematologia
Ospedale di Magenta

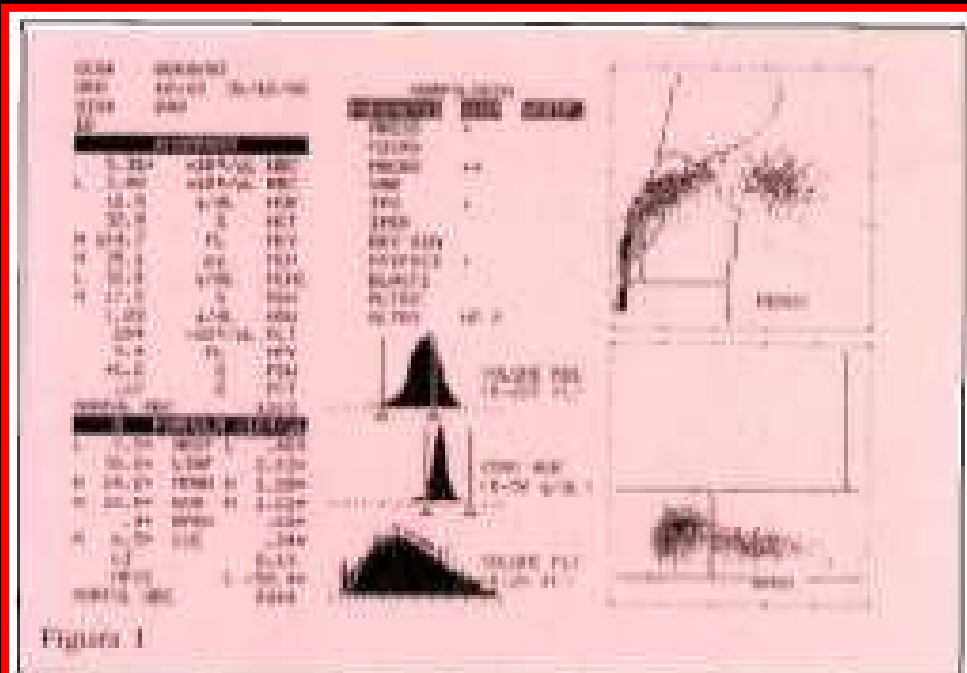


Figure 1

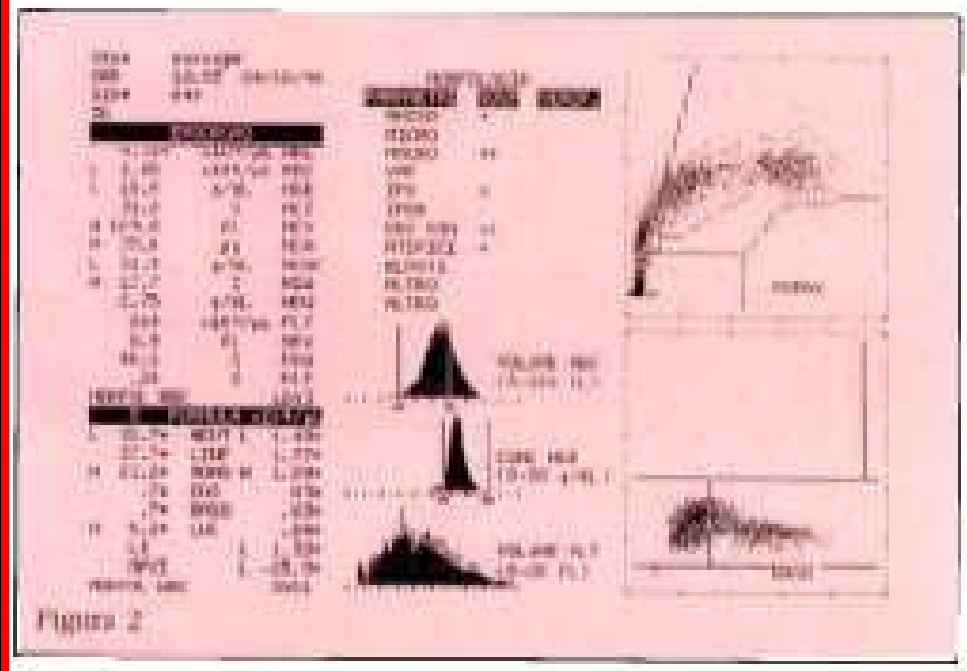


Figure 2

Sindromi mielodisplastiche: utilità dell'analisi morfometrica del citogramma del canale della perossidasi nella valutazione preliminare della dismielopoiesi

G.L. Devoto, D. Tanca, M. Venturini

Laboratorio di analisi Ospedale Civile
USL 4 Chiavarese, Lavagna (Genova)

Figura 1 - Caso 1:
Anemia refrattaria.

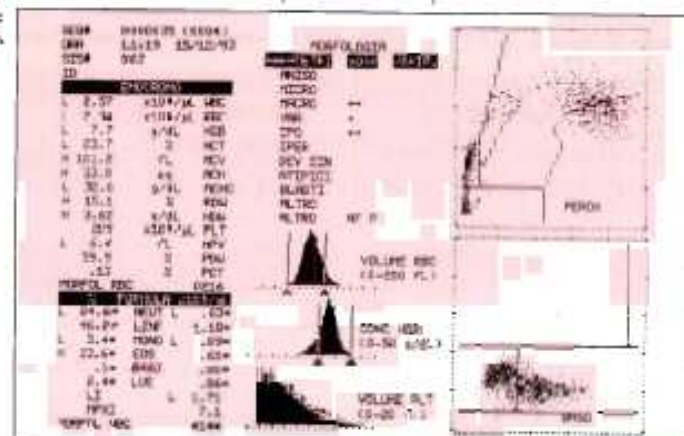


Figura 2 - Caso 2:
Anemia refrattaria
con eccesso
di blasti in
trasformazione.

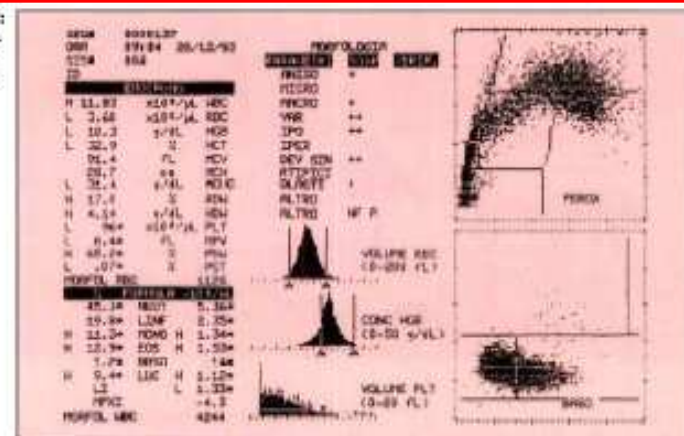
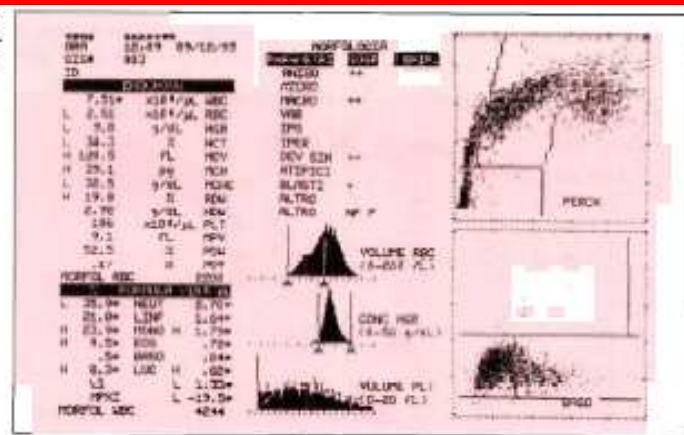


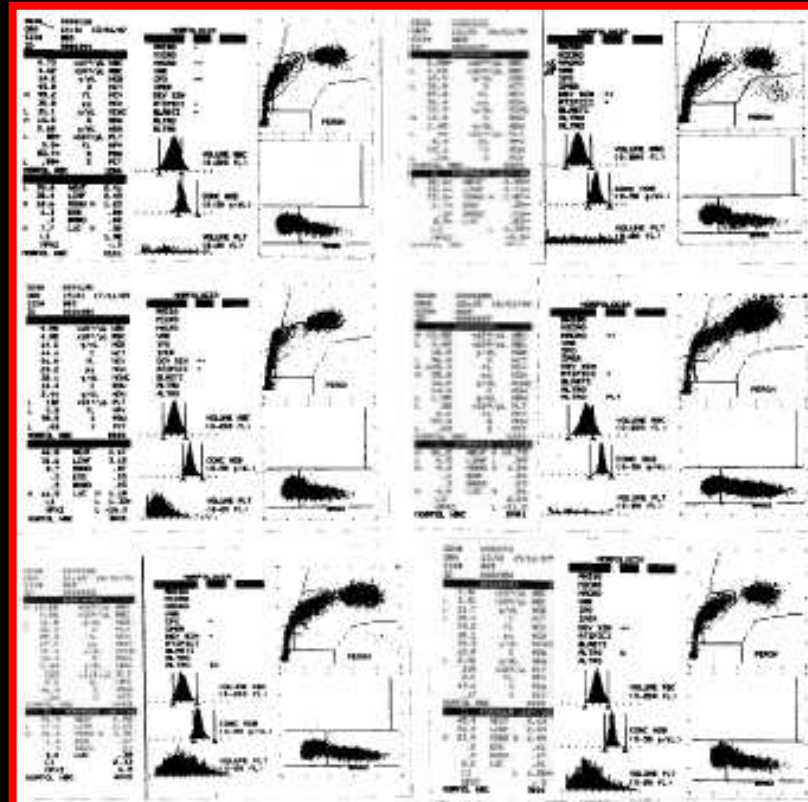
Figura 3 - Caso 3:
Leucemia mielomonocitica
cronica.



La citoematologia automatizzata nelle sindromi mielodisplastiche

P. Cappelletti, B. Milanesi*, D. Signori,
P. Doretto, F. Falcomer

Servizio di Chimica e Microscopia Clinica. Ospedale di Pordenone - USL N°11 *1° Laboratorio di Analisi chimico-cliniche e microbiologia - Ospedali Civili- Brescia.



Chronic myeloblastic leukemias: peripheral blood morphology and cell distribution in automated cytochemistry

P. Salutari, G. d'Onofrio, G. Zini, M. Tommasi, G. Leone

Center of Research for the Development and Clinical Evaluation of Automated Methods in Hematology, Department of Hematology, Università Cattolica del Sacro Cuore, Rome, Italy

Figure 1. Cytograms obtained from H-1 system in a case of chronic granulocytic leukemia (left), chronic myeloblastic leukemia (center), and chronic myelomonocytic leukemia (right). For an explanation of morphology see Table 4.

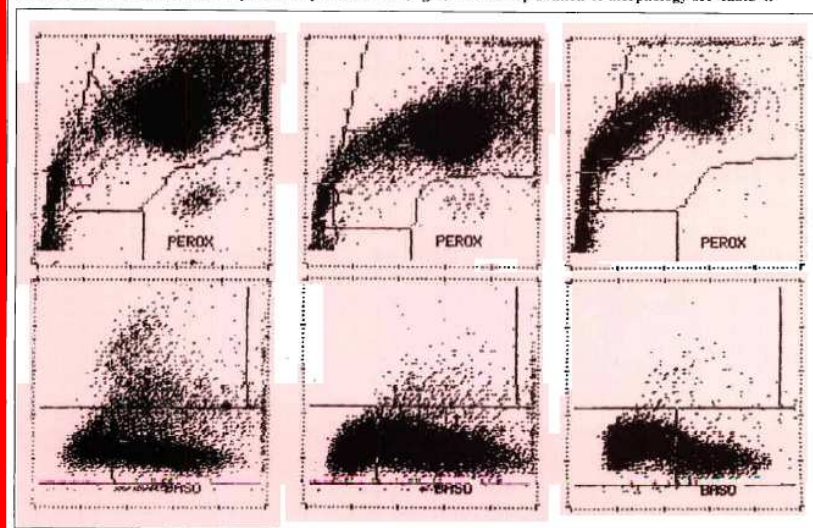


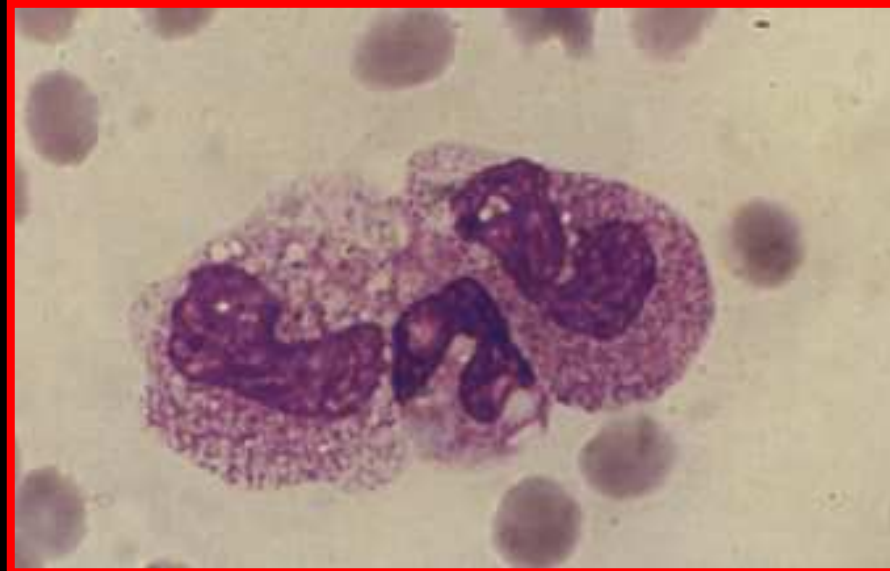
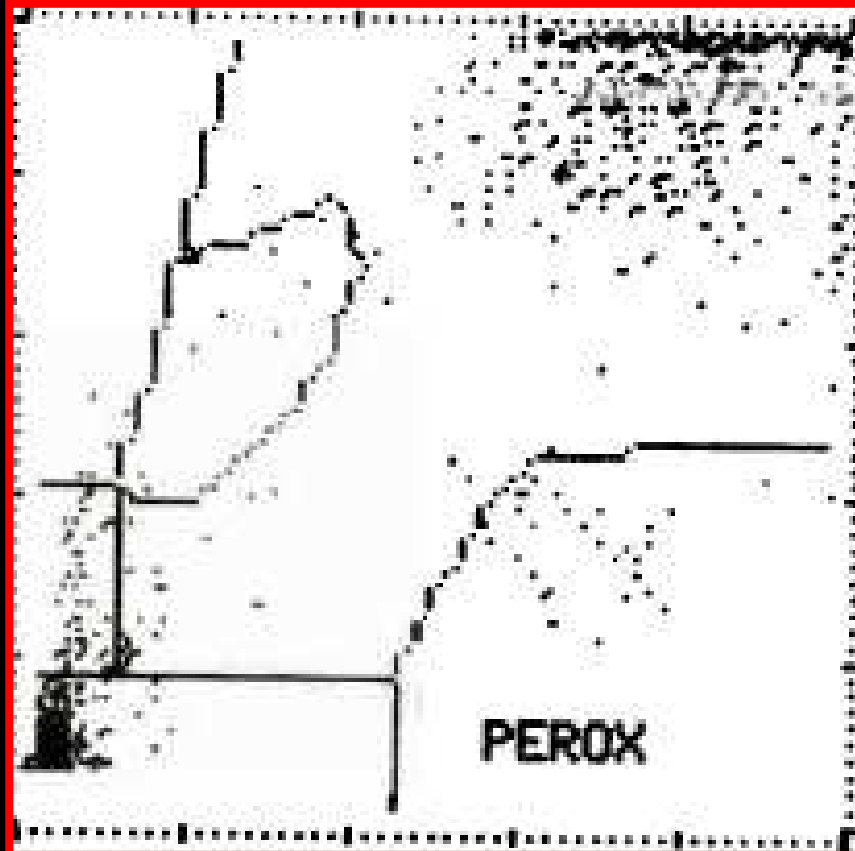
Table 4. Morphology of cytograms from H¹ systems in different types of chronic myeloblastic leukemia.

	Dispersion of neutrophils	Lymphocytes/neutrophils separation	LUC peak	Monocyte cluster	"True" basophil cluster
CGL	+++	+	-	-	+
aCML	++	-	-	+	-
CMML	+	-	±	+++	-

Anomalie ultramorfologiche dei granulociti neutrofili nelle infezioni da virus HIV

G. d'Onofrio, G. Zini, M. Tommasi, S. Valentini,
G. Mauro*, E. Tamburrini*, L. Ortona*

Servizio di Ematologia (Istituto di Semeiotica Medica) e
Istituto di Clinica delle Malattie Infettive*, Università
Cattolica del Sacro Cuore, Roma



d'Onofrio - Zini



Morfologia del Sangue

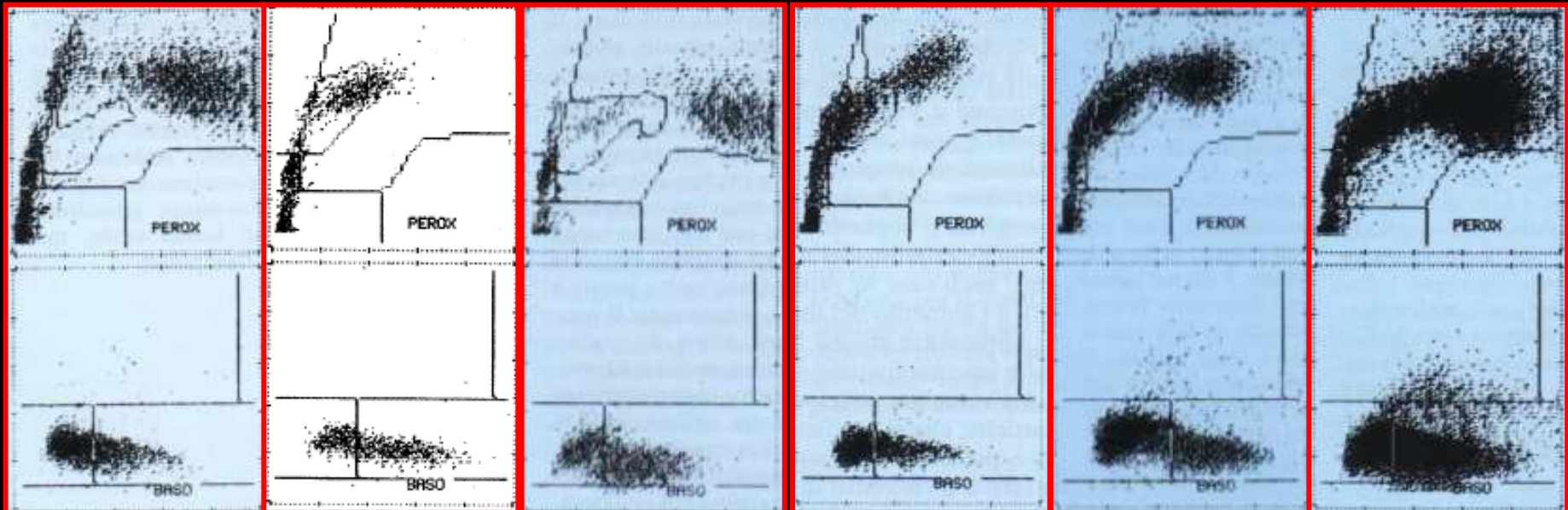


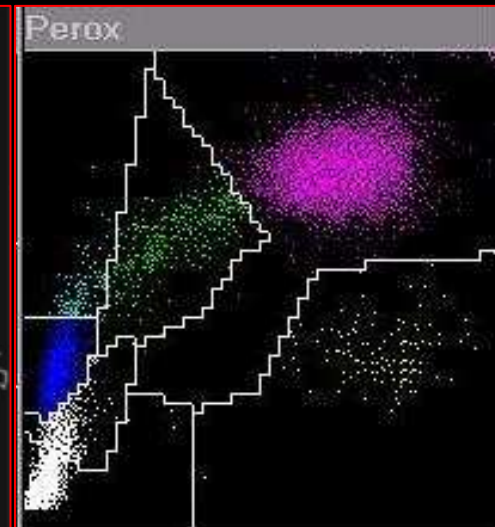
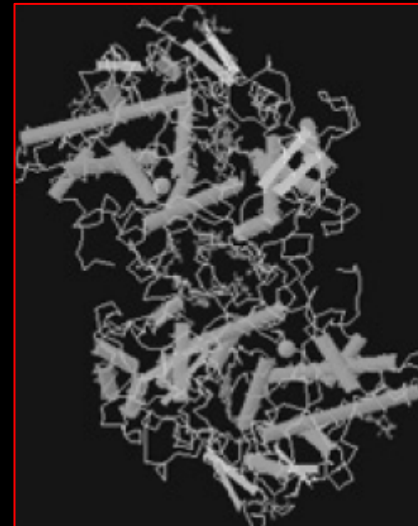
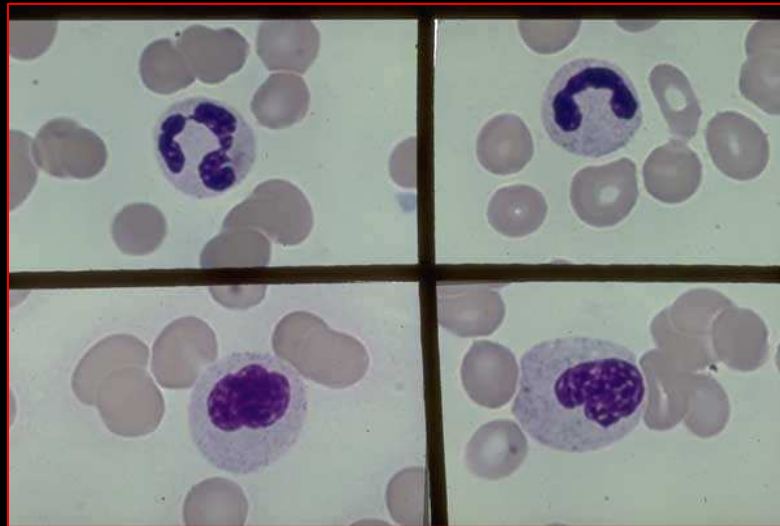
Table 1 Morphological signs of dysplasia in peripheral blood

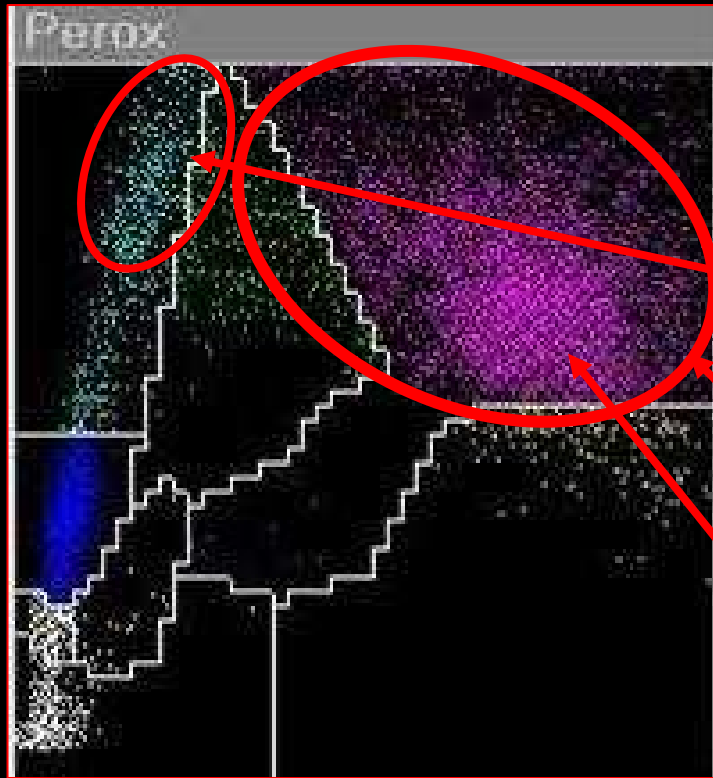
Signs in peripheral blood

Pseudo-Pelger cells, blasts, hypo/degranulated white precursors, left shift of white precursors

Giant platelets, anisometry of platelets

Anisocytosis, poikilocytosis, dimorphic red cells, polychromasy, hypochromasy, megalocytes, basophilic stippling, red nucleated precursors, tear drop cells, ovalocytes, fragmentocytes



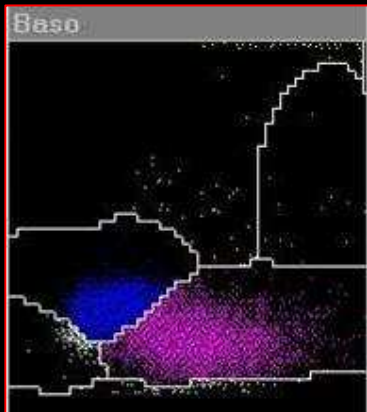


1° Pattern-algoritmo

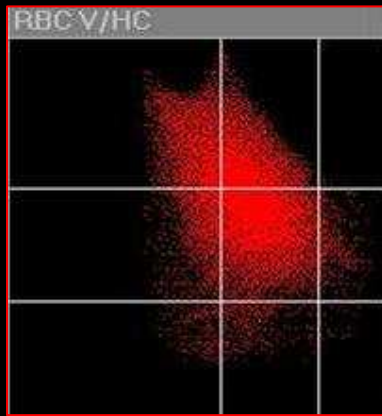
LUC ↑

HPX ↑

Neutro Y ↓



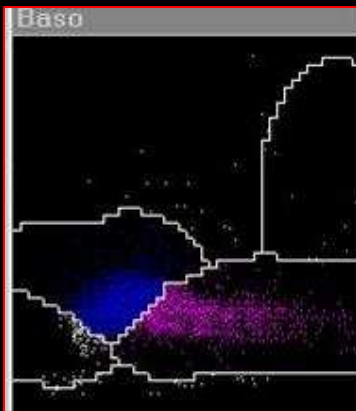
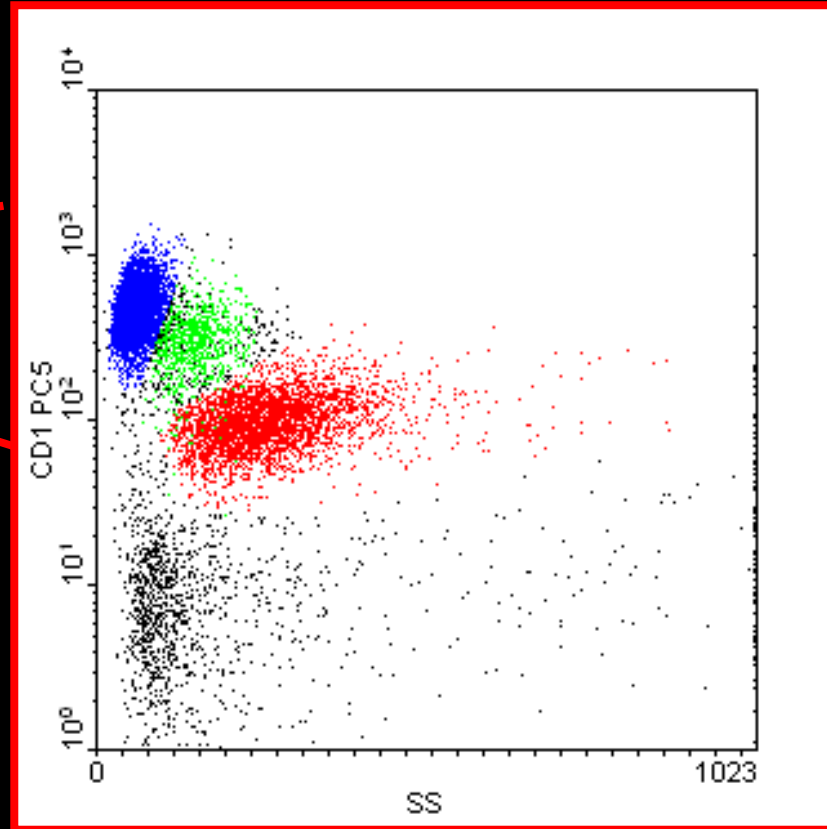
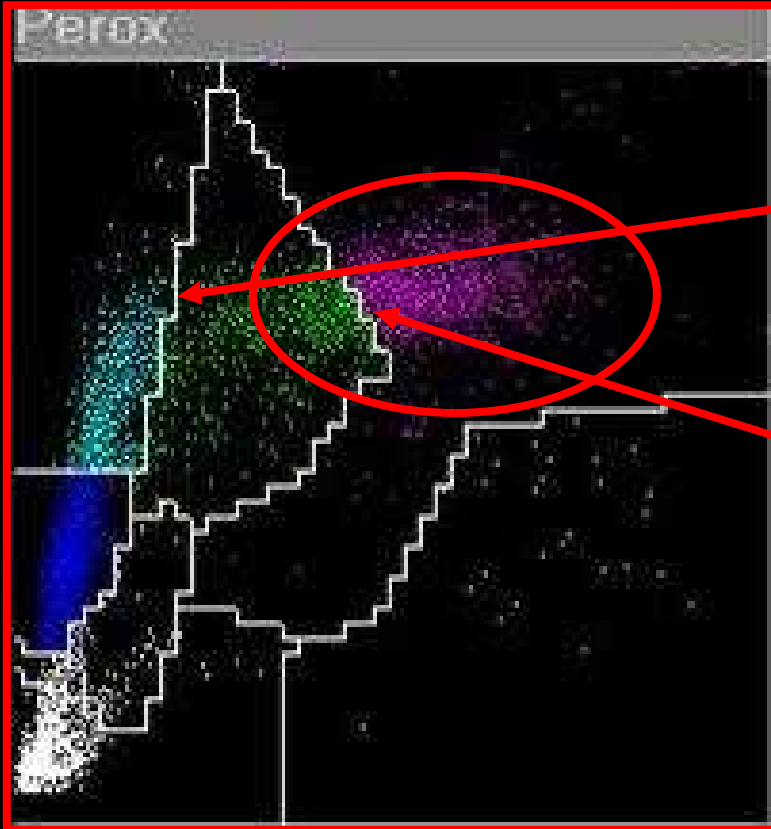
Allarme "Blasti"
60% dei casi



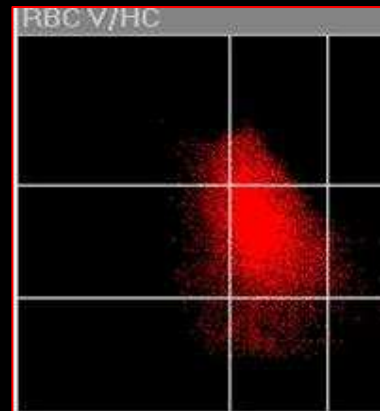
RDW elevato
100% dei casi



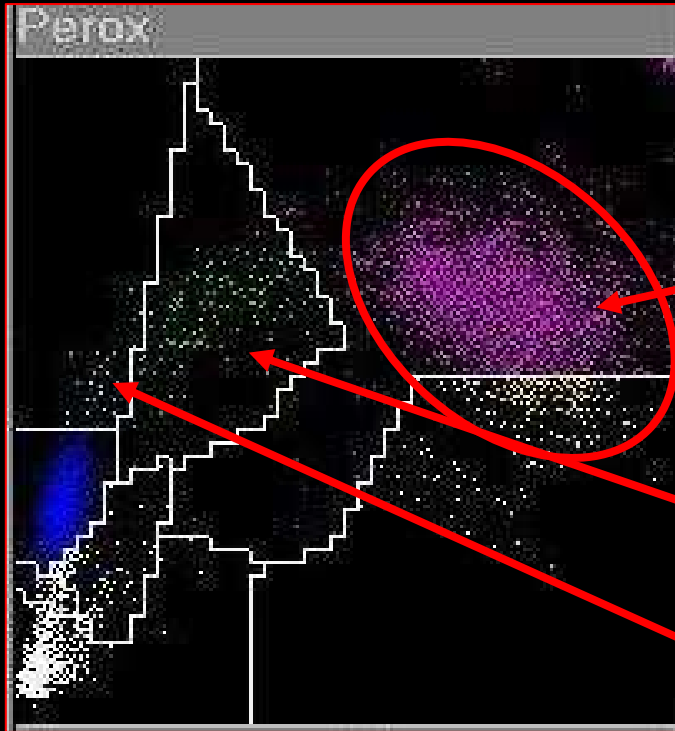
Piastrinopenia
60% dei casi



Allarme "Blasti"
53,5% dei casi



RDW elevato
93,7% dei casi



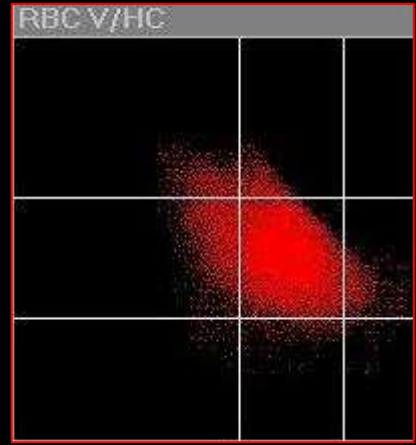
3° pattern-algoritmo

Neutro X ↑

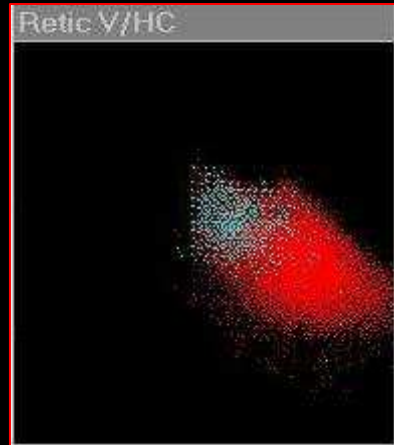
Neutro Y ↓

Mono =/↓

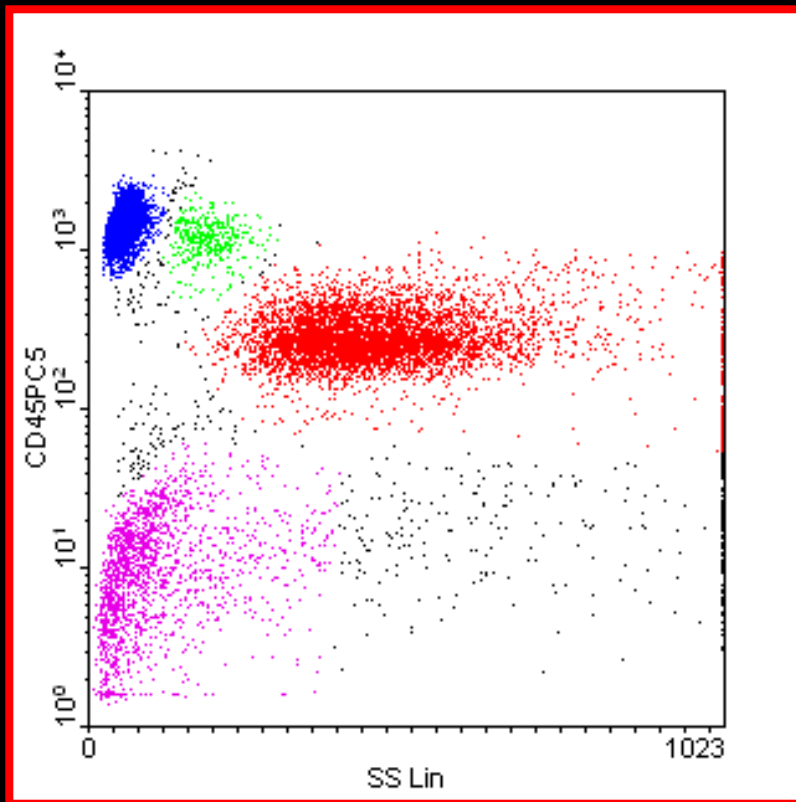
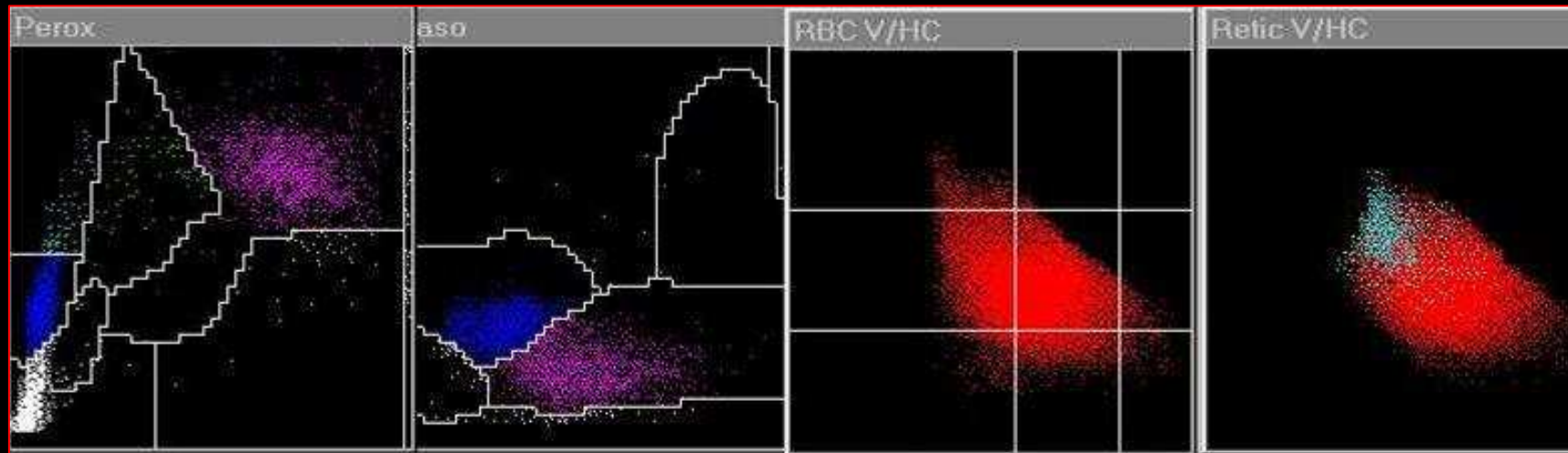
LUC =

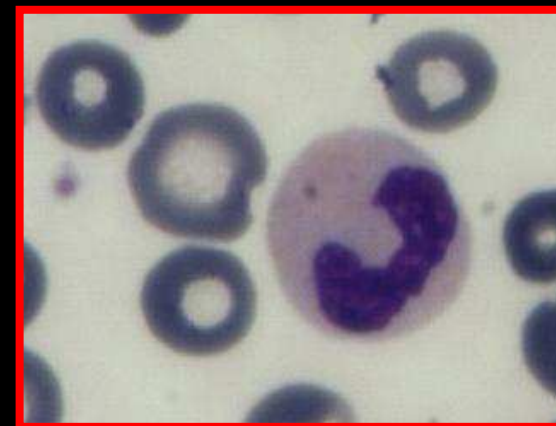
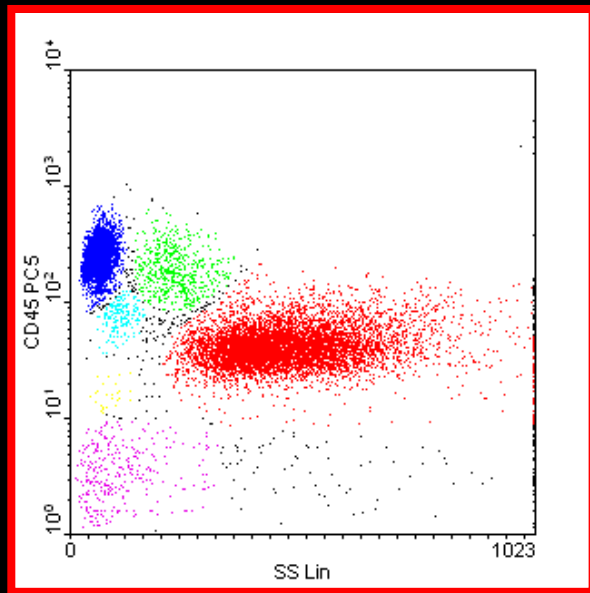
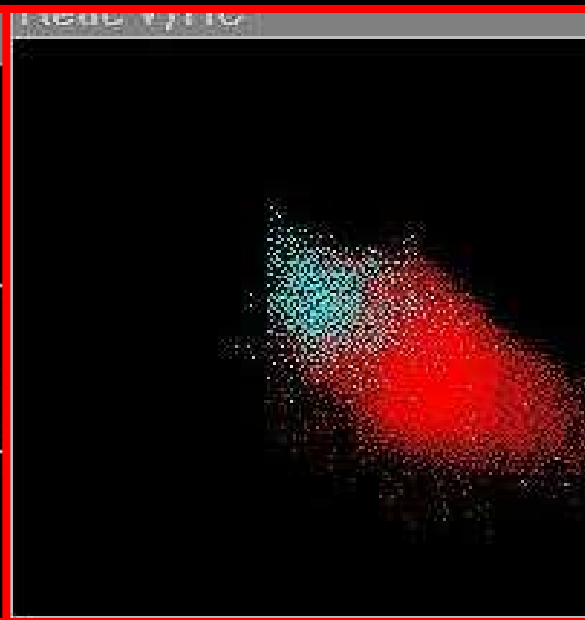
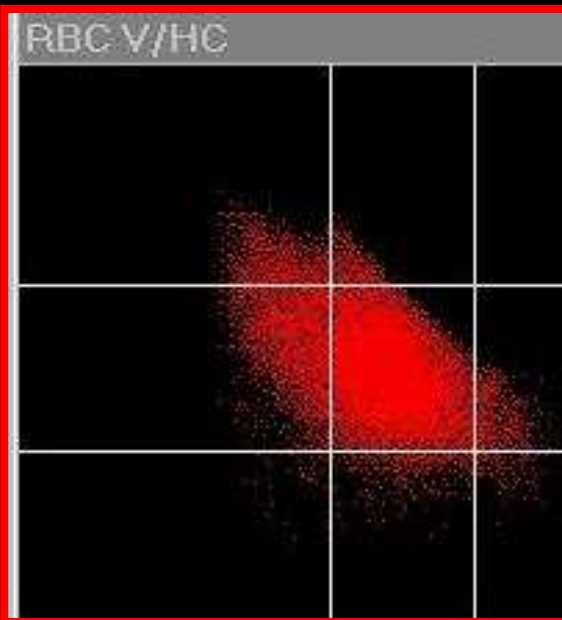
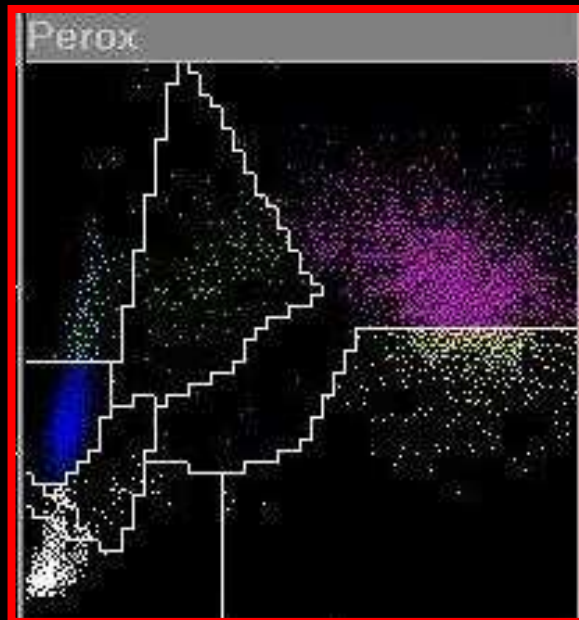


Emazie ipocromiche (>4%) da carente apporto di ferro



CHCMr costantemente ridotto (media 25,8 g/dL) per eritropoiesi da carente apporto di Ferro.





Rapporti tra Perossidasi e Ferro

J Mol Med (1998) 76: 676–681

REVIEW

Francesco Lanza

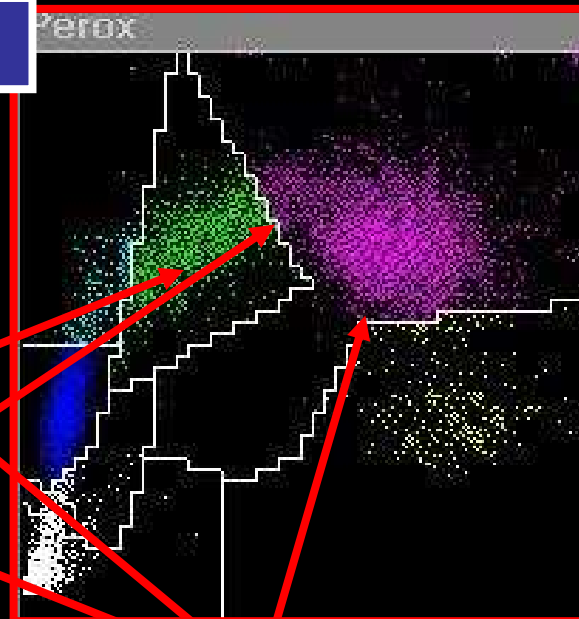
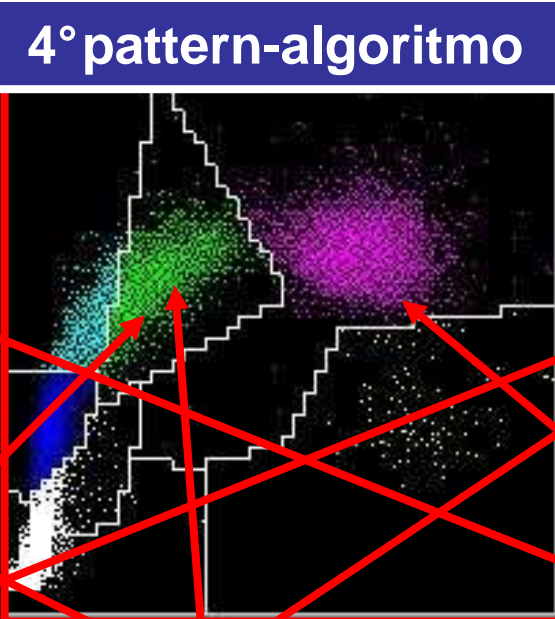
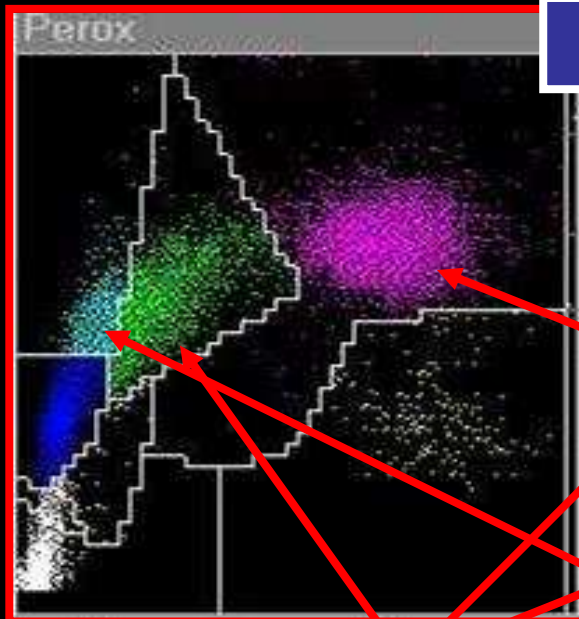
Clinical manifestation of myeloperoxidase deficiency

자동혈구분석기 H*2를 이용한 철결핍성빈혈 환자의 Mean Peroxidase Index 분석

(Korean J Lab Med 2002)

“Lo MPXI è ridotto nella Anemia da Carenza di Ferro e sembra essere inficiato da varie condizioni dei pazienti. Lo MPXI aumenta dopo terapia marziale, il che suggerisce che l’attività MPO è tornata normale”.

4° pattern-algoritmo

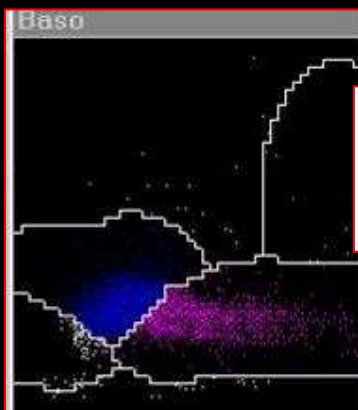


Mono ↑

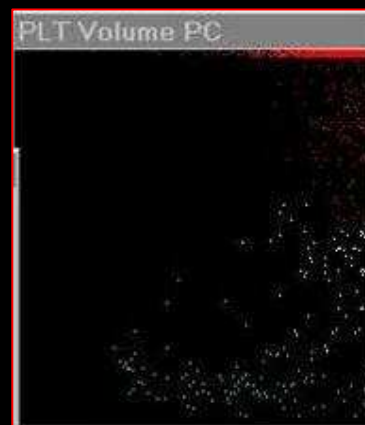
Perox Mono ↓/=/↑

Neutro Y ↓

LUC ↑/=



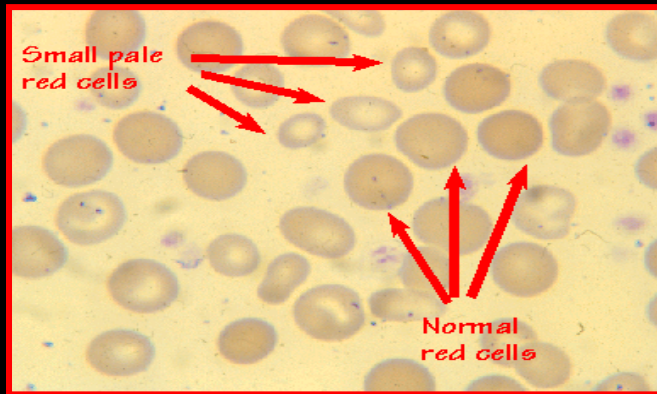
Allarme "Blasti"
62% dei casi



Piastrinopenia
64% dei casi

RARS

Morfologia del Sangue Periferico

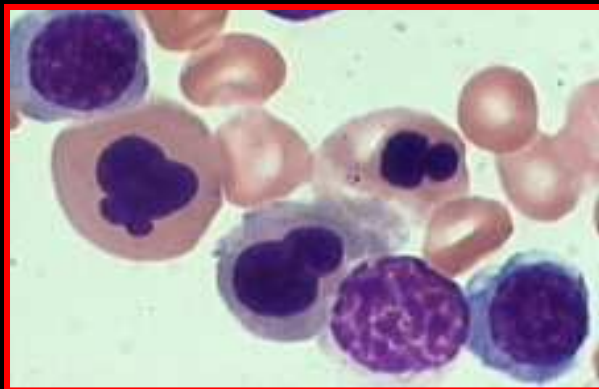


“pattern dimorfico allo striscio di sangue periferico”:

- ***maggioranza dei globuli rossi normocromici***
- ***2a popolazione ipocromica”***

RARS

Morfologia Midollare (oltre ai sideroblasti)



“diseritropoiesi con anomalità nucleari (alterazioni megaloblastoidi)”

h

[haematologica]

2004;89:1446-1453

Hypochromic red blood cells in low-risk myelodysplastic syndromes: effects of treatment with hemopoietic growth factors

THERESE LJUNG
RUNE BÄCK
EVA HELLSTRÖM-LINDBERG

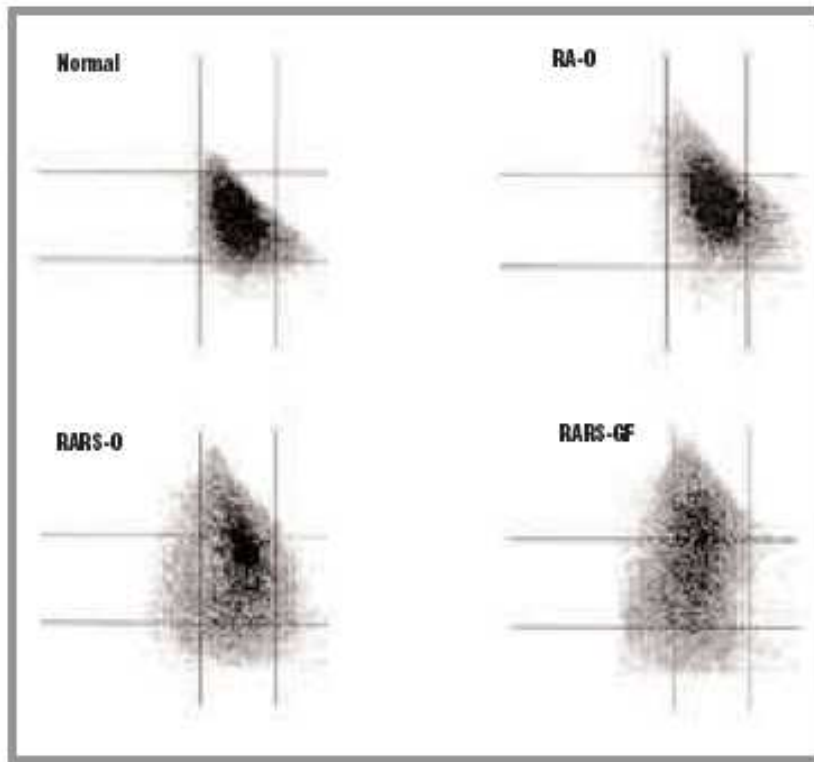


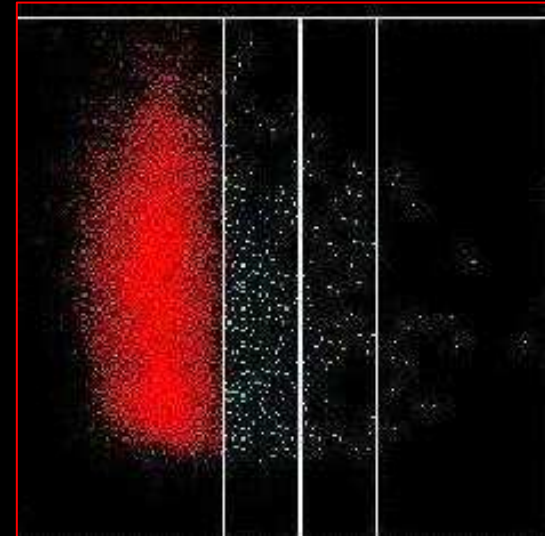
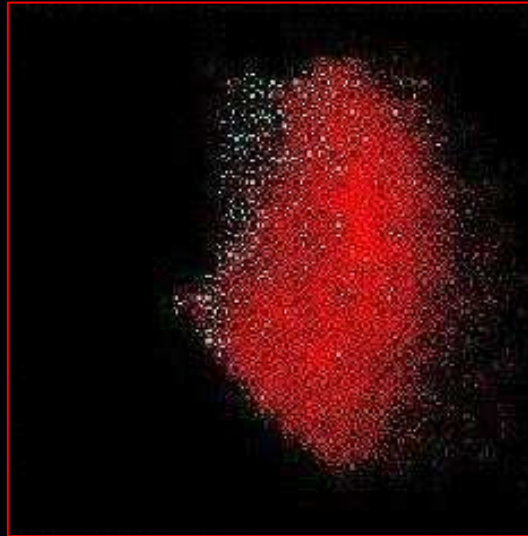
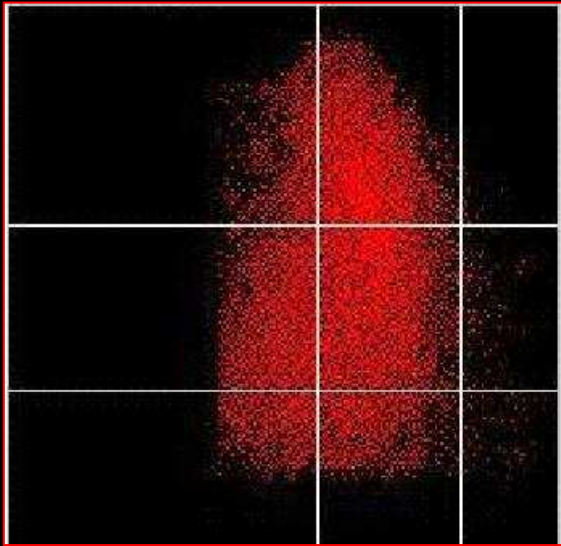
Figure 2. Scattergrams showing the erythrocyte population of one normal control, one RA-O, one RARS-O and one RARS-GF. Normal: normal control; RA-O: RA patient with no treatment; RARS-O: RARS patient with no treatment; RARS-GF: RARS patient treated with growth factors (EPO±G-CSF).

*“gli eritrociti e i reticolociti dei pazienti RARS non trattati mostrano un’**umentata percentuale di cellule con ridotta concentrazione emoglobinica**”*

*“lo scattergramma eritrocitario da pazienti con RARS mostra un’**umentata percentuale d cellule ipocromiche che, in combinazione con adeguati depositi di ferro, potrebbe essere usato come marker diagnostico**”.*

*“La maggioranza dei pazienti RA non hanno un’**umentata percentuale di cellule ipocromiche, ma è molto interessante che quei pochi pazienti RA che hanno questo pattern hanno delle anomalità cromosomiche, come la monosomia 7 e la trisomia 8**”.*

5° pattern-algoritmo

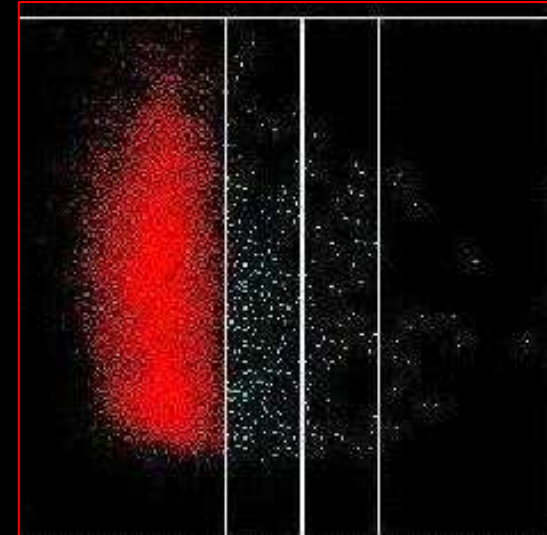
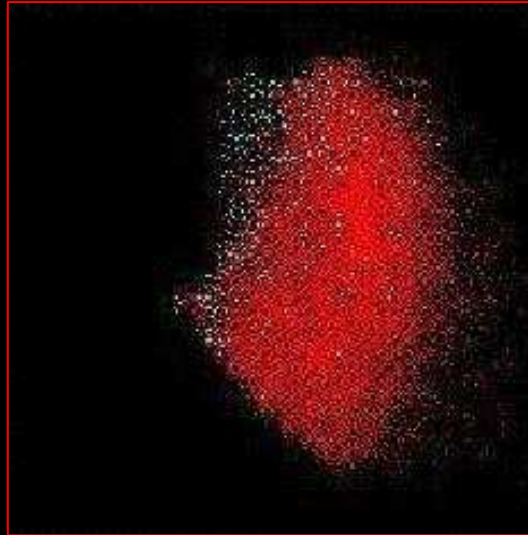
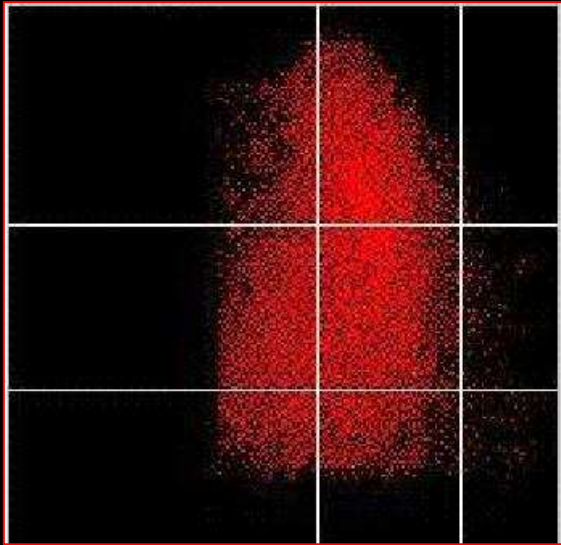


Il citogramma V-HC presenta almeno 2 popolazioni molto evidenti:

- **Macrocitica con lieve ipocromia**
- **Microcitica-Ipocromica**

che si traduce anche in dati numerici caratteristici:

MCV	RDW	HDW	CHDW	% IPO	MCVr	CHCMr	RDWr	HDWr	CHDWr
↑	↑↑↑↑	↑↑	↑↑↑	↑↑	↑	↓↓	↑↑↑	↑↑	↑↑↑



Due popolazioni di globuli rossi che derivano da 2 diversi tipi di eritropoiesi:

- 1. Megaloblastoide**
- 2. Da carente apporto di Ferro.**



Nocera Inferiore



Benevento



Palermo



Reggio Emilia



Bologna



Lavagna

3 diversi laboratori hanno provveduto a raccogliere i file da 5 diversi analizzatori di:

- 600 soggetti sani non pediatrici
- 150 soggetti sani di età superiore a 65 anni

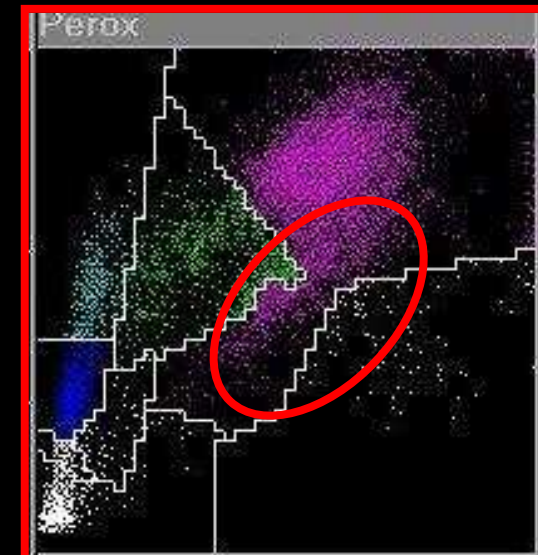
	Neutro X (media)	Neutro Y (media)
Soggetti sani non pediatrici	62,14	72,66
Soggetti sani > 65 anni	63,15	71,81
	n.s.	n.s.

Nessuno dei campioni di soggetti sani esaminati ha mostrato un positività agli algoritmi per “displasia”.

Stabilità nel tempo dei parametri utilizzati per gli algoritmi

Un laboratorio ha provveduto all'esame di 20 campioni normali conservati a temperatura ambiente e di altri 20 campioni normali conservati a 4°C ai tempi 0, 6, 10 e 24 h.

Non sono state rilevate variazioni significative dei parametri controllati eccetto per la percentuale della popolazione di **globuli rossi macrociti e ipocromici** per le quali si hanno variazioni significative a 10 ore dal primo test.



Nocera Inferiore

Benevento

Palermo

80.000 file (raw-data)

**Routine e Urgenza
Ematologica**

Reggio Emilia

Bologna

Lavagna

Nocera Inferiore

Benevento

Palermo

1° lab	0,9%
2° lab I analizzatore	1,4%
2° lab II analizzatore	1,7%
3° lab	1,8%
4° lab I analizzatori	1,9%
4° lab II analizzatore	2,1%
5° lab	2,3%
6° lab I analizzatore	2,5%
6° lab II analizzatore	2,5%
media	1,9%

1,9%

Reggio Emilia

Bologna

Lavagna

Nocera Inferiore

Benevento

Palermo

Dopo riesame dei raw-data su un
unico analizzatore:

Allarme “*displasia*” **1,6%**
(1280/80.000)

Reggio Emilia

Bologna

Lavagna

Nocera Inferiore

Benevento

Palermo

Allarme "*displasia*" 1,6% (1280/80.000)

756/1280 con allarme "*blasti*"

Aumento del carico di lavoro morfologico = **0,7%**

Reggio Emilia

Bologna

Lavagna

Nocera Inferiore

Benevento

Palermo

Controllo delle Diagnosi sui campioni “allarmati”:

MDS primitive: 499/1280 (39% dei positivi)

Non MDS primitive: 781/1280 (61% dei positivi) in gran parte “oncologici”

Reggio Emilia

Bologna

Lavagna

828 campioni di 244 pazienti con diagnosi di MDS primitiva

Sottotipi MDS	n.
AR	78
ARSA	123
RCMD	127
RCMD-RS	44
AREB 1 e 2	201
Sindrome 5q-	21
MDS-U	84
CMML	150

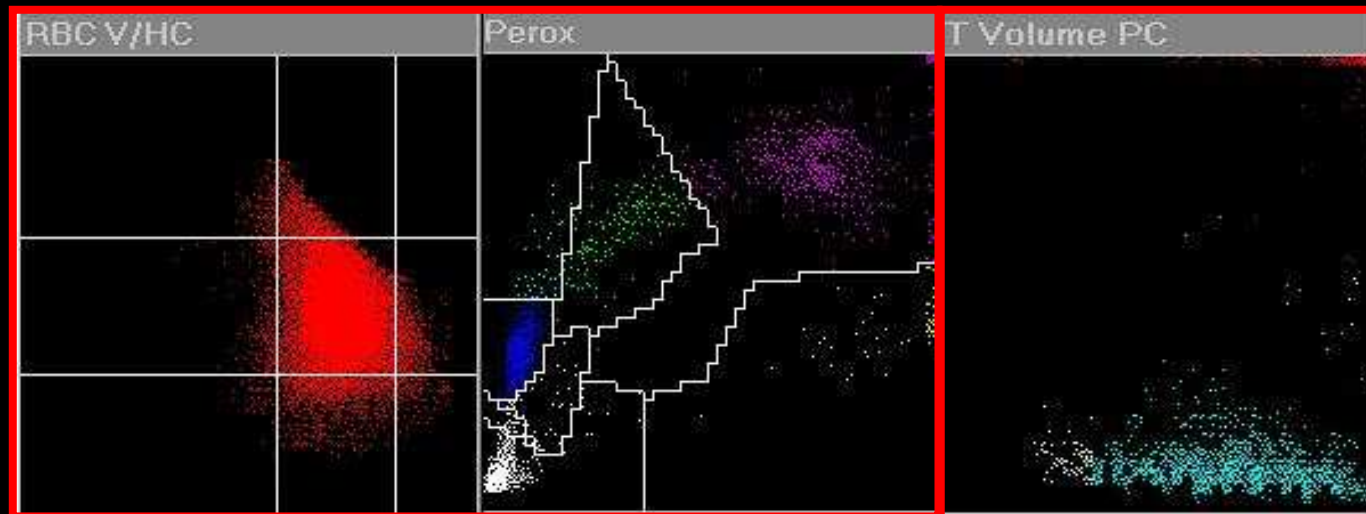
Veri Positivi = 83%

L'analisi dei file (raw-data) di 1256 campioni di pazienti con malattia ematologica non MDS ha fornito come risultato 78 positivi (6,2%).

AML	37/68
ALL	2/8
CLL	2/240
IDA trasfusa	1
Deficit vitamine	5/14
Anemia emolitica acuta	1/13
MCL-B atipica	7/77
Mielofibrosi	10/23
Meloproliferativa	5/69
CMLa	2/10
Mieloma	4/21
Mononucleosi con def perox	1
Iposplenismo	1/13

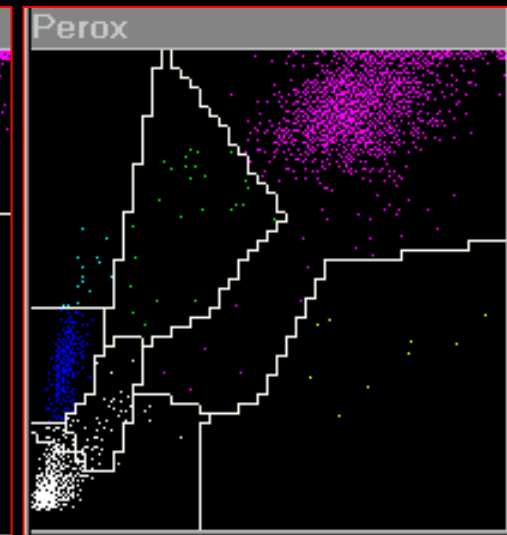
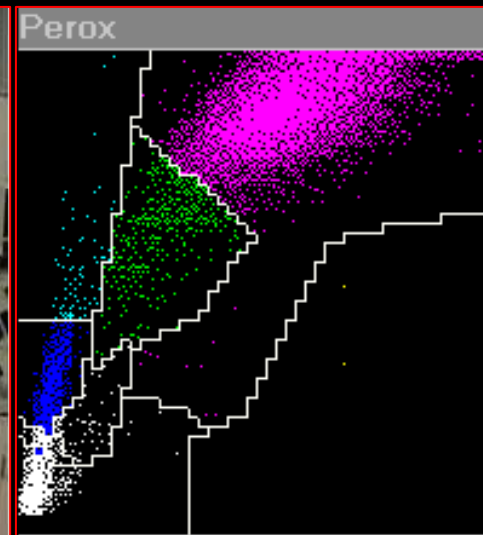
File di 400 campioni di pazienti provenienti dal Reparto di Oncologia o dal DH Oncologico

30 di questi campioni hanno dato un risultato positivo all'allarme "displasia" con un percentuale di positività (9,5%) significativamente più elevata dei campioni della routine ematologica (1,6%).



40 campioni di pazienti con Sepsis o SIRS provenienti da 2 Reparti di Rianimazione.

Nessuno dei 40 campioni esaminati ha fatto rilevare un allarme “displasia” secondo gli algoritmi utilizzati.



**È utile un allarme
“*displasia*” su ADVIA
2120?**

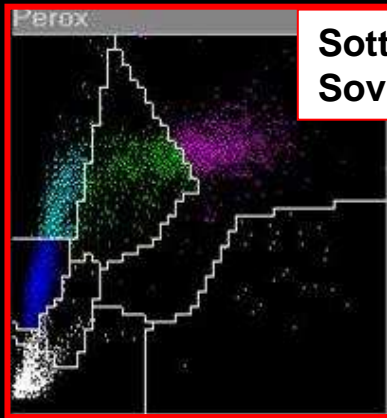
Quality counts: new parameters in blood cell counting

C. BRIGGS

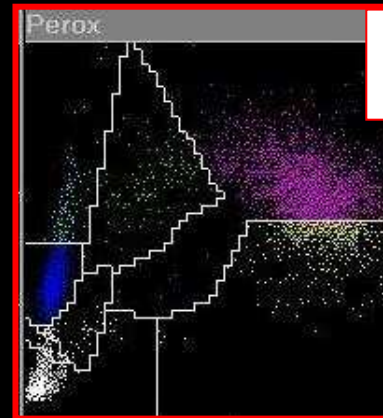
*Currently, there are **no accredited EQAS** for these parameters.*

*For a number of parameters, on some instruments, there is **no Internal Quality Control**, which brings into question whether these parameters should be used for a clinical decision making*

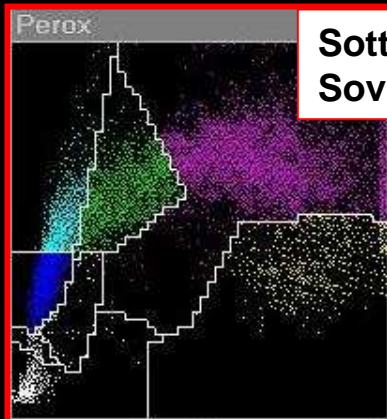
Controllo Microscopico di campioni con dati non precisi e accurati



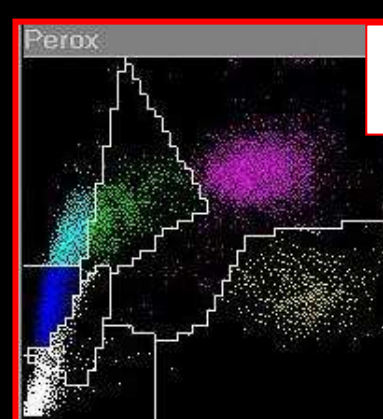
Sottostima dei Neutrofili
Sovrastima dei Monociti



Sottostima dei Neutrofili
Sovrastima degli Eosinofili



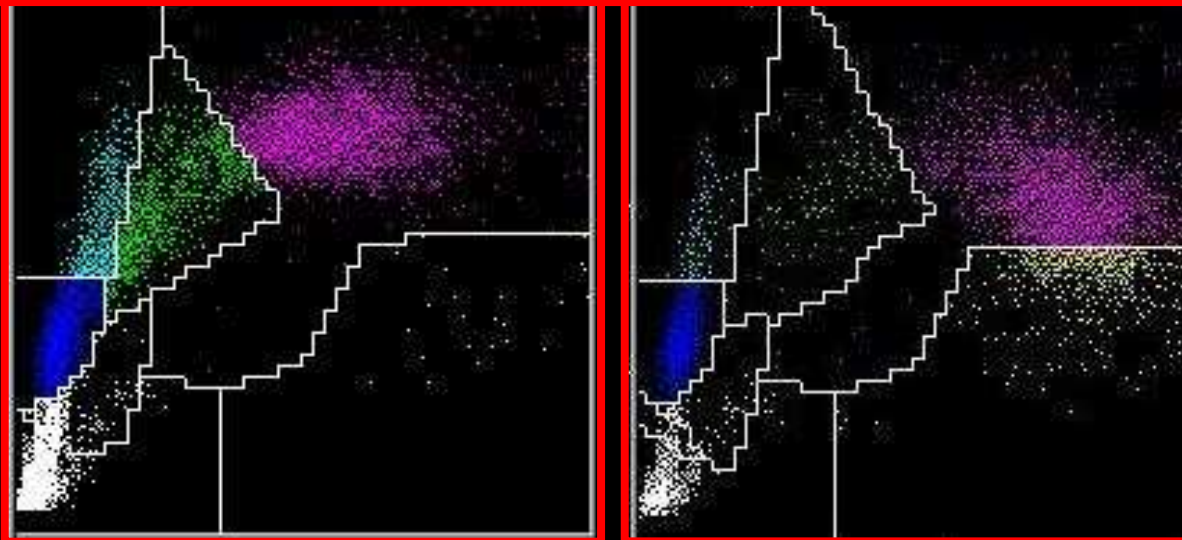
Sottostima dei Monociti
Sovrastima dei Neutrofili

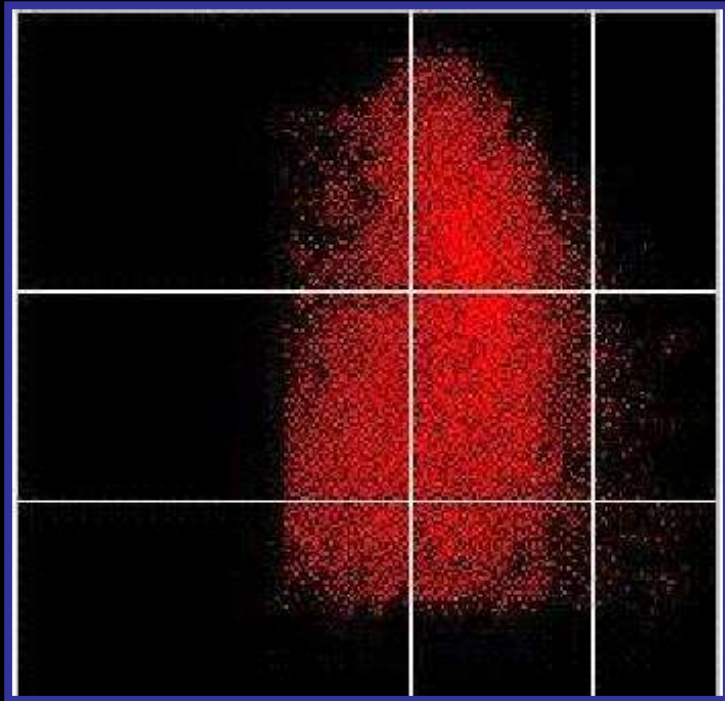


Sottostima dei
Monociti

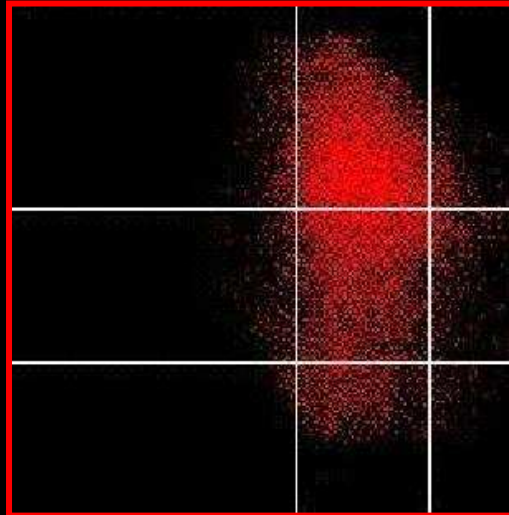
Sottotipi MDS	n.	Falsi Negativi
AR	78	51 (65,3%)
ARSA	123	8 (6,5%)
RCMD	127	13 (10,2%)
RCMD-RS	44	3 (6,8%)
AREB 1 e 2	201	16 (7,9%)
Sindrome 5q-	21	18 (85,7%)
MDS-U	84	7 (8,3%)
CMML	150	24 (16%)

È possibile che questa tecnologia, basata sul contenuto di perossidasi dei WBC, abbia una **sensibilità maggiore** rispetto alla morfologia classica **per le alterazioni displastiche**?

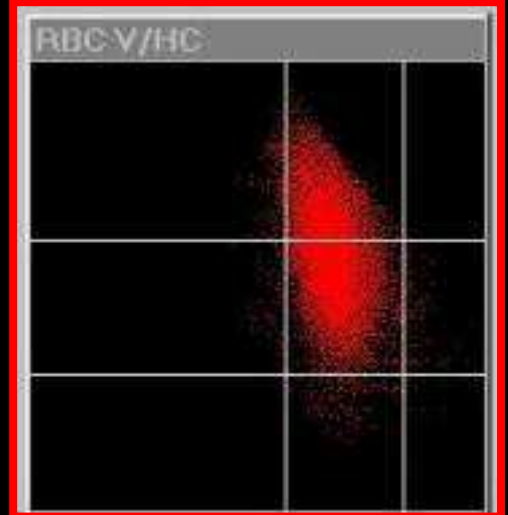




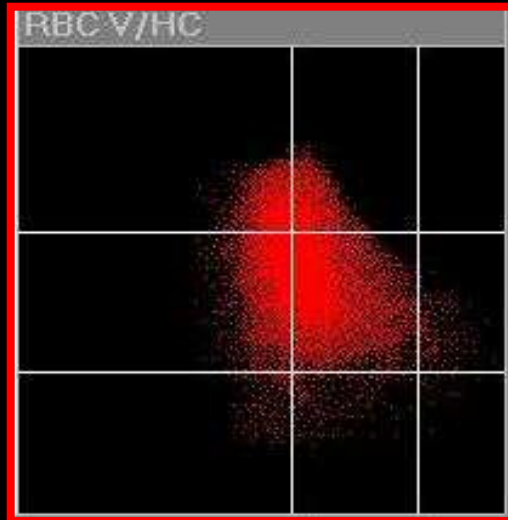
SARS



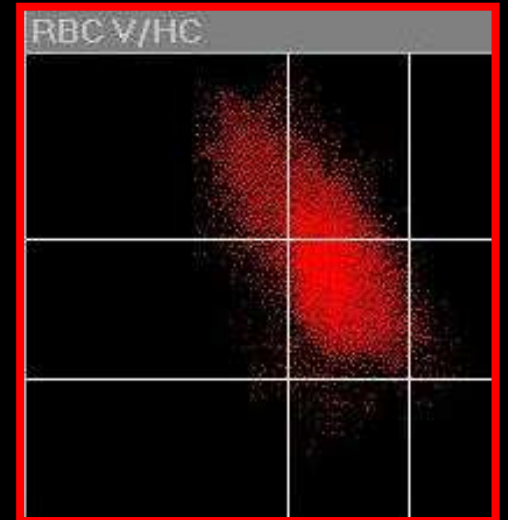
Megaloblastica



Target-cell



Stomatociti



Reticolocitosi

Table 2. Proposal for updated WHO classification of myelodysplastic/myeloproliferative neoplasms.

Disease	Blood findings	Bone marrow findings
Chronic myelomonocytic leukemia (CMML)	Peripheral blood monocytosis $> 1 \times 10^9/L$ No BCR/ABL-1 fusion gene < 20% blasts	Dysplasia in one or more myeloid lineage* < 20% blasts. Blasts include myeloblasts, monoblasts and promonocytes. No rearrangement of <i>PDGFRA</i> or <i>PDGFRB</i>
Atypical chronic myeloid leukemia, BCR-ABL1 negative (aCML)	Leukocytosis, Neutrophilia Neutrophilic dysplasia Neutrophil precursors $\geq 10\%$ of leukocytes Blasts < 20% No BCR-ABL1 fusion gene No rearrangement of <i>PDGFRA</i> or <i>PDGFRB</i> Minimal basophilia Monocytes < 10% of leukocytes	Neutrophil dysplasia with or without other dysplastic lineages < 20% blasts
Juvenile myelomonocytic leukemia (JMML)	Peripheral blood monocytosis $> 1 \times 10^9/L$ < 20% blasts Usually WBC $> 10 \times 10^9/L$ < 20% blasts	< 20% blasts. Blasts include myeloblasts, monoblasts, and promonocytes.

Refractory anemia with marked sideroblasts associated with marked thrombocytosis (RARS-T**) (provisional entity)**

Persistent Thrombocytosis $> 450 \times 10^9/L$

Anemia

BCR<ABL1 negative

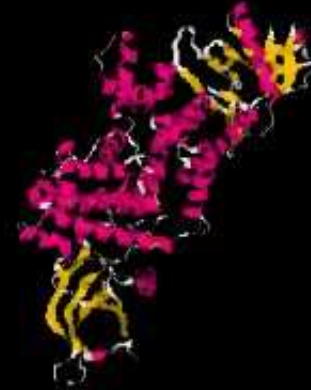
Cases with t(3;3)(q21;q26) and isolated 5q- are excluded

* If myelodysplasia is minimal or absent, CMML can still be diagnosed if the other requirements are met and there is an acquired clonal cytogenetic or molecular genetic abnormality present in the hematopoietic cells, or the monocytosis has persisted for at least 3 months and all other causes of monocytosis have been excluded.

† Provisional entity. In WHO classification from 2002 the cut-off for platelet count was $600 \times 10^9/L$. In the WHO 2008 classification the cut-off value is $450 \times 10^9/L$, to be in line with the classification of essential thrombocythemia. Around 50% of cases with RARS-T carry the JAK2 mutation, but this is not a diagnostic criterion.



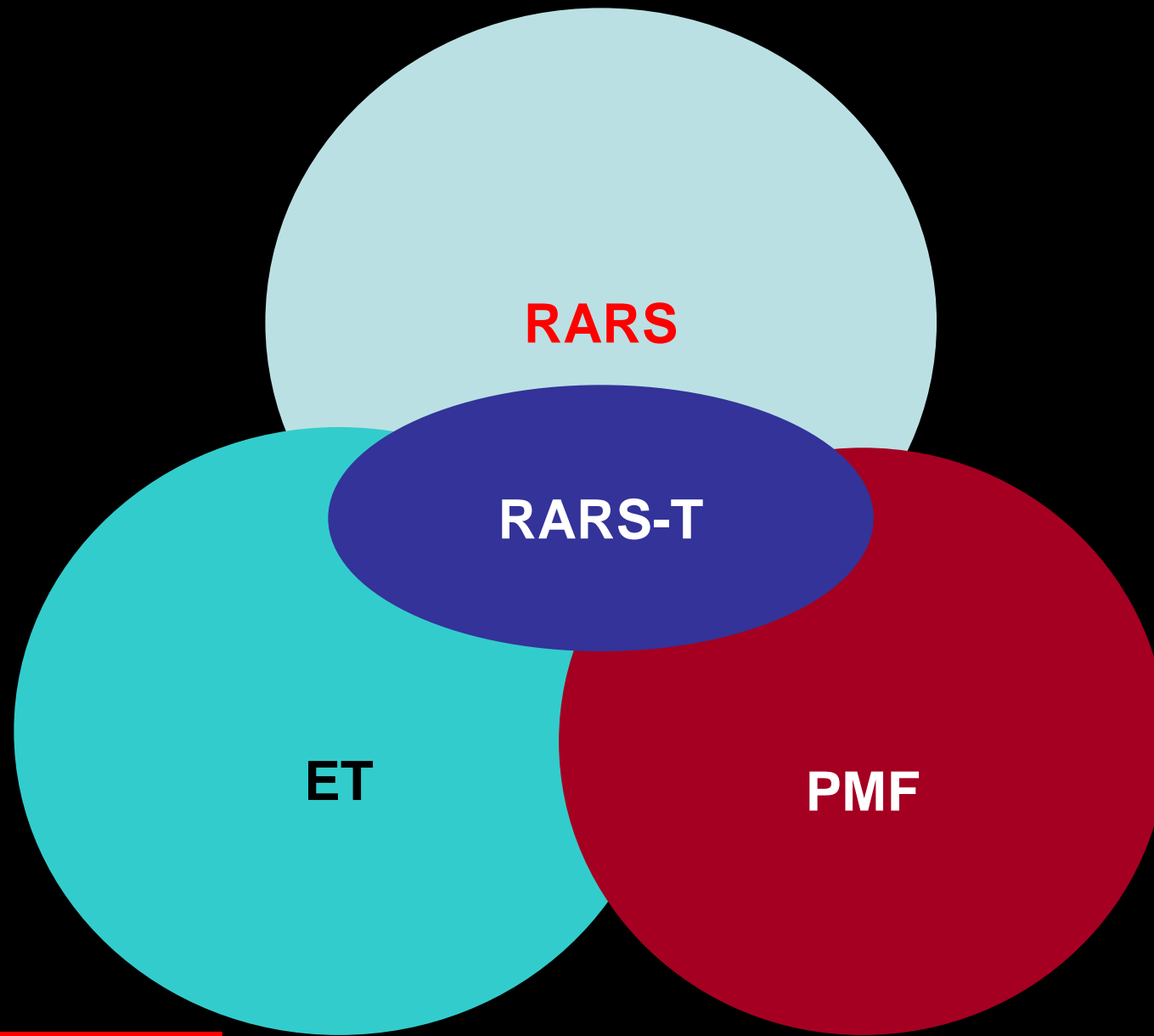
JH1: Kinase domain
 JH2: Pseudokinase domain
 JH7-4: FERM domain
 JH4-3: SH2 domain



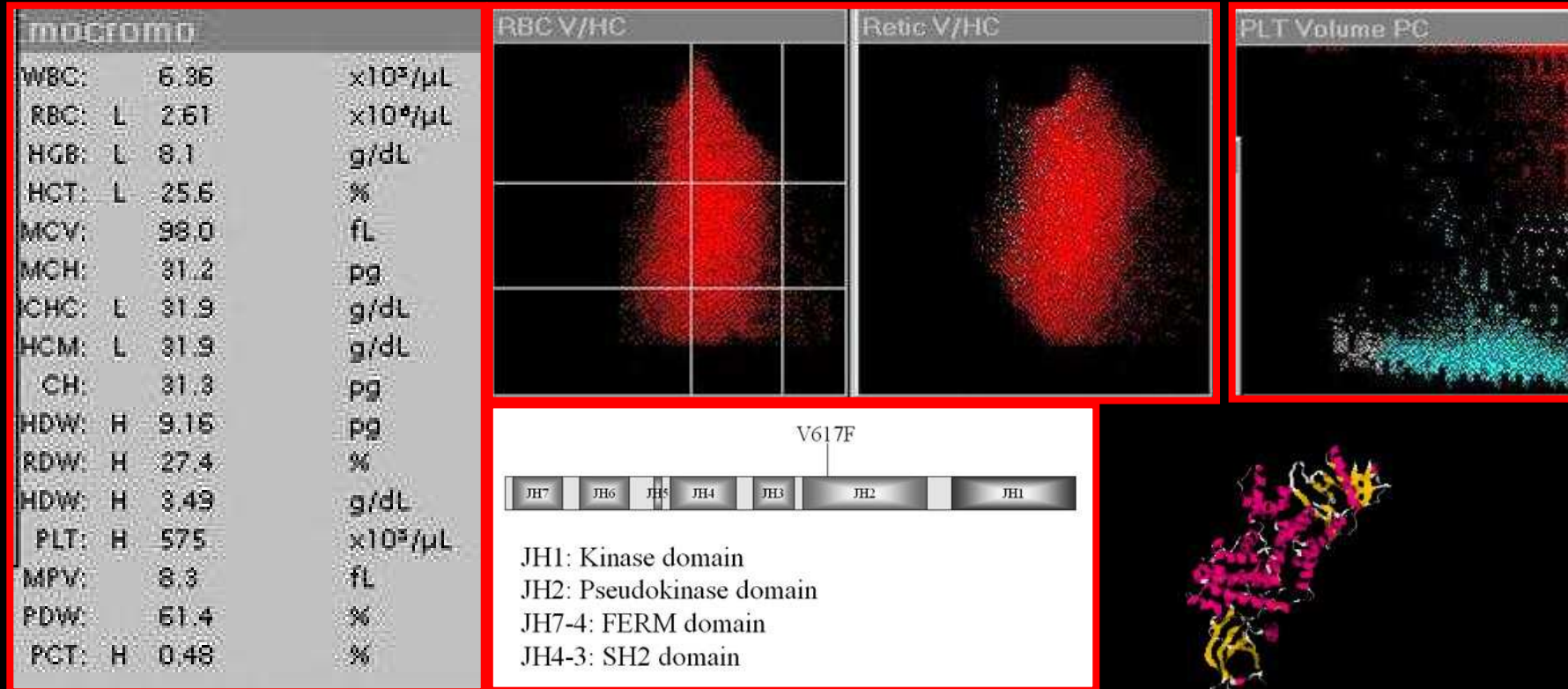
La mutazione JAK2-V617F è presente:

- in ~95% con Policitemia Vera;
- In circa il 50% dei pazienti con ET e Mielofibrosi Primitiva,
- solo ad una bassa frequenza nelle MDS (fra 52 pazienti con SARS la mutazione è stata rilevata solo in 2 casi).

Recentemente, la mutazione JAK2 è stata trovata in 6/9, 5/16, 3/3, 4/6 e 5/7 pazienti con RARS-T, suggerendo che una ampia percentuale dei casi RARS-T sono disordini correlati alla mutazione JAK2.



Malcovati e Coll.



Il rilevamento di questo pattern/algoritmo può far prevedere la:

- **RARS**
- **RARS-T**
- **mutazione JAK-2**

I monociti tra reattività e displasia

RIMeL / IJLaM 2008; 4 (Suppl.)

G. Da Rin

Dipartimento di Medicina dei Servizi, S.C. Medicina di Laboratorio, ASL n. 3, Bassano del Grappa (VI)

Flow Cytometric Analysis of Monocytes as a Tool for Distinguishing Chronic Myelomonocytic Leukemia From Reactive Monocytosis

Am J Clin Pathol 2005;124:799-806

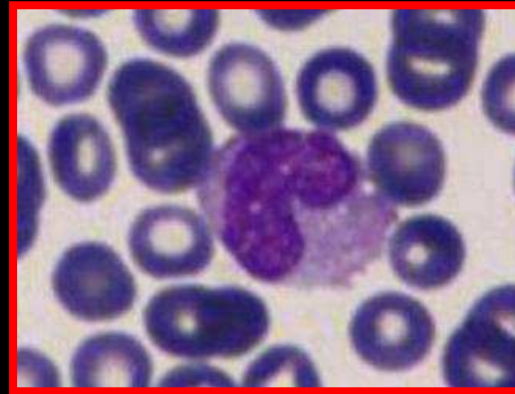
*Yin Xu, MD, PhD, Robert W. McKenna, MD, Nitin J. Karandikar, MD, PhD, Alex J. Pildain, MD,
and Steven H. Kroft, MD*

Anche nella CMML, eccetto nei casi con notevole displasia o immaturità, ci sono delle difficoltà nel rilevare le alterazioni morfologiche dei monociti.

Citando **D'Onofrio e Zini**:

“l'aspetto dei monociti spesso non è diverso da quello normale.....”

- dimensioni maggiori
- nucleo di forma irregolare, spesso iperlobulato
- anomalie delle granulazioni e del citoplasma



original paper

Haematologica 1997; 82:25-30

*Advances in the Cytobiology of Leukemias**

MYELODYSPLASTIC SYNDROMES WITH MONOCYTIC COMPONENT: HEMATOLOGIC AND CYTOGENETIC CHARACTERIZATION

GIAN MATTEO RIGOLIN, ANTONIO CUNEO, MARIA GRAZIA ROBERTI, ANTONELLA BARDI,
GIANLUIGI CASTOLDI

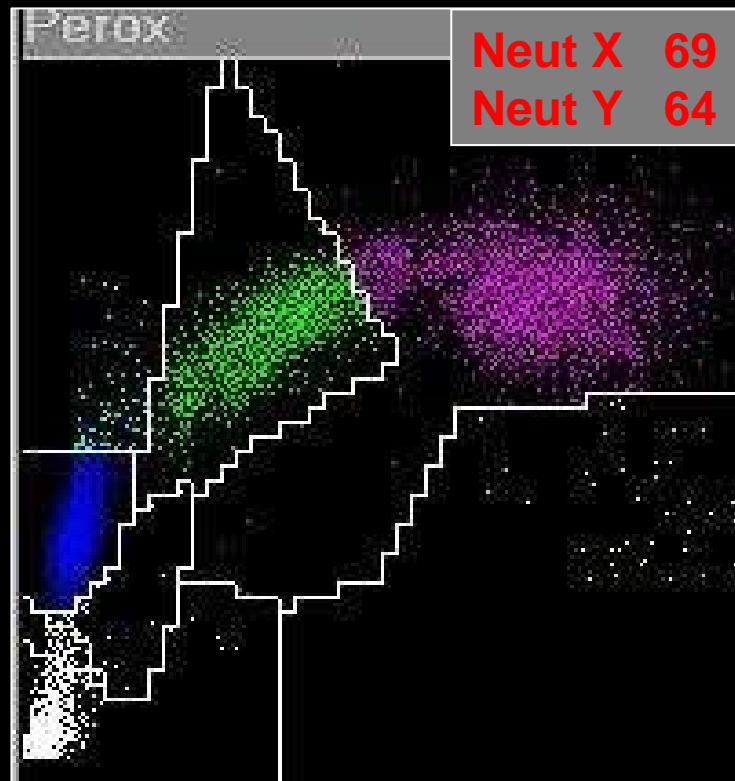
Dipartimento di Scienze Biomediche e Terapie Avanzate, Sezione di Ematologia, Università di Ferrara, Italy

Conclusion e Perspectives.

“..... relative or absolute increase in the number of monocytes may represent the first event in the subsequent progression of the disease towards acute leukemia”.

Emocromo		
WBC:	5,72	$\times 10^3/\mu\text{L}$
RBC:	5,46	$\times 10^6/\mu\text{L}$
HGB: L	11,7	g/dL
HCT: L	36,3	%
MCV: L	66,4	fL
MCH: L	21,5	pg
MCHC:	32,4	g/dL
CHCM: L	32,8	g/dL
CH:	21,6	pg
CHDW: H	2,67	pg
RDW: H	14,5	%
HDW:	2,81	g/dL
PLT:	166	$\times 10^3/\mu\text{L}$

Formula WBC		
	%	$\times 10^3/\mu\text{L}$
WBC:		5,72
Neut: L	35,4	2,02
Linfo:	38,7	2,21
Mono: H	22,8	1,30
Eos:	0,8	0,05
Baso:	0,4	0,02
LUC:	1,9	0,11



Collega di anni 56

Monocitosi da 1 anno. Assenza di altre patologie rilevanti.

Assenza di Blasti e presenza di qualche Monocita “atipico” allo striscio di sangue periferico.

Viene posto il sospetto di CMML confermata su Midollo in un Centro Ematologico di Riferimento (tra l’altro Monociti Midollari CD56+, citogenetica negativa).

Table 4. Reported series of CMML

	Onida	Catalano	Groupe de Fran- cais	Fenaux
Date	1966-1999	1982-1993	1984-1988	1971-1986
N	213	77	100	107
Male:female	2.4:1	2.3:1	1.8:1	2.1:1
Median age (range)	65 (20-88)	71 (21-83)	73.5	70.3 (23-89)
Hb (g/dL)	10.2 (5.2-15.6)	8.8 (3-14.2)	10.9	
WBC $\times 10^9/L$	20.4 (2.1-35.2)	18.5	15.6	10.3 (2.9-180)
PLT $\times 10^9/L$	46 (3-81)	90 (1-980)	136.5	120
Mono $\times 10^9/L$	4.2 (1-162)	5 (0-30%)	3.63	2.6 (1-43)
BM blast %	4 (0-19)	5 (0-30%)	NS	
LDH	783 (270-5310)	370 (116-3300)	NS	
Polyclonal HG	NS	66%		63%
Splenomegaly	29%	54.5%	40.5%	25%
Hepatomegaly	18.9%	77%	30%	16%
Lymph nodes	13.9%	13%	14.3%	
Skin/gingival	NS	1.3%	4%/2%	
Serositis	NS	5.6%		
Fever	NS	13%	19.8%	
Night sweats	NS	NS	14.4%	

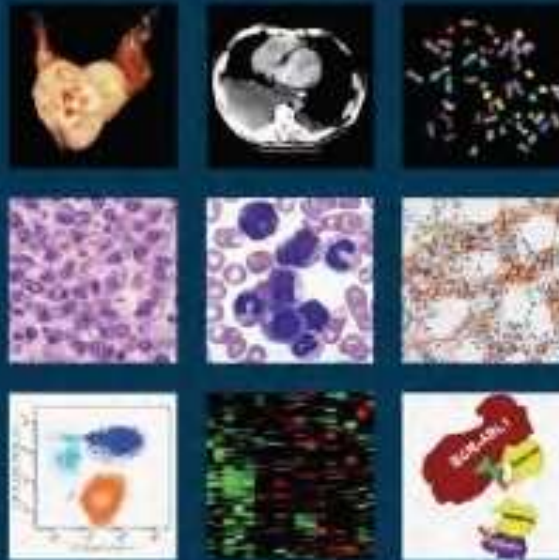
Median survival 12 (0-154) 17 (2-108) 36 30 (1-81)
(range)

AML evolution 19% 14% 26% 17%

AML, acute myeloid leukemia; BM, bone marrow; c/s, cytogenetics; Hb, hemoglobin; HG, hemoglobin; LDH, lactate dehydrogenase; Mono, monocyte; PLT, platelets; WBC, white blood cell.

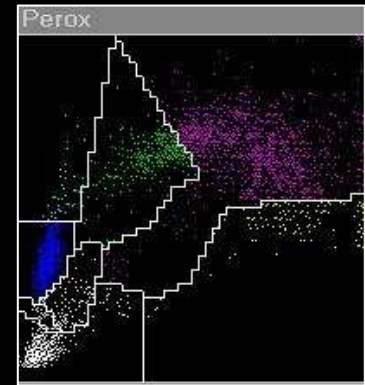
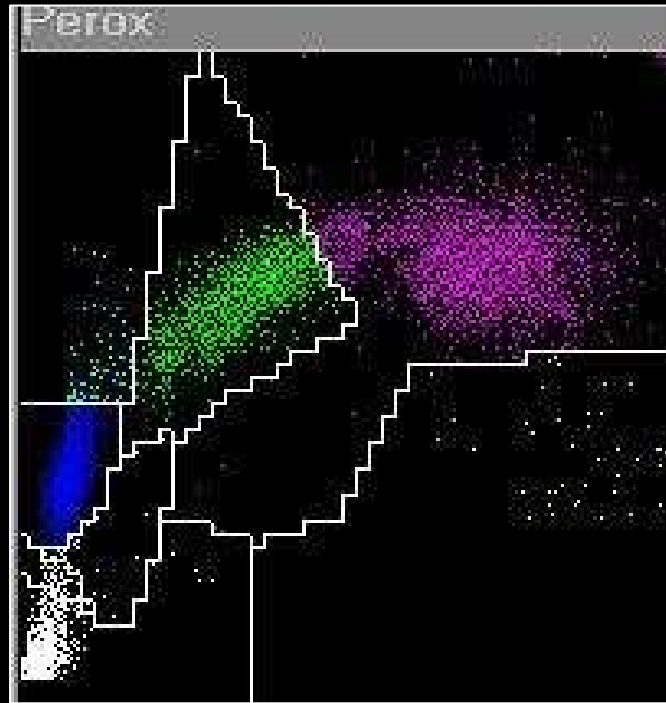
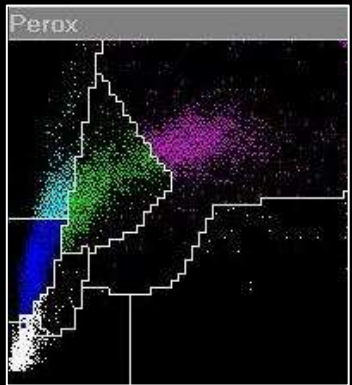
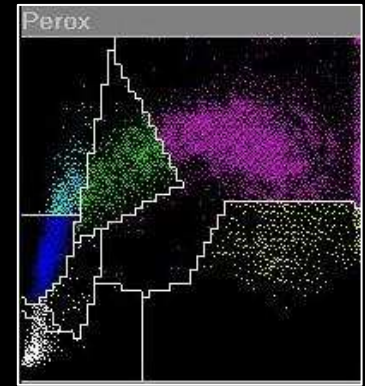
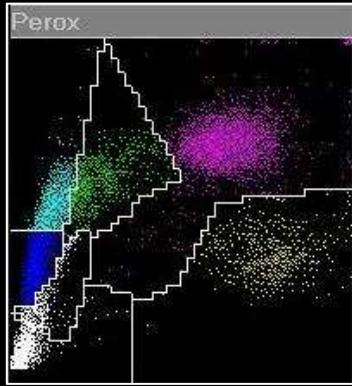
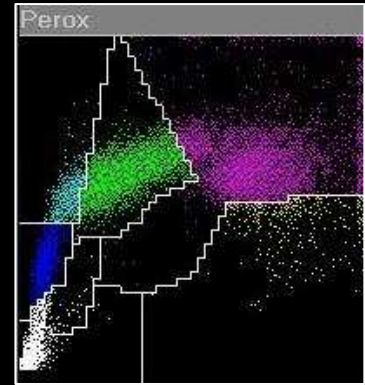
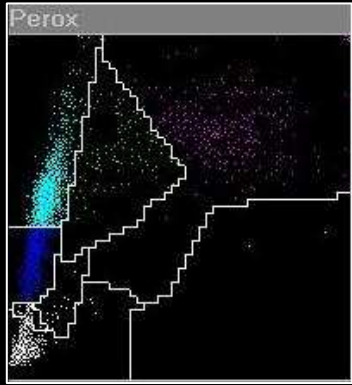
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Edited by Steven H. Swerdlow, Elias Campo, Nancy Lin Harris, Elaine S. Jaffe,
Suzanne A. Pileri, Harald Stein, Jürgen Thiele, James W. Wright



WHO

*Survival of patients with CMML is reported to vary from **1** to more than **100** months, but the median survival time in most series is **20-40** months.*



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