

The background of the slide is a microscopic image of a blood smear. It shows numerous white blood cells (leukocytes) with large, dark purple nuclei and varying amounts of light purple cytoplasm. Some cells have prominent nucleoli. The background is a light pinkish-purple color.

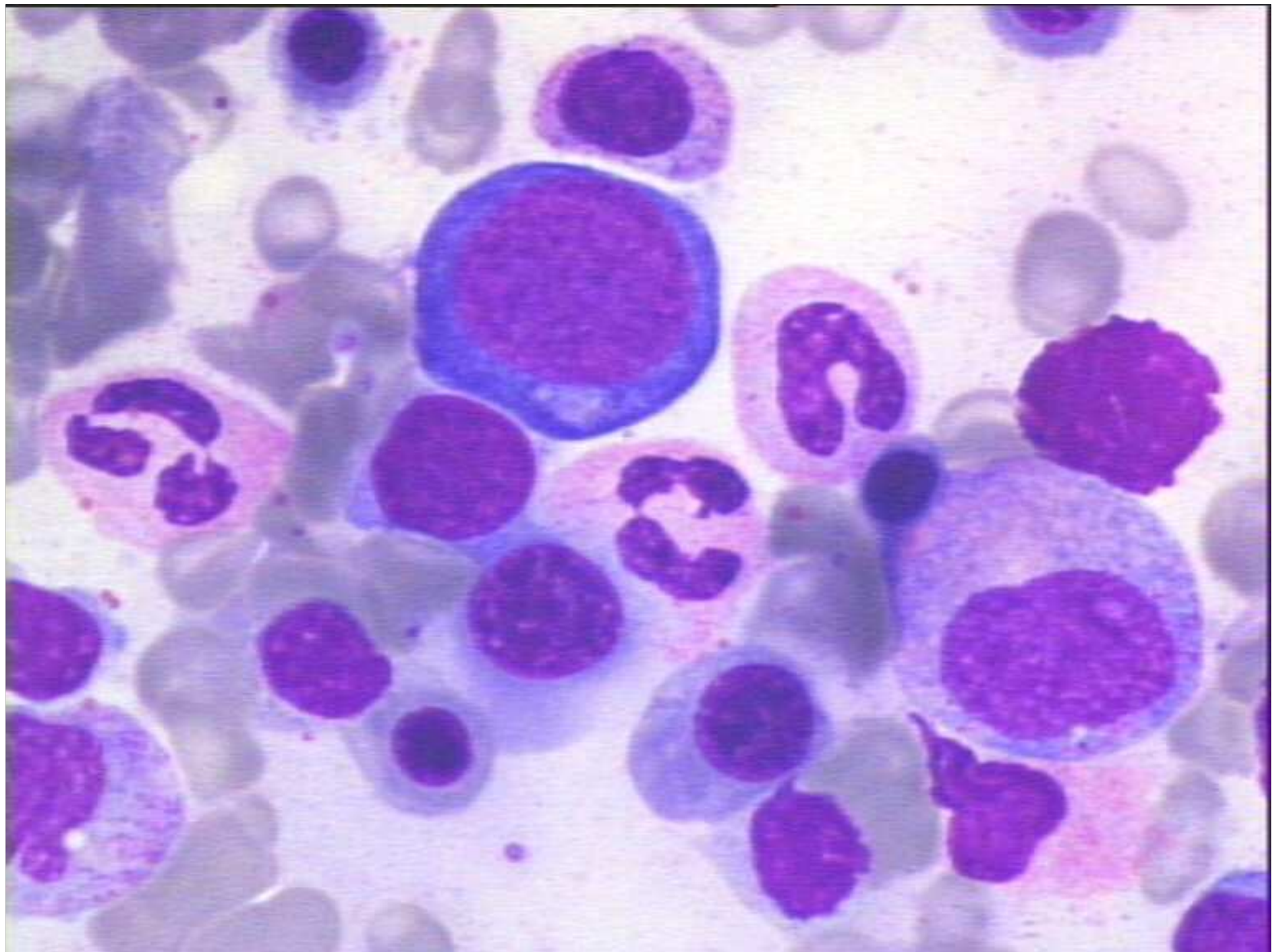
Milano 11 novembre 2010
Corso di aggiornamento in
Ematologia di Laboratorio

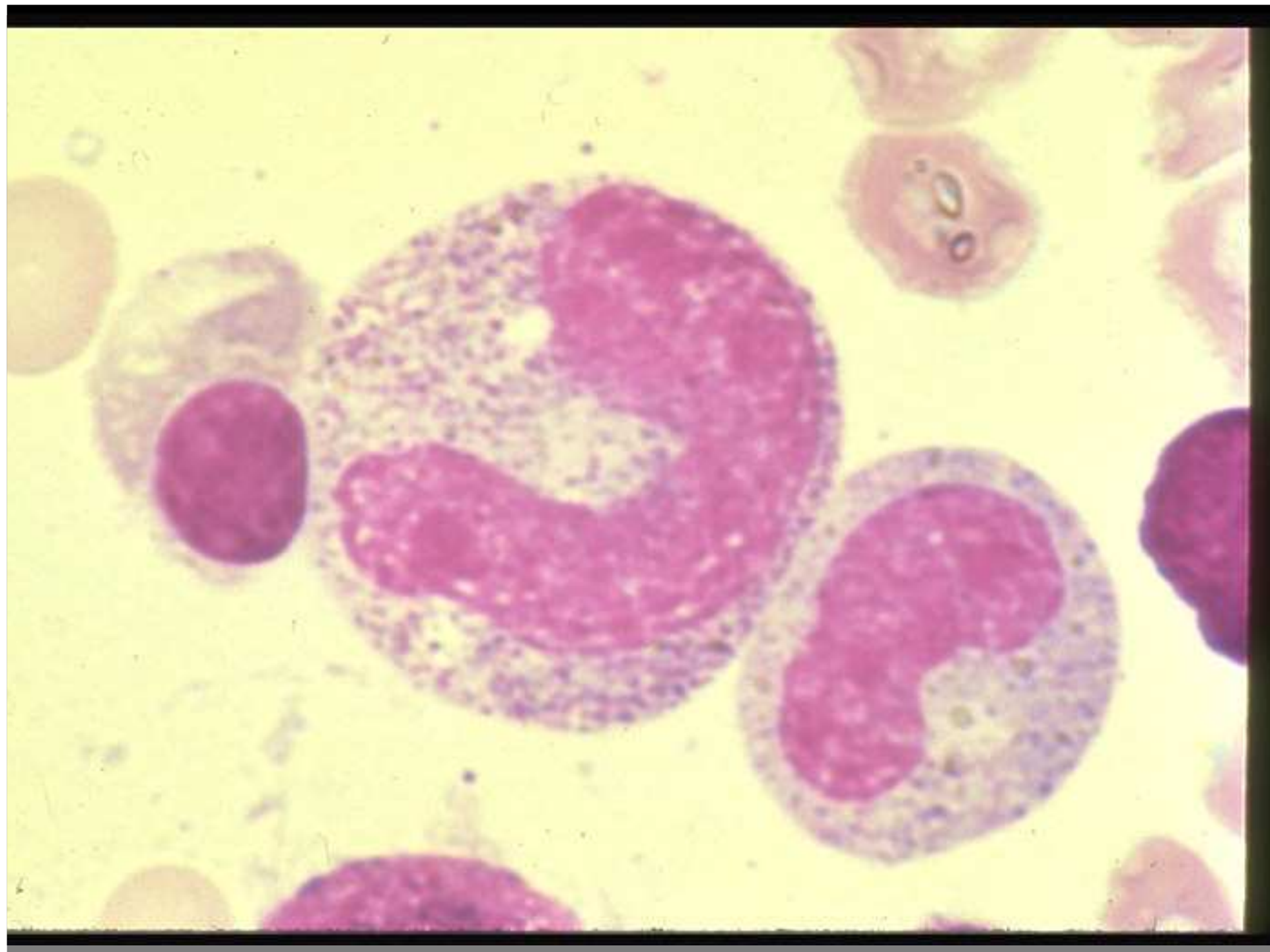
**Formula leucocitaria in
ematologia automatizzata**

Diagnosi citomorfologica

Caratteri generali

- dimensioni
- forma
- rapporto nucleo-citoplasmatico
- ordine
- sincronismo maturativo
- contesto citologico





Nucleo (1)

- forma
- posizione
- profilo
- segmentazione
- numero

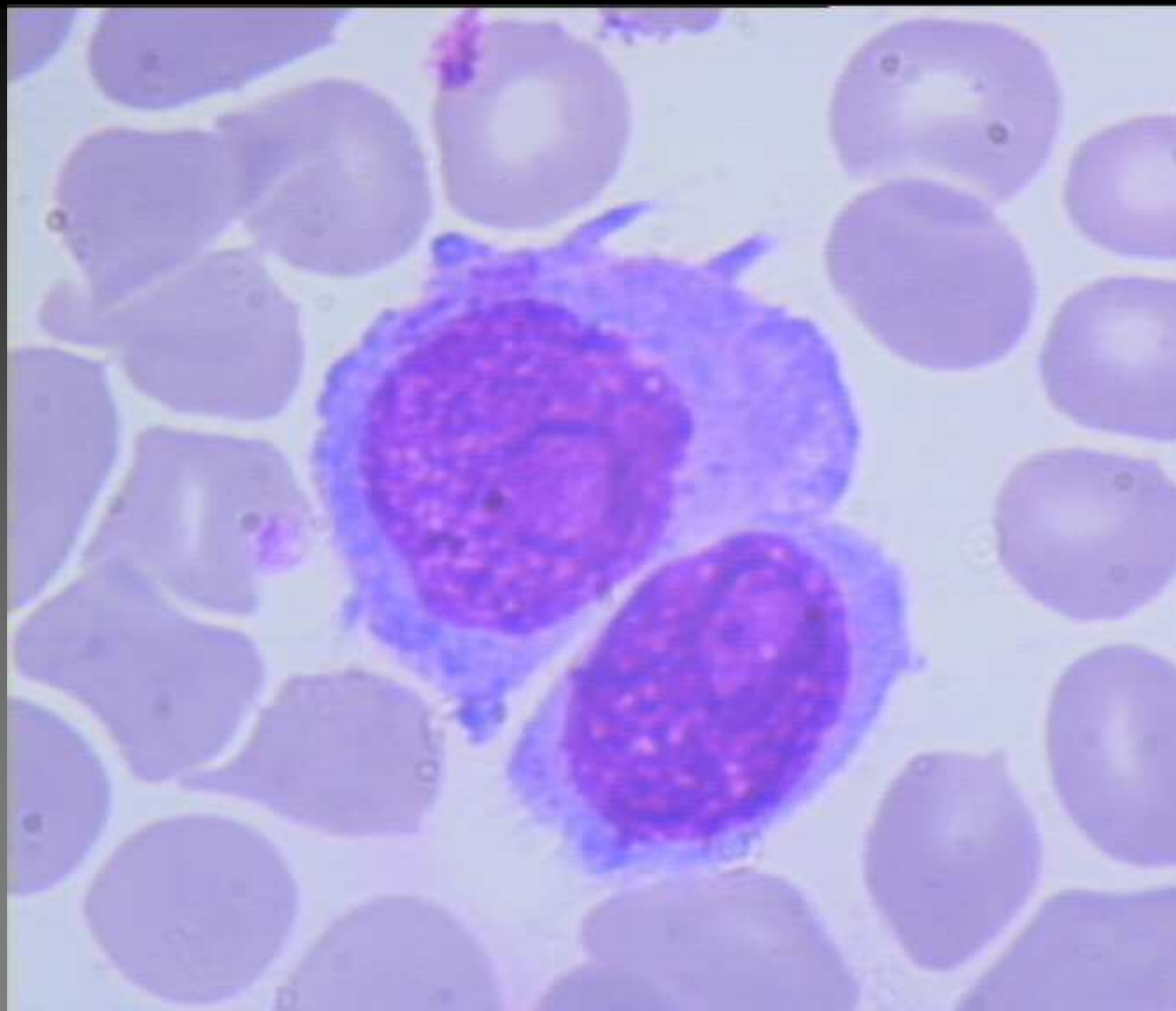
Nucleo (2): la cromatina

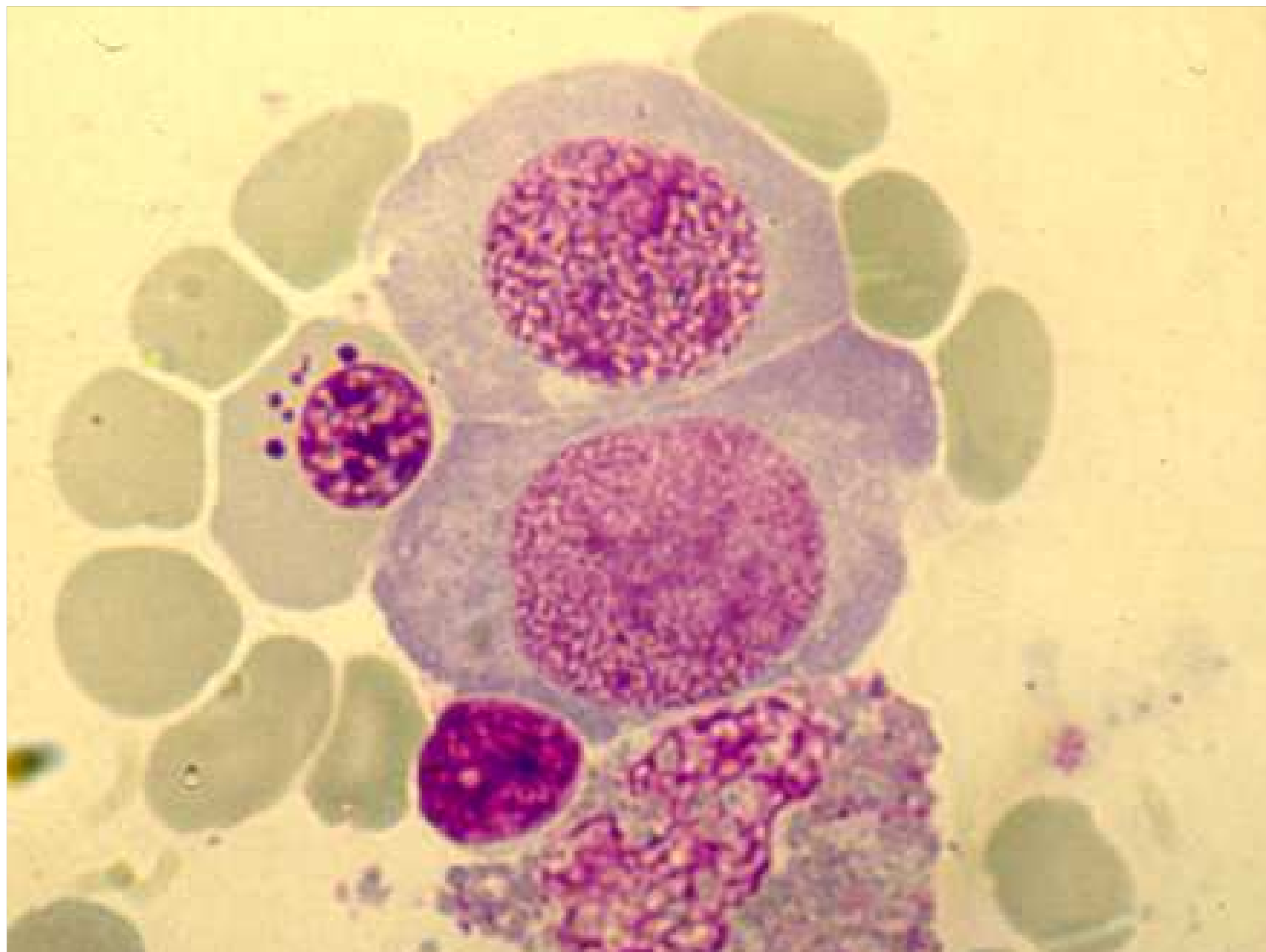
- condensazione
- omogeneità

Nucleo (3): i nucleoli

- prominenza
- numero
- rinforzo



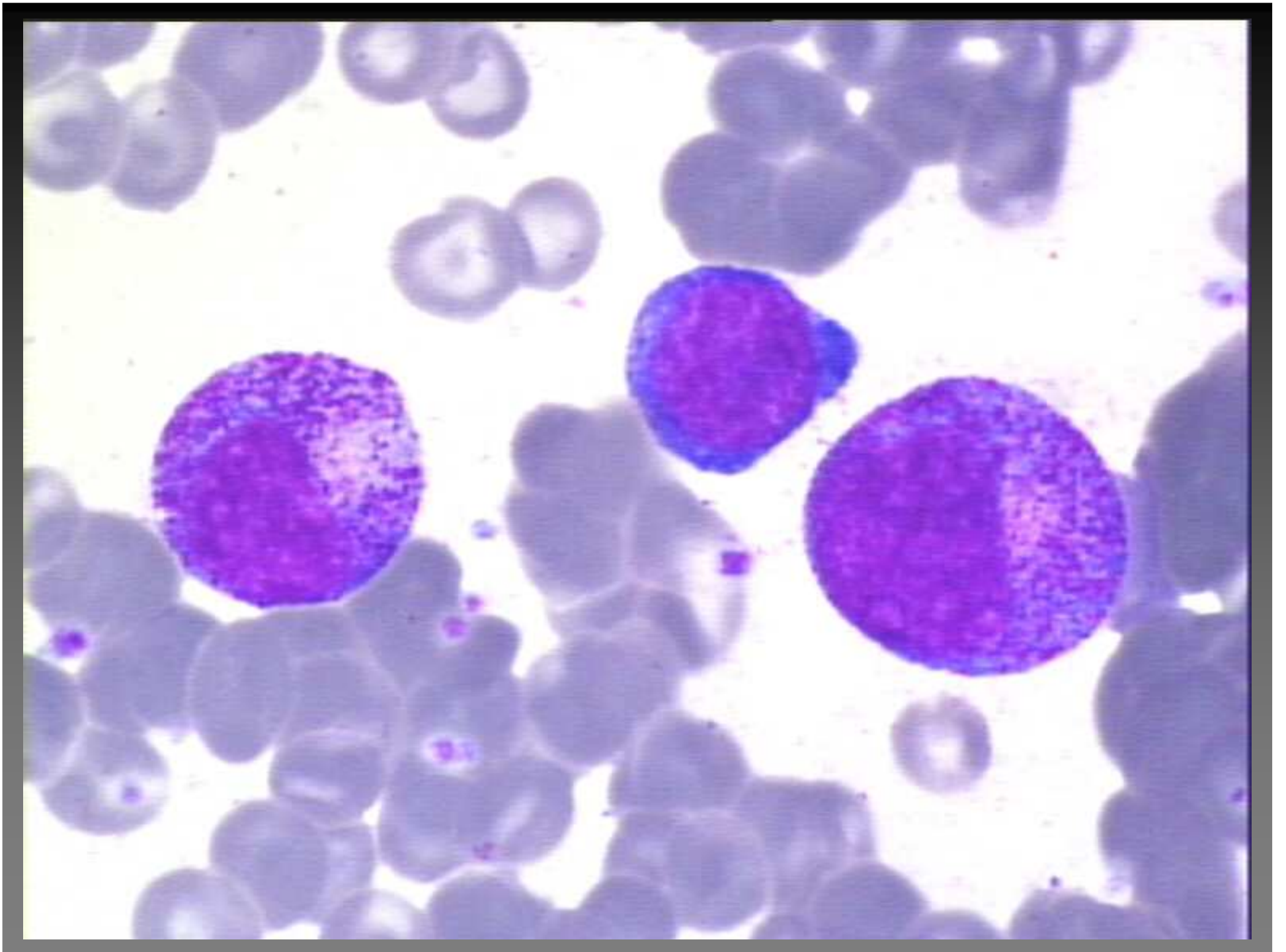


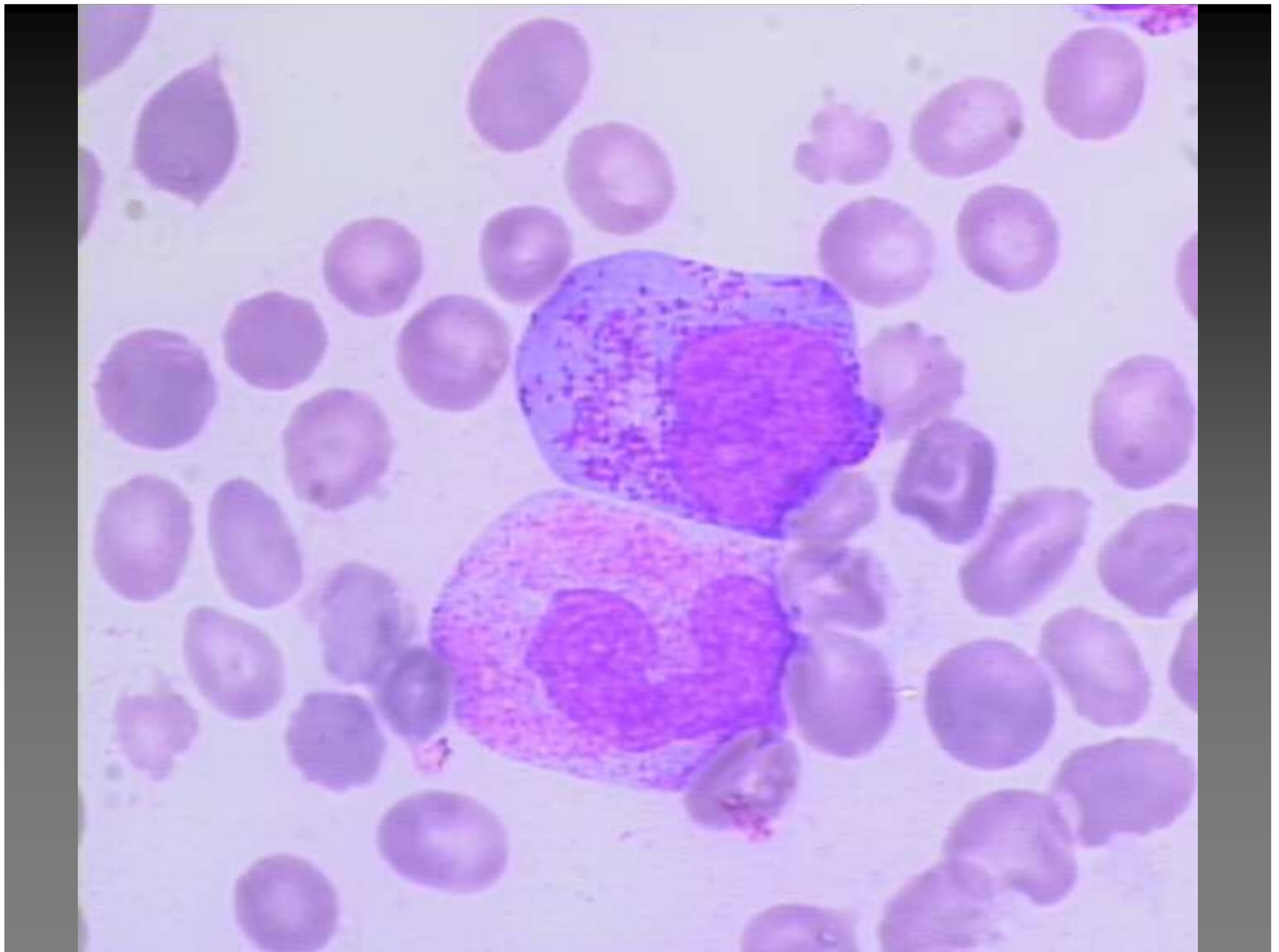


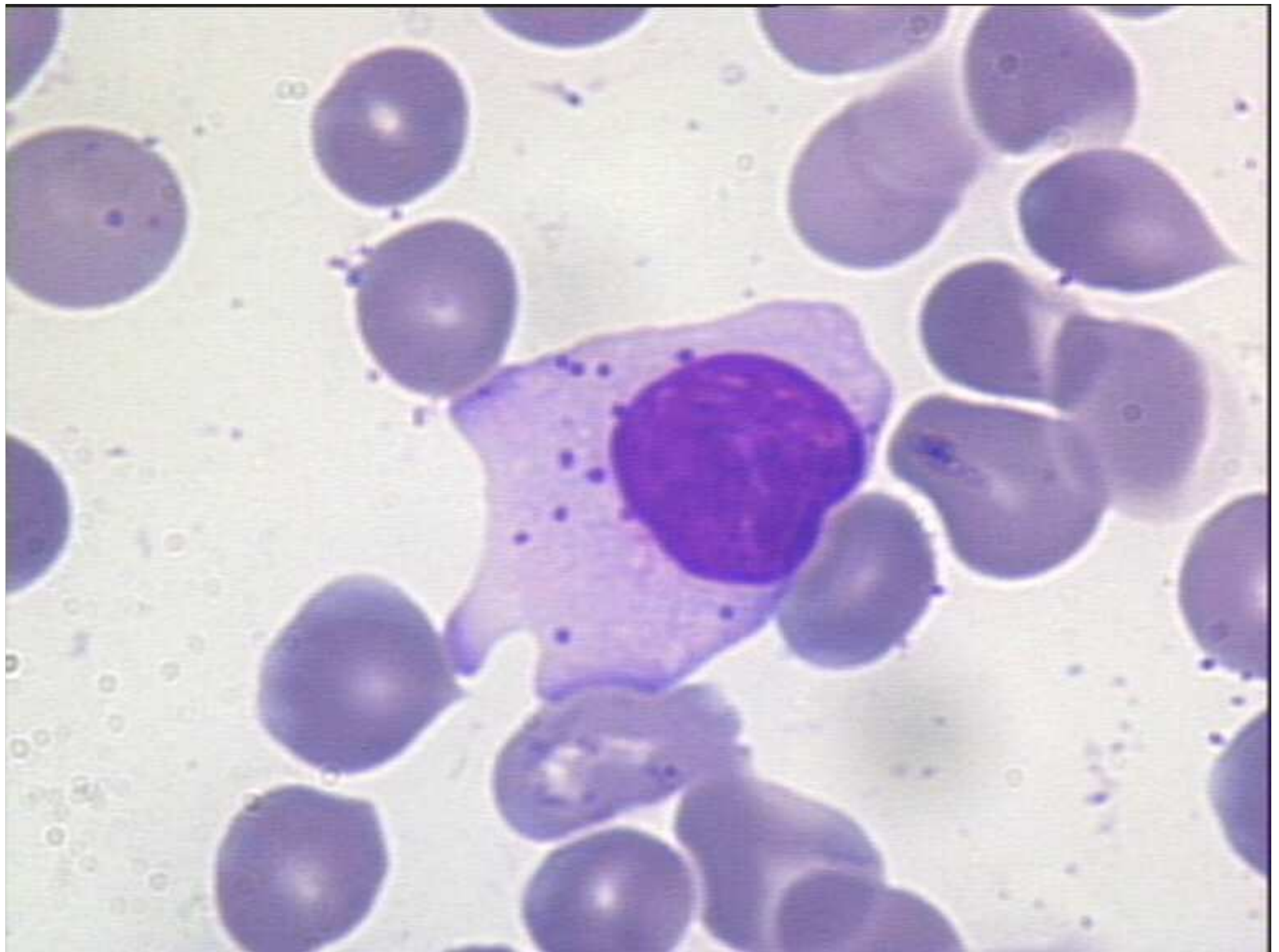
Citoplasma (1): il fondo

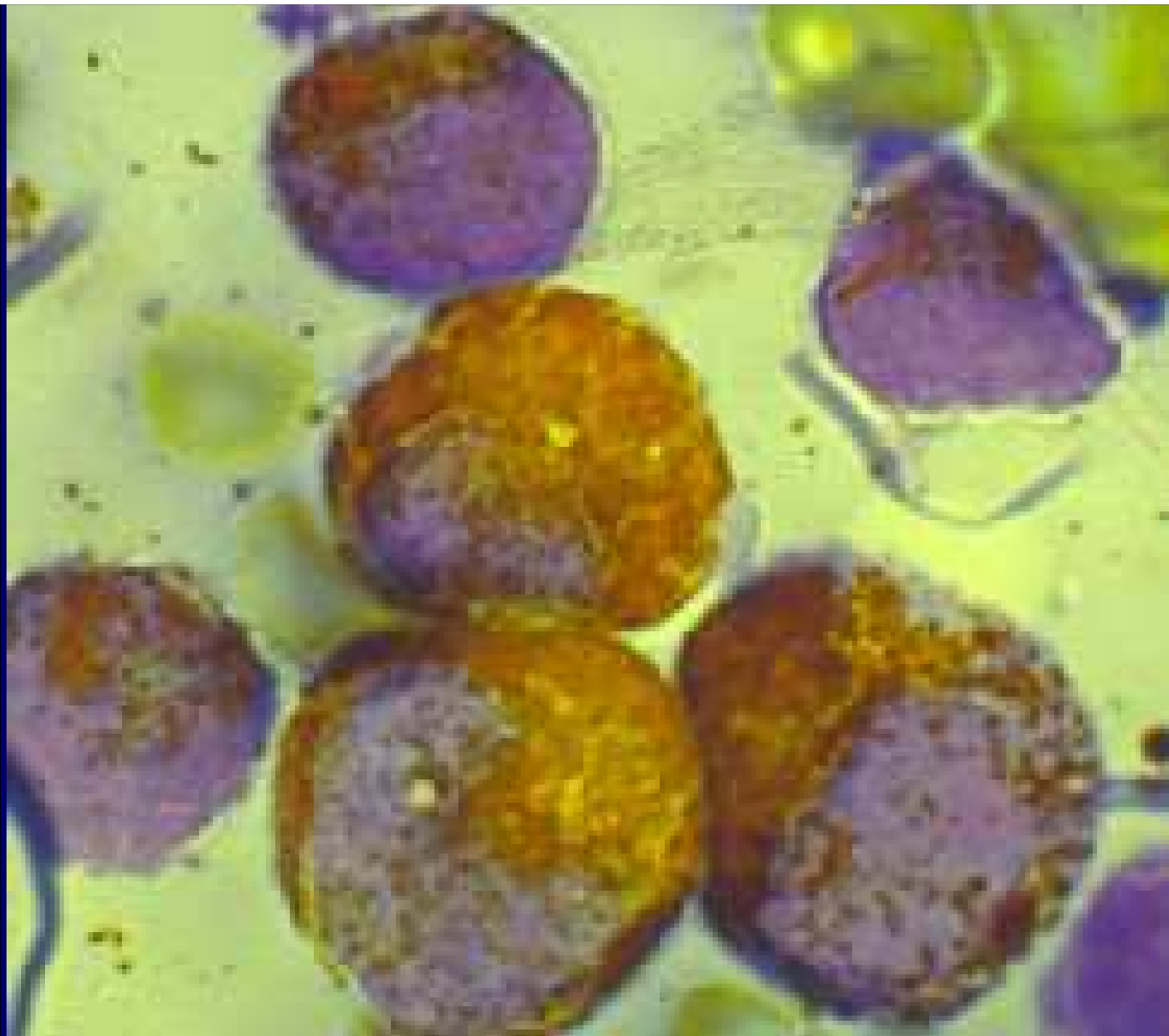
Citoplasma (2): il centrosoma

Citoplasma (3): i granuli



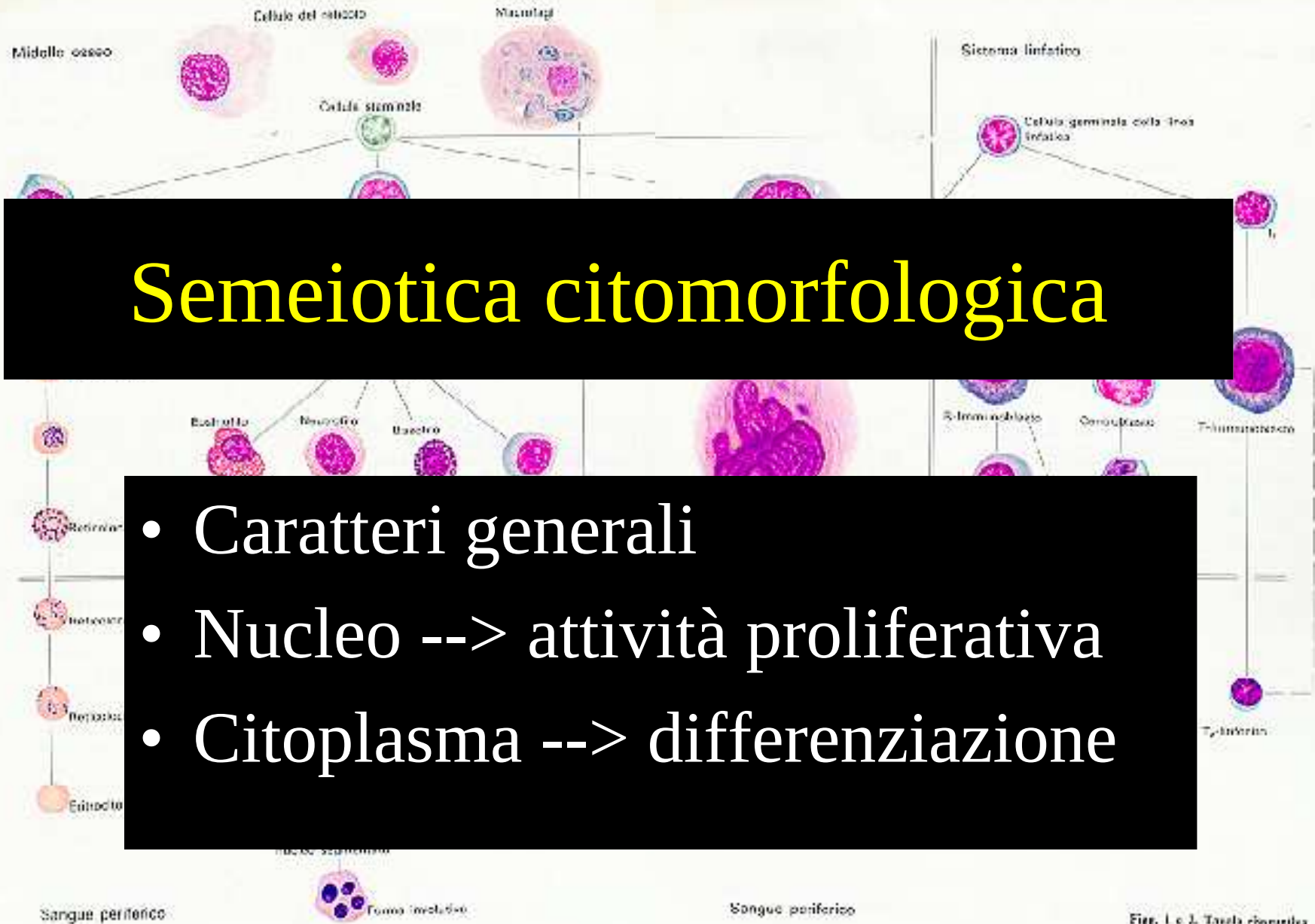






Semeiotica citomorfologica

- Caratteri generali
- Nucleo --> attività proliferativa
- Citoplasma --> differenziazione



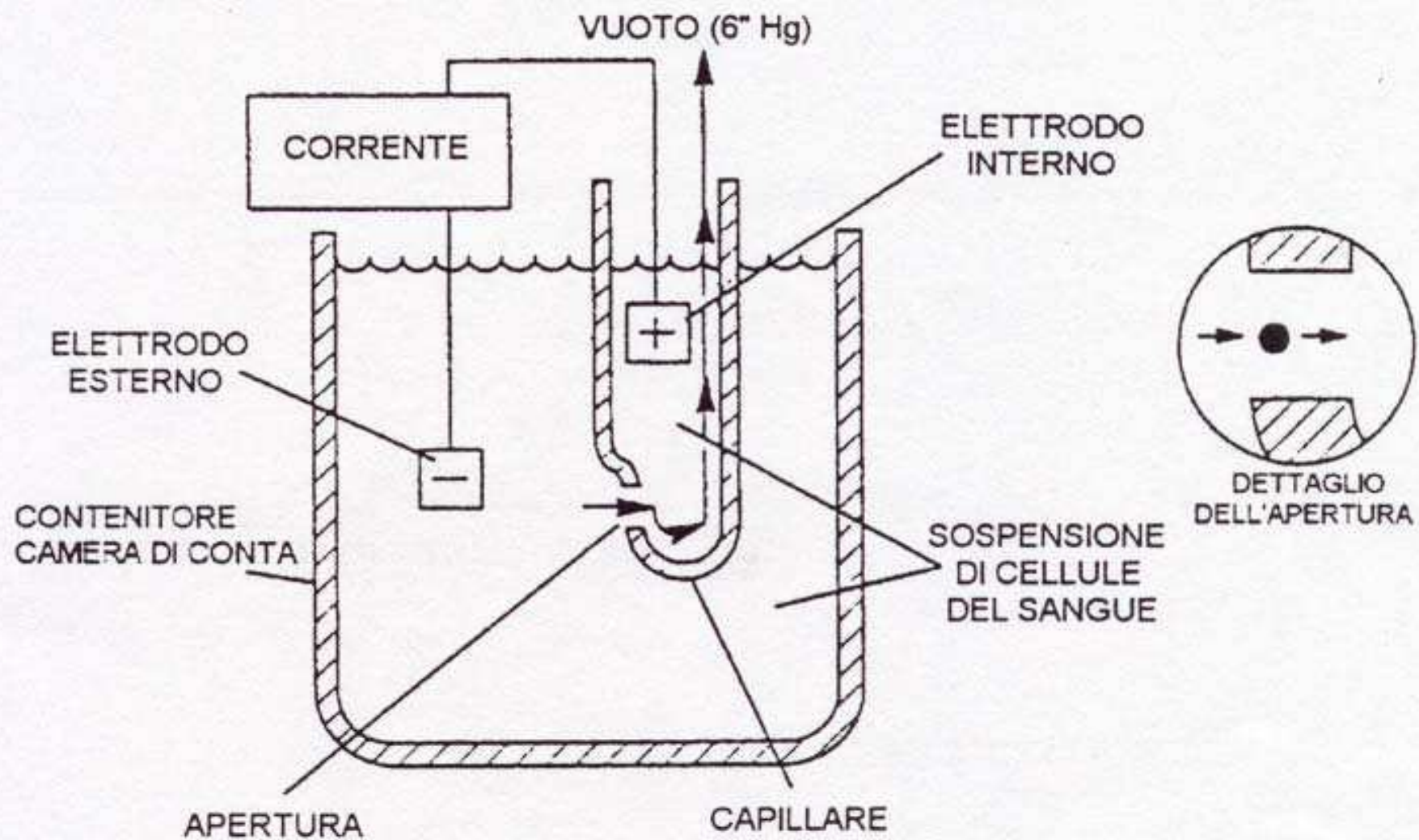
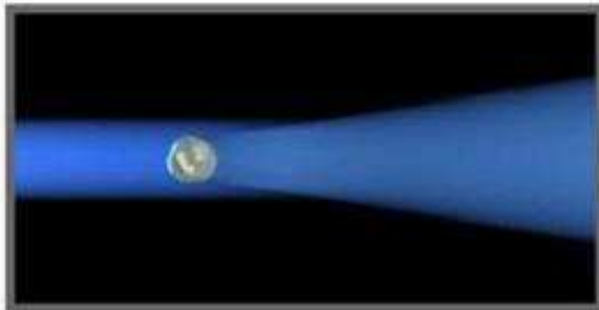
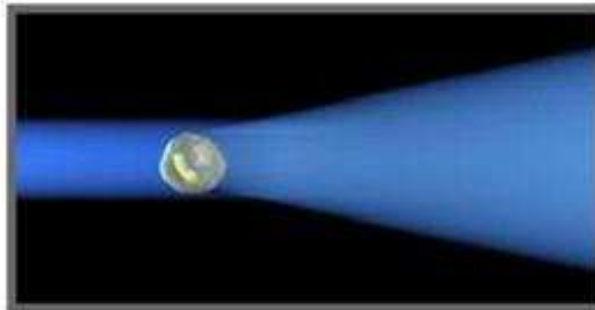


Figura 1: Metodo Coulter per il conteggio e il dimensionamento

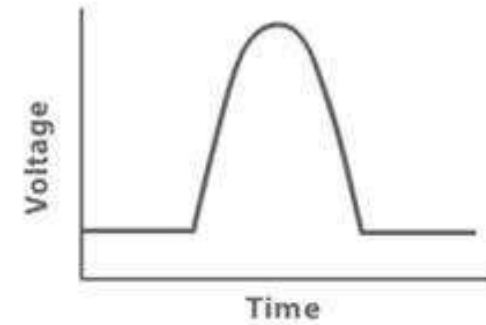
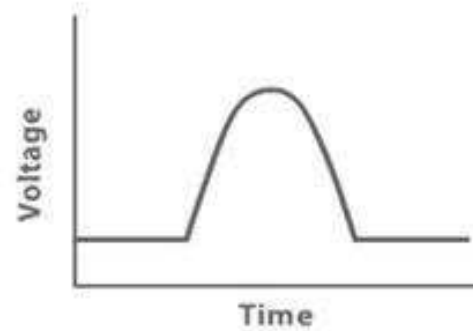
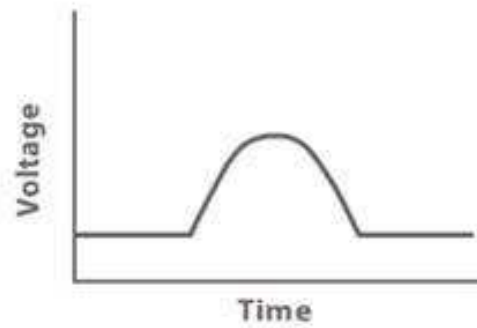
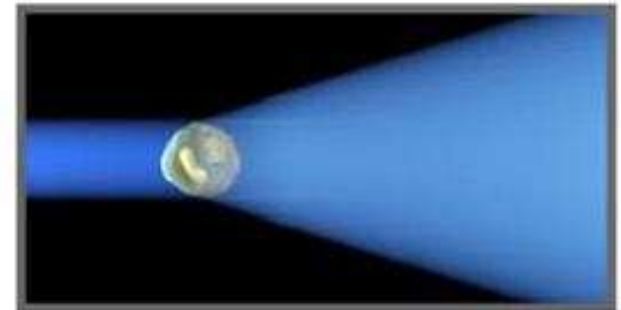
Small

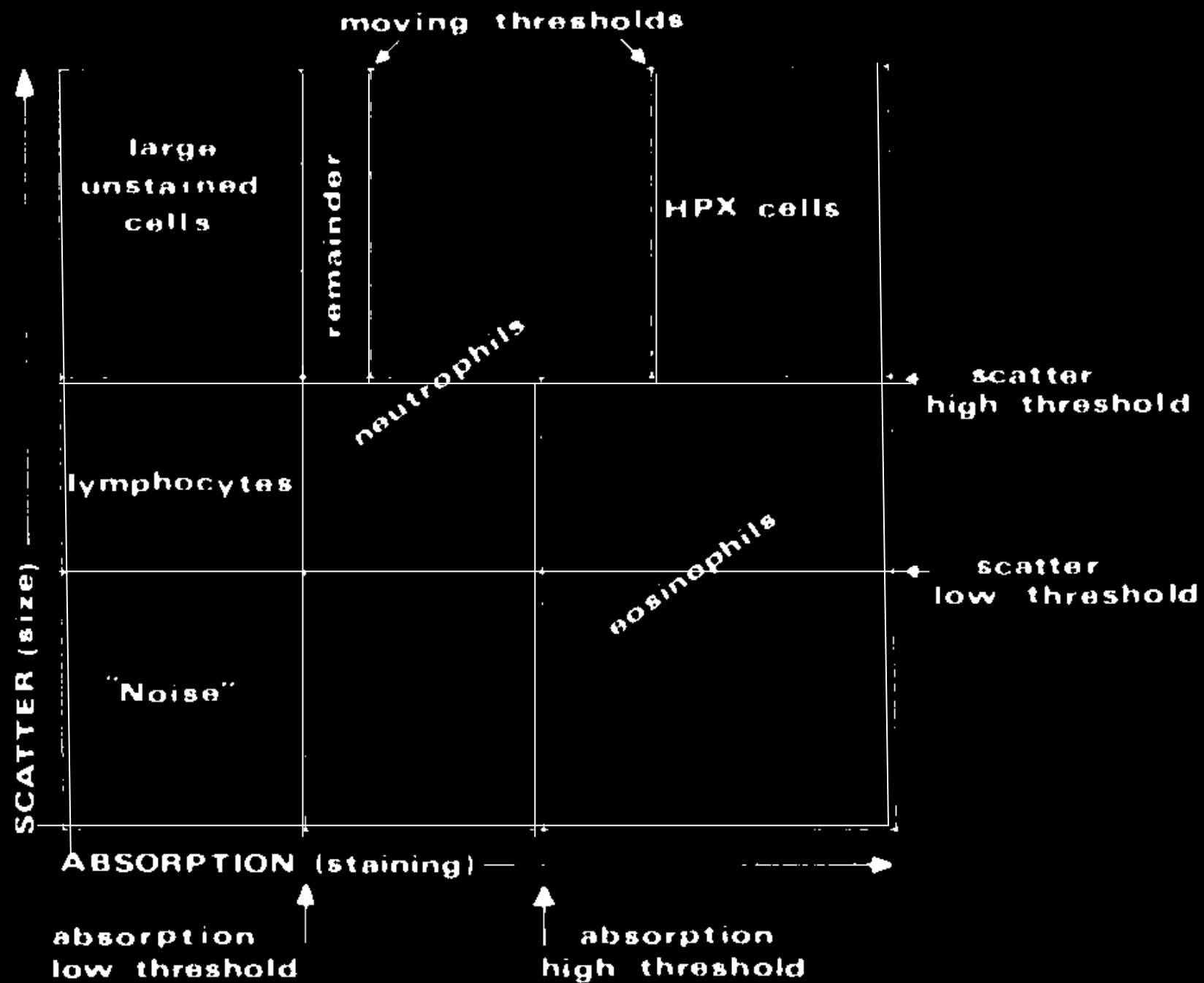


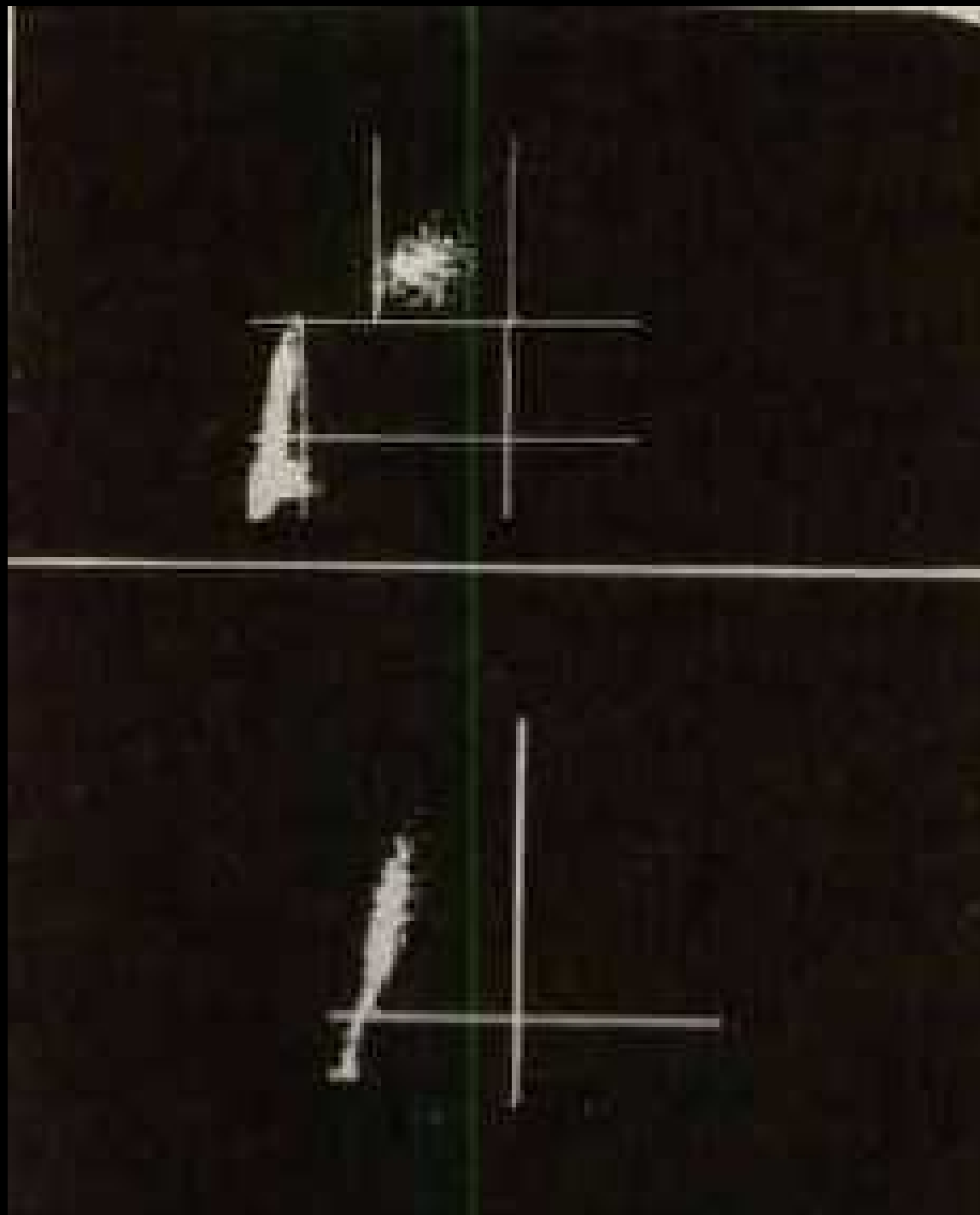
Medium



Large







TECHNICON H6000/H601

DATE: 12/10

SEQ: 004 ID#:

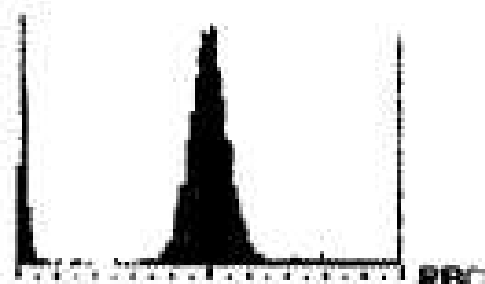
D.M.A.

CBC

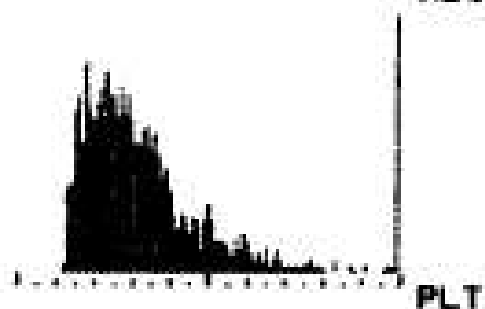
	9.00	$\times 10^3$	WBC
L	2.49	$\times 10^3$	RBC
	12.6	g/dL	Hgb
L	22.0	%	HCT
	88.2	μ^3	MCV
H	58.6	pg	MCH
H	57.3	g/dL	MCHC
	16.9		RDW
H	498	$\times 10^3$	PLT
	6.9	μ^3	MPV
	43.7		PDW
	0.35	%	PCT

DIFFERENTIAL

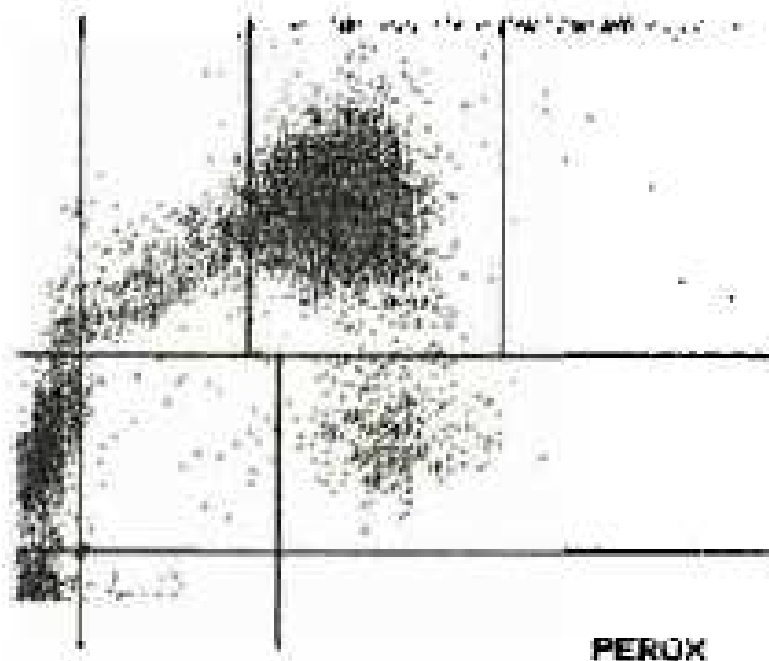
%	TYPE	$\times 10^3$
62.8	NEUT	5.70
28.4	LYMP	2.58
3.9	MONO	.36
3.0	EOS	.27
.7	BASO	.06
1.1	LUC	.10



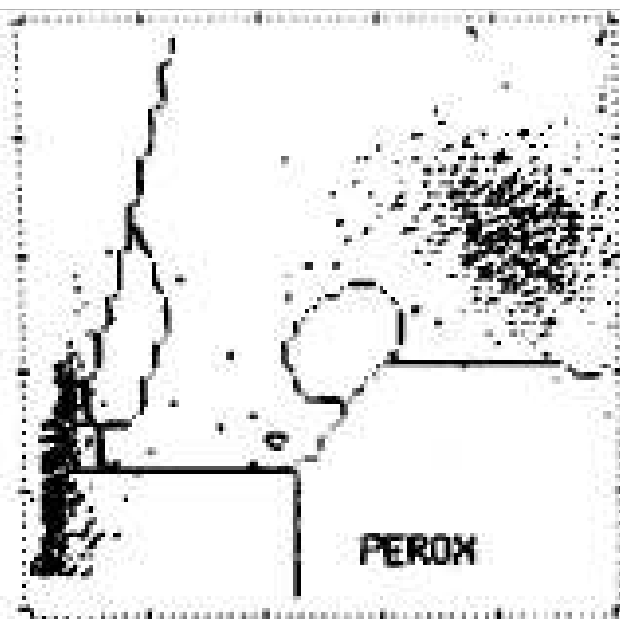
RBC



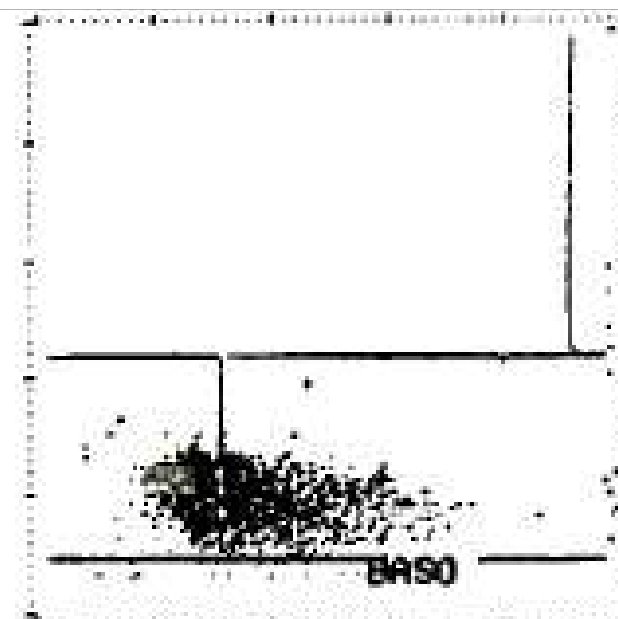
PLT



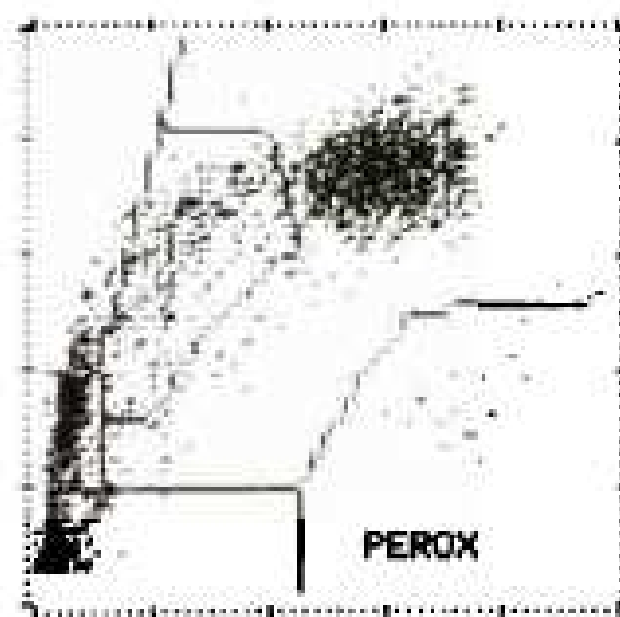
PEROX



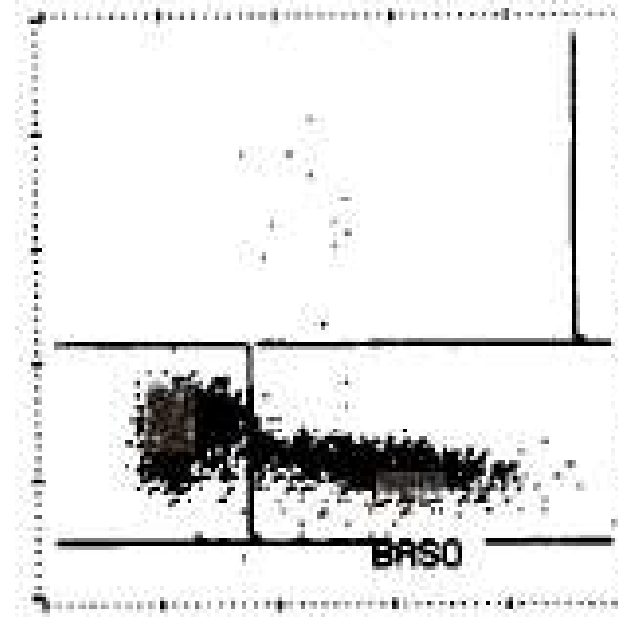
a



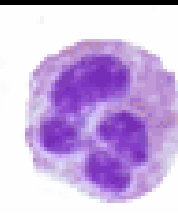
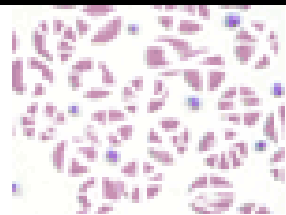
c



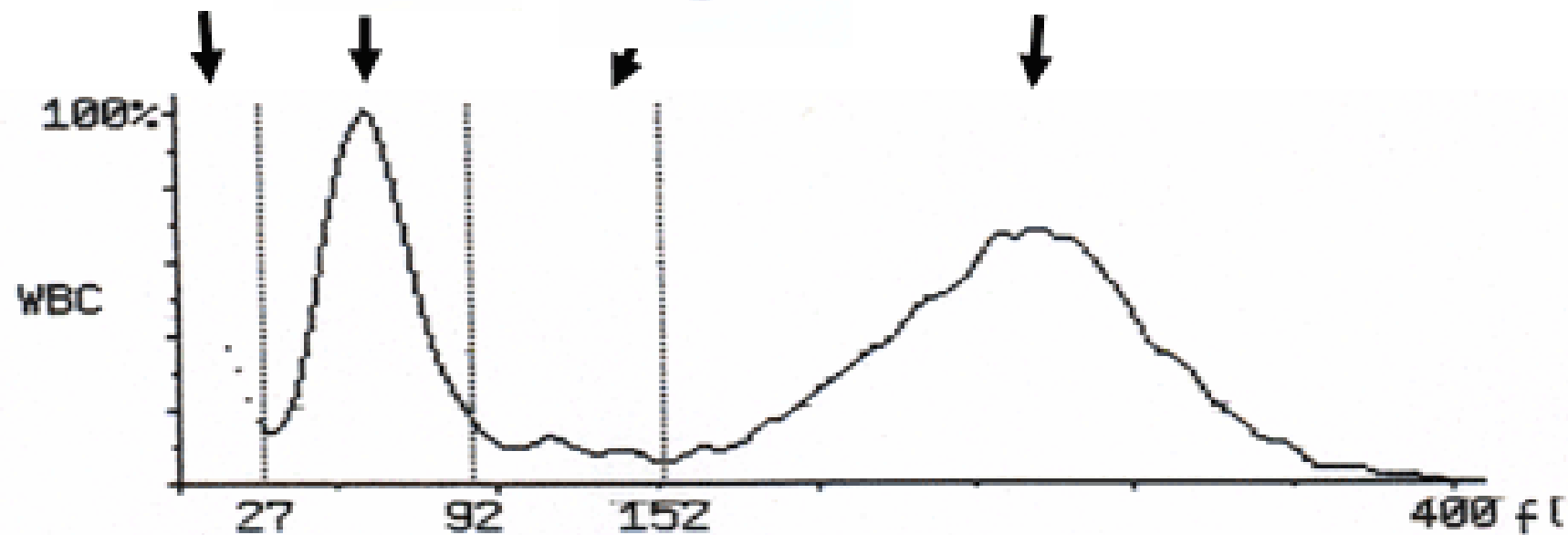
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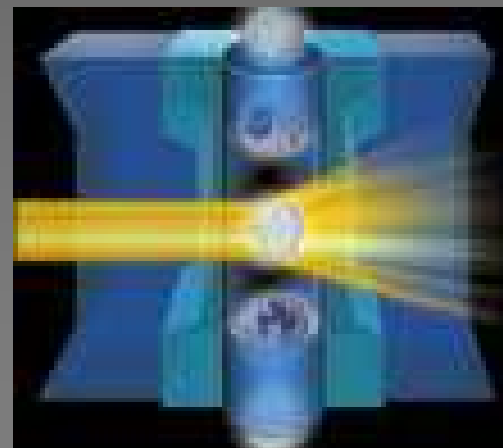
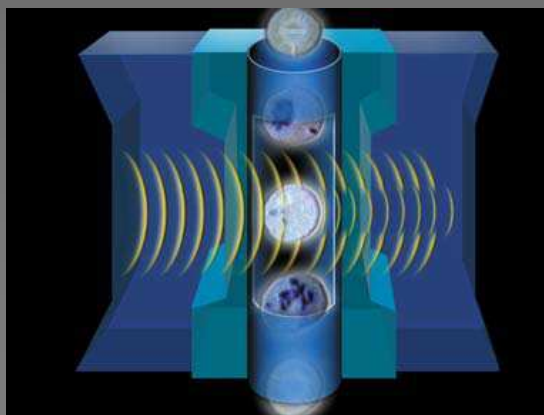
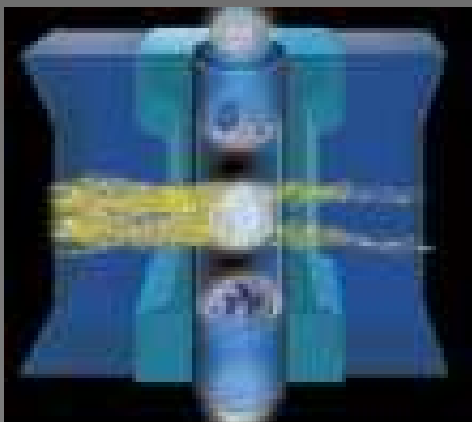
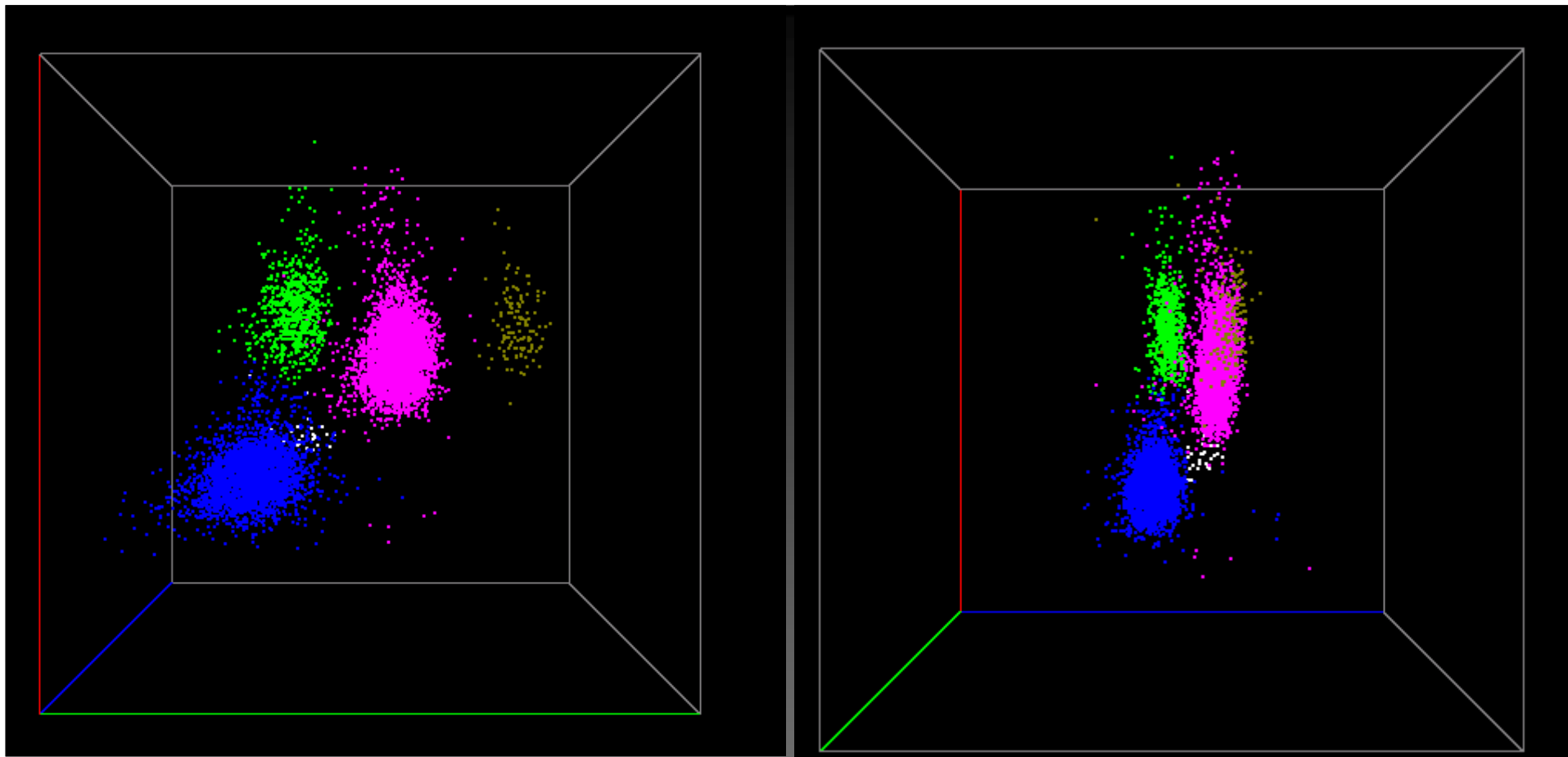


d

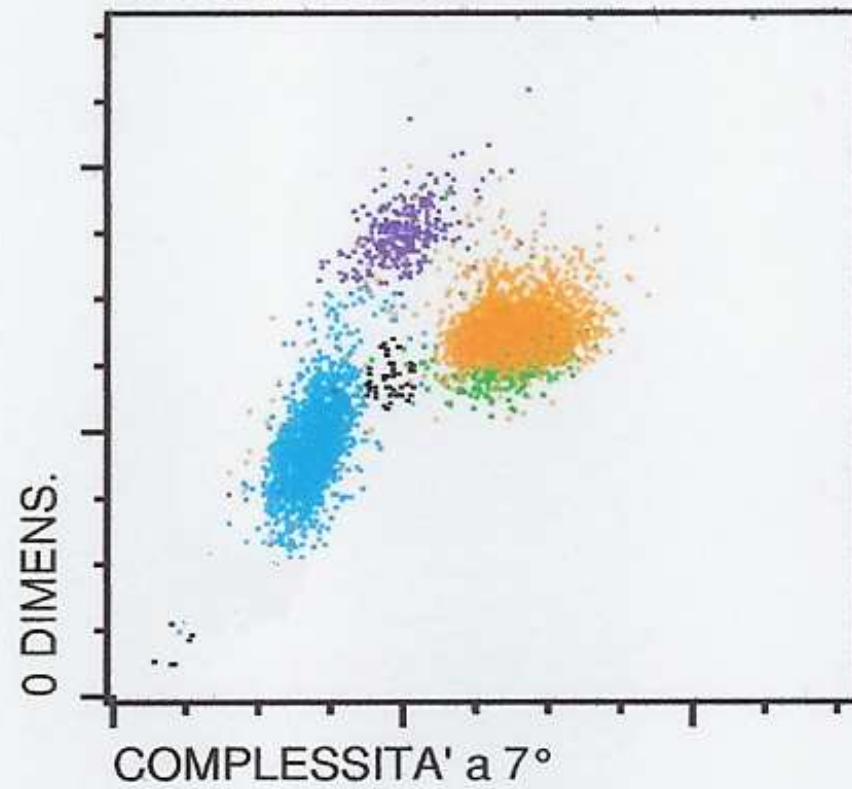


Classification of
WBC types
based on size

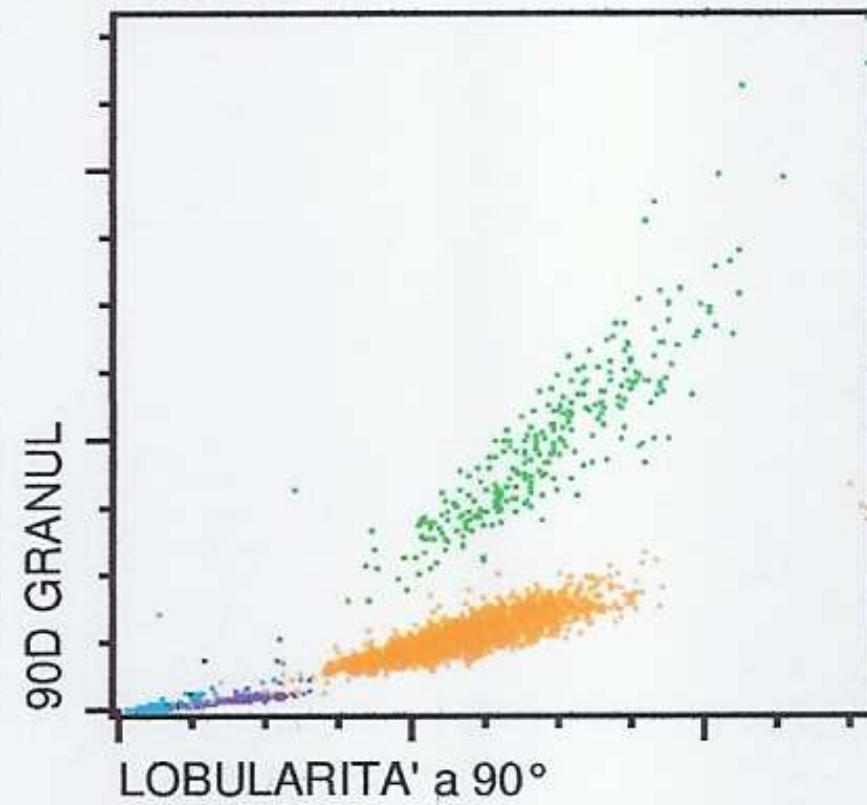


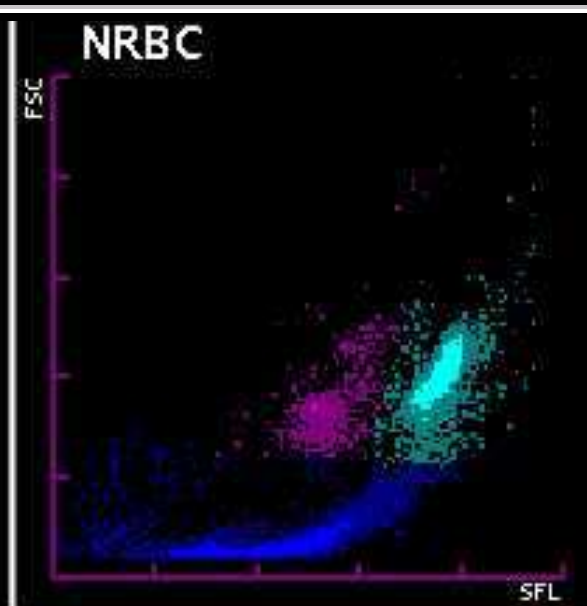
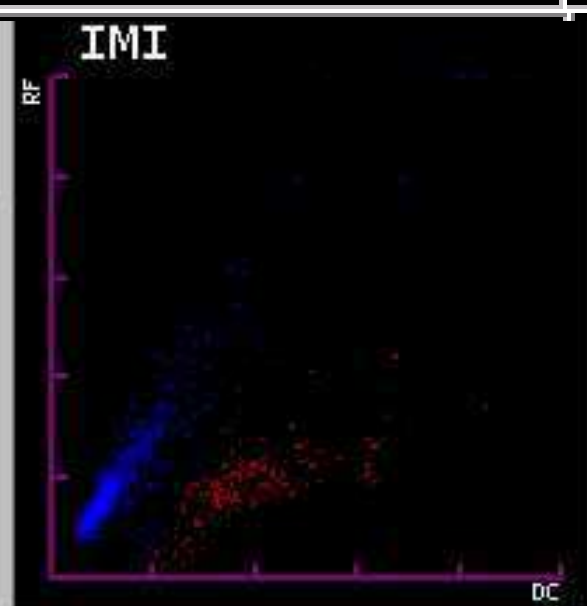
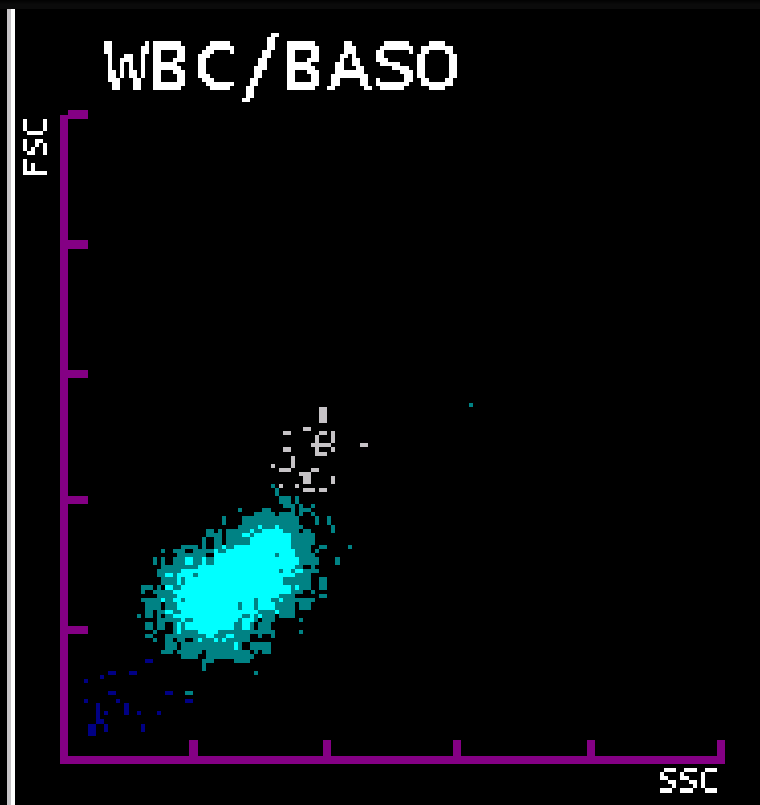
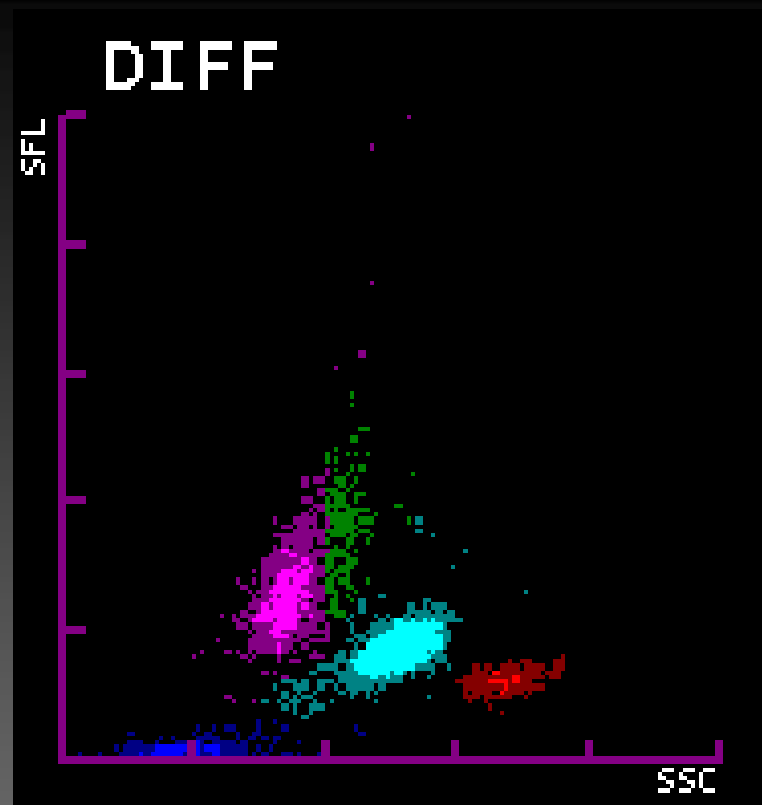


Formula leucocitaria



NEU - EOS



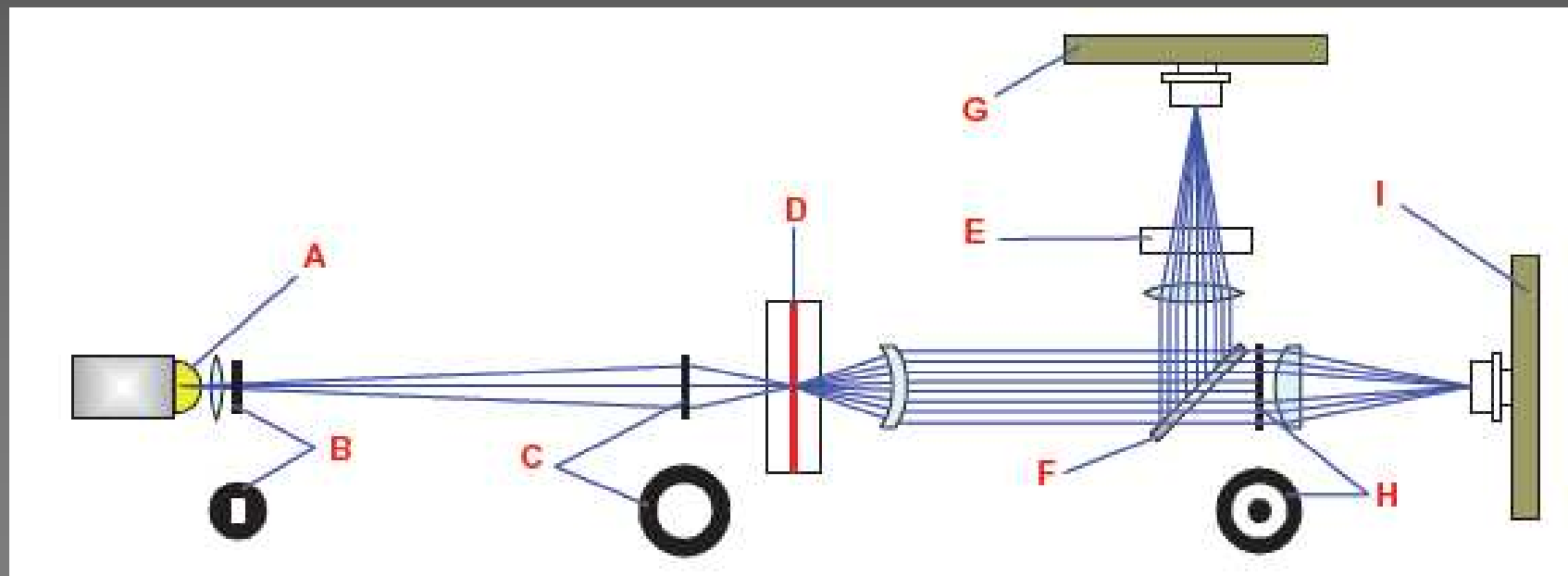
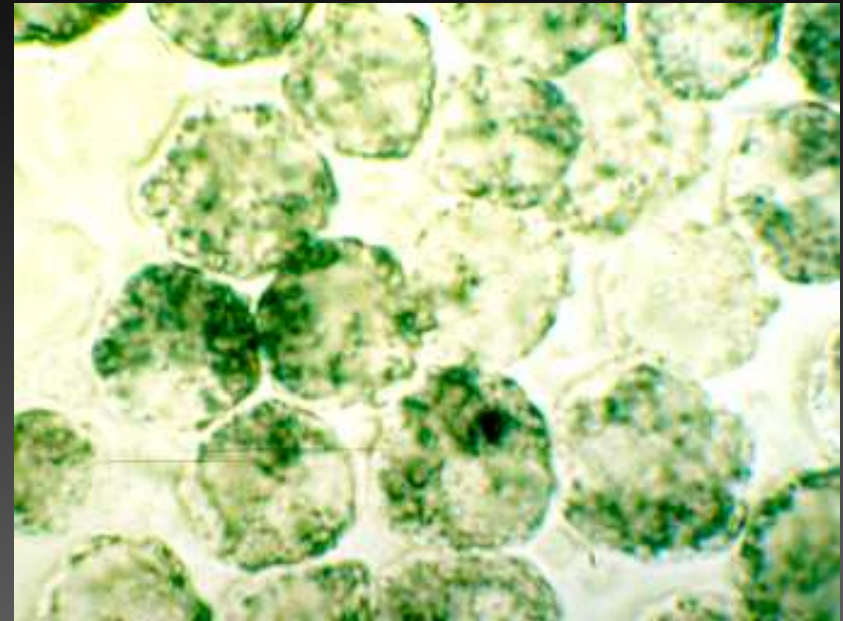


Perox

Baso

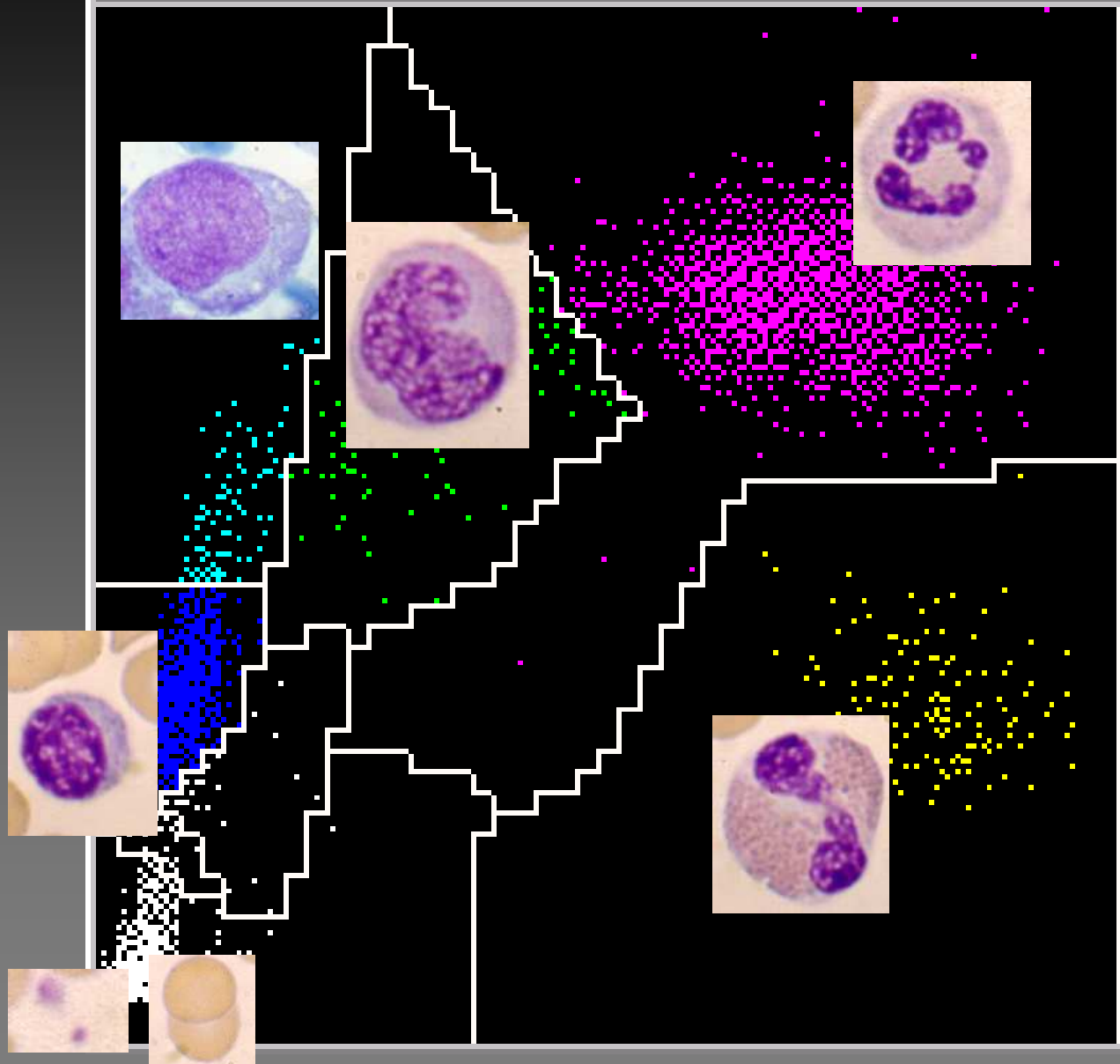
Semeiotica citomorfologica

- Caratteri generali
- Nucleo --> attività proliferativa
- Citoplasma --> differenziazione

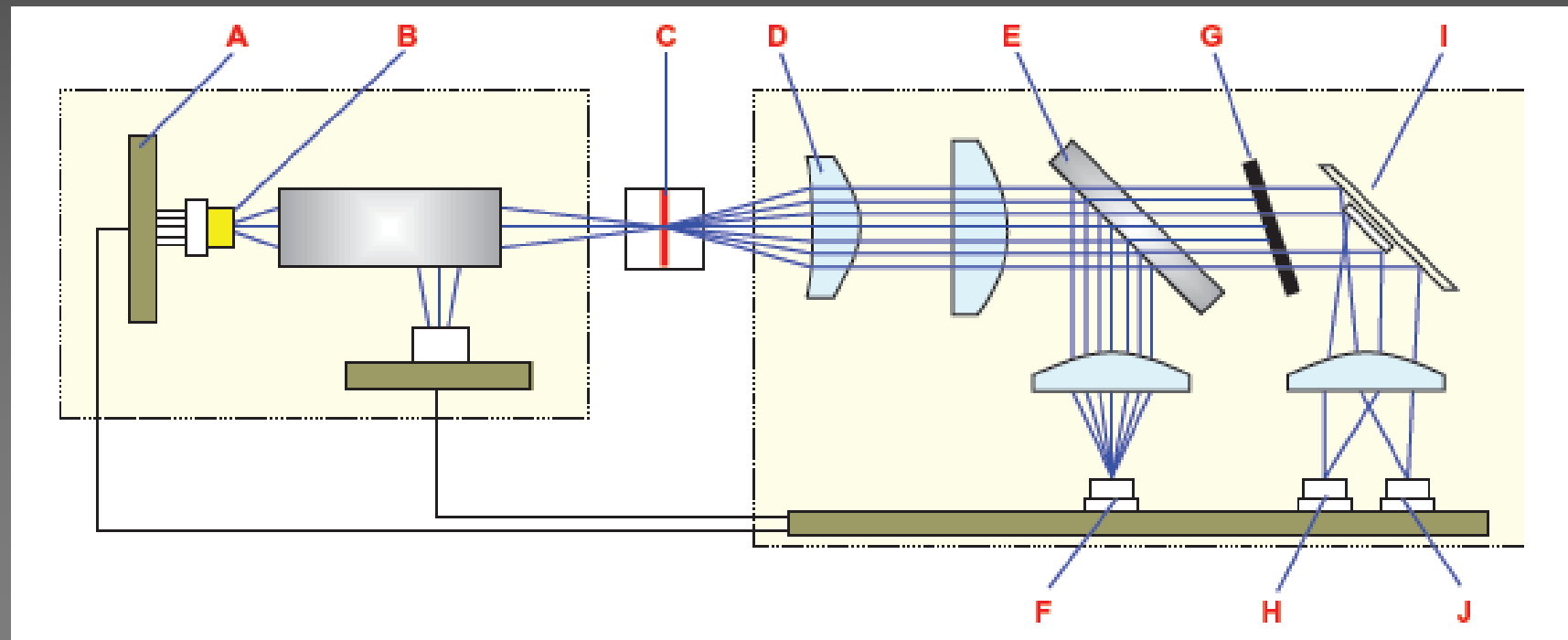
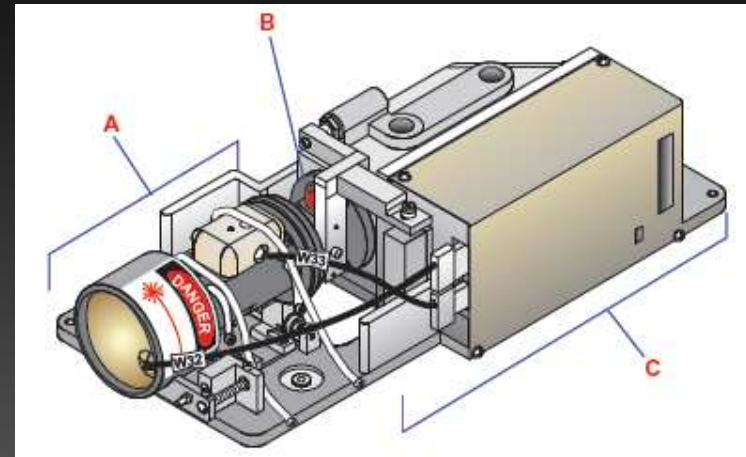
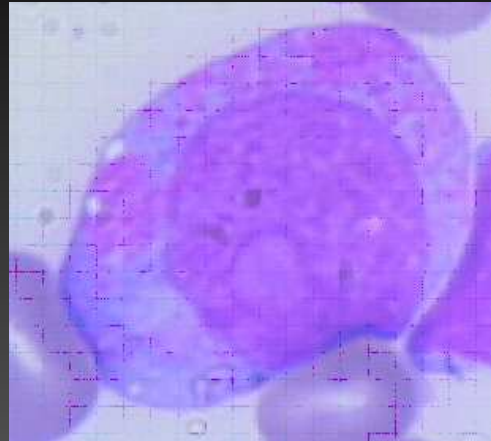


Perox

Cell
Size

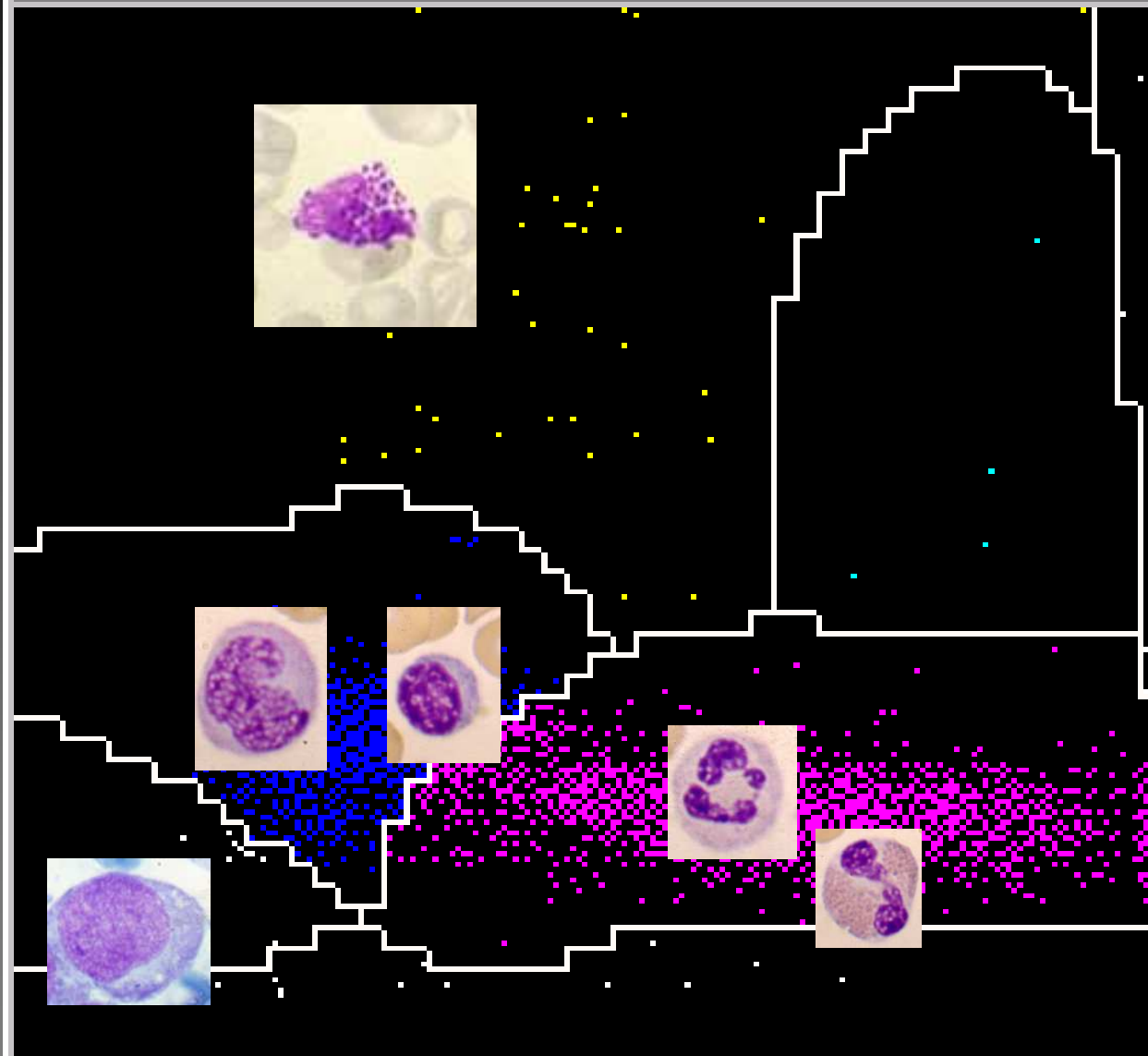


Peroxidase Activity in the Cytoplasm



Baso

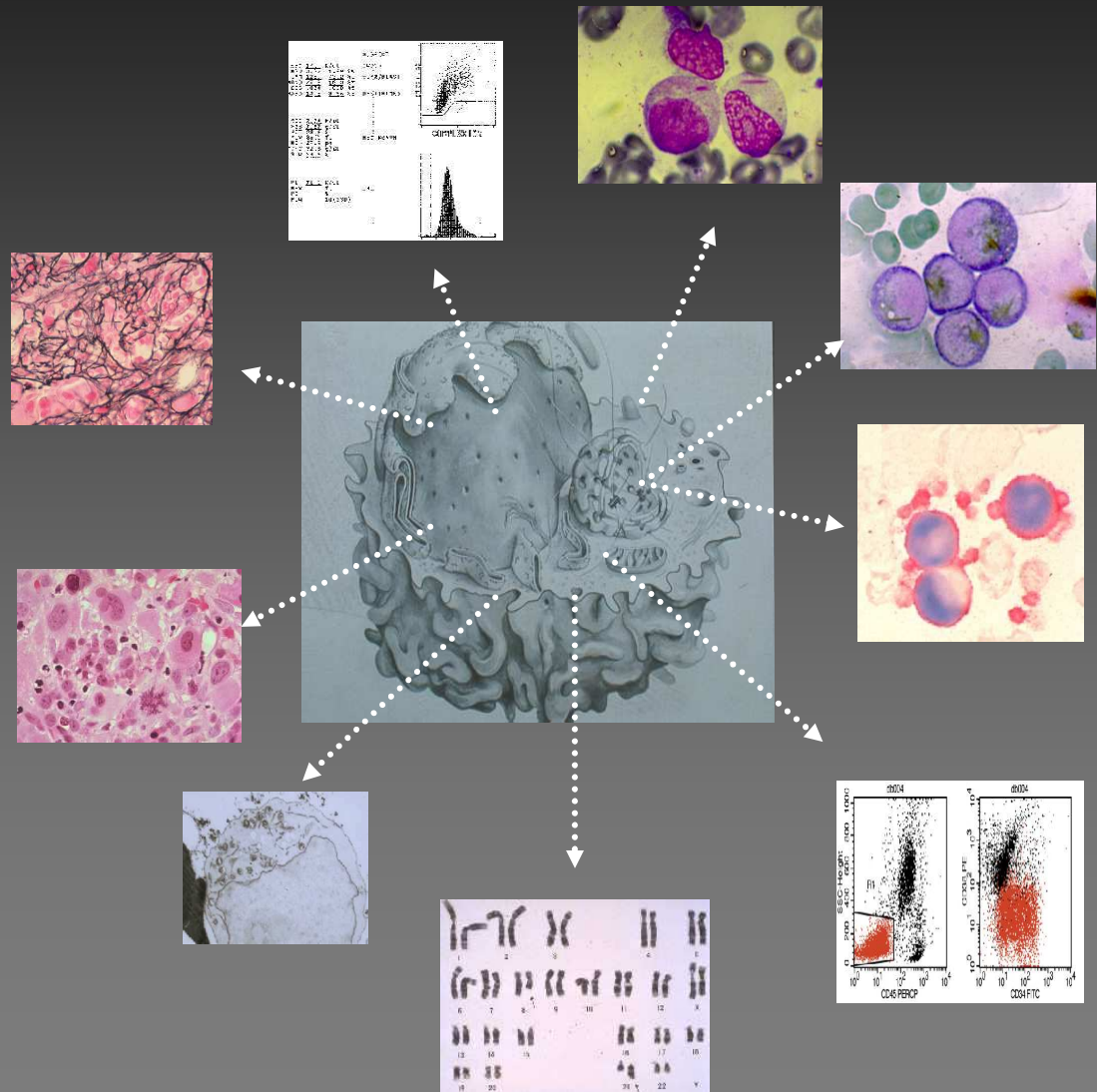
Cell
Size



Nuclear Shape and Density

Caratteristiche diagnostiche fondamentali delle sindromi mielodisplastiche, sia nell'epoca FAB e che nell'era WHO

1. ematopoiesi inefficace
2. displasia morfologica
3. possibile aumento dei blasti



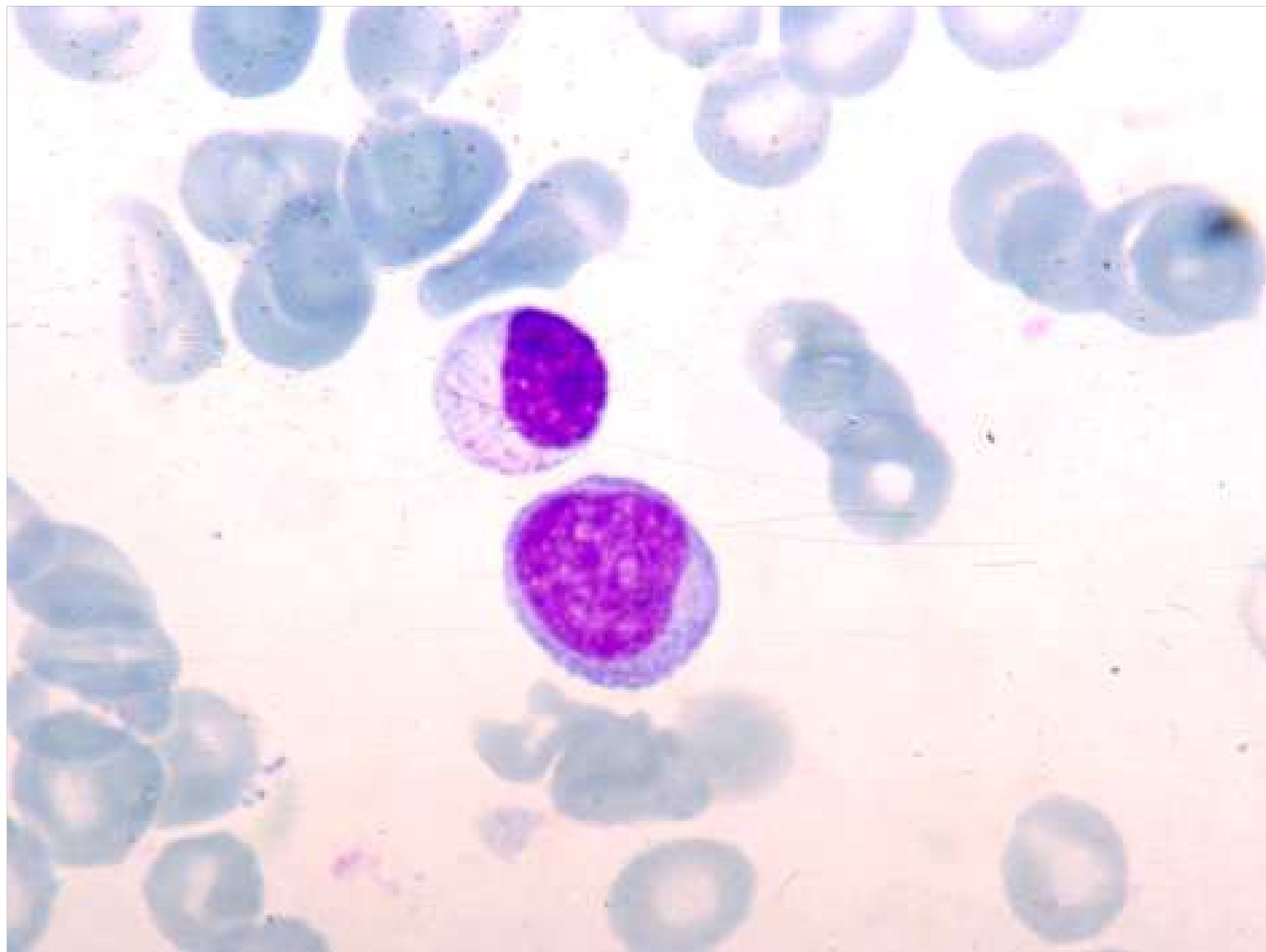
WHO 2008 MDS Classification - Adults

Disease	Blood findings	Bone marrow findings
Refractory cytopenias with unilineage dysplasia (RCUD) Refractory anaemia (RA); Refractory neutropenia (RN); Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia ¹ No or rare blasts (<1%) ²	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
Refractory anaemia with ring sideroblasts (RARS)	Anaemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%) ² No Auer rods <1x10 ⁹ /L monocytes	Dysplasia in ≥10% of the cells in ≥ two myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer rods ±15% ring sideroblasts
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts ² No Auer rods <1x10 ⁹ /L monocytes	Unilineage or multilineage dysplasia 5-9% blasts ² No Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts Auer rods ± ³ <1x10 ⁹ /L monocytes	Unilineage or multilineage dysplasia 10–19% blasts Auer rods ± ³
Myelodysplastic syndrome – unclassified (MDS-U)	Cytopenias ≤1% blasts ²	Unequivocal dysplasia in less than 10% of cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS (See Table 5.04) <5% blasts
MDS associated with isolated del(5q)	Anaemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

¹ Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U.

² If the marrow myeloblast percentage is <5% but there are 2-4% myeloblasts in the blood, the diagnostic classification is RAEB 1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS, U.

³ Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB 2.







SEQ# 0000025
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 SIS# 000
 ID 0127

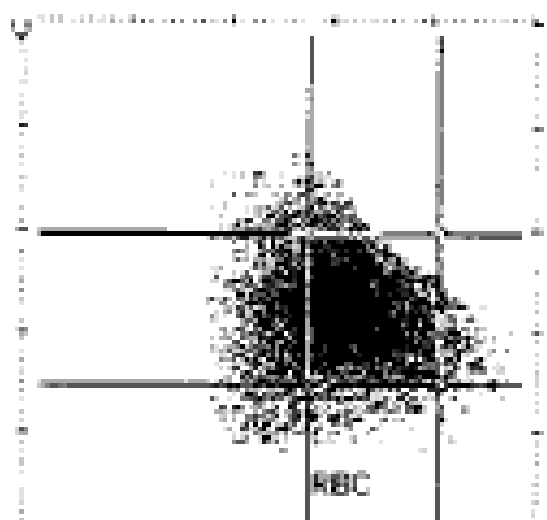
ENDOPHIO

	6.04	$\times 10^3/\mu\text{L}$	WBC
L	3.42	$\times 10^3/\mu\text{L}$	RBC
L	9.4	g/dL	HGB
L	29.7	%	HCT
	86.9	fL	MCV
	27.5	pg	MCH
L	31.7	g/dL	MCHC
H	18.2	%	RDW
H	4.06	g/dL	HDI
	325*	$\times 10^3/\mu\text{L}$	PLT
L	6.1*	fL	MPV
	61.0*	%	PDW
	.21*	%	PCT

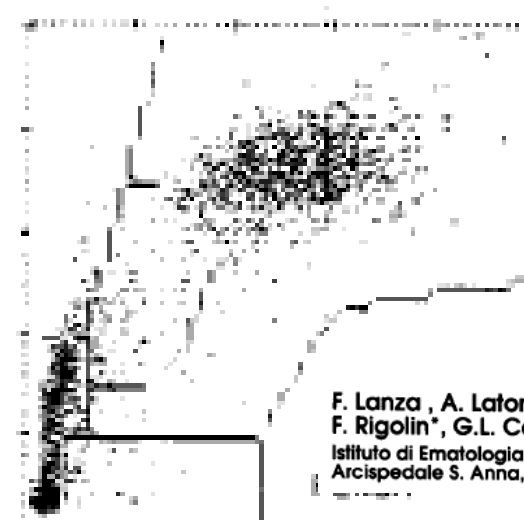
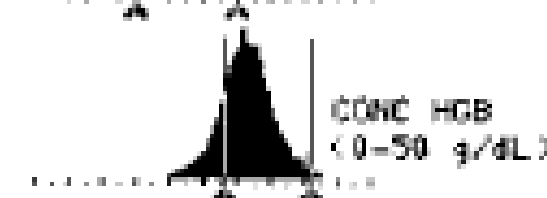
MORFOL RBC

%	FORMULA	$\times 10^3/\mu\text{L}$
60.5	NEUT	3.66
27.6	LINF	1.67
7.2	MONO	.44
.3	EOS	.02
1.2	BASO	.07
3.2	LUC	.19
LI	L	1.19*
MPXI	L	-14.0

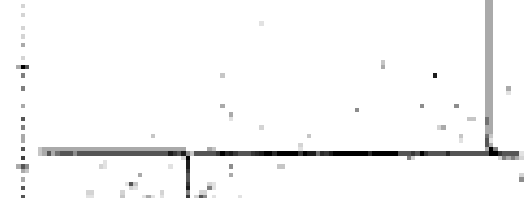
MORFOL WBC



HGB



WBC

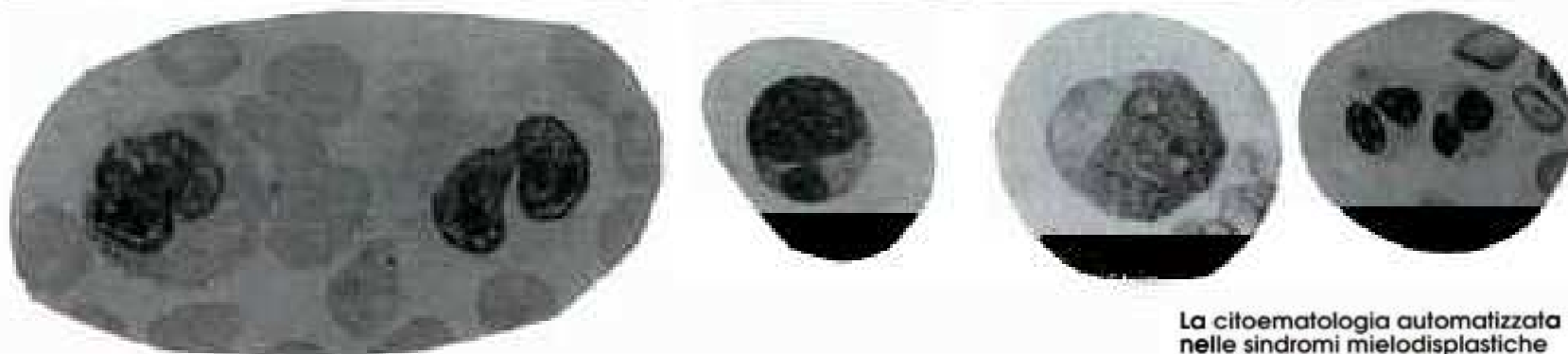
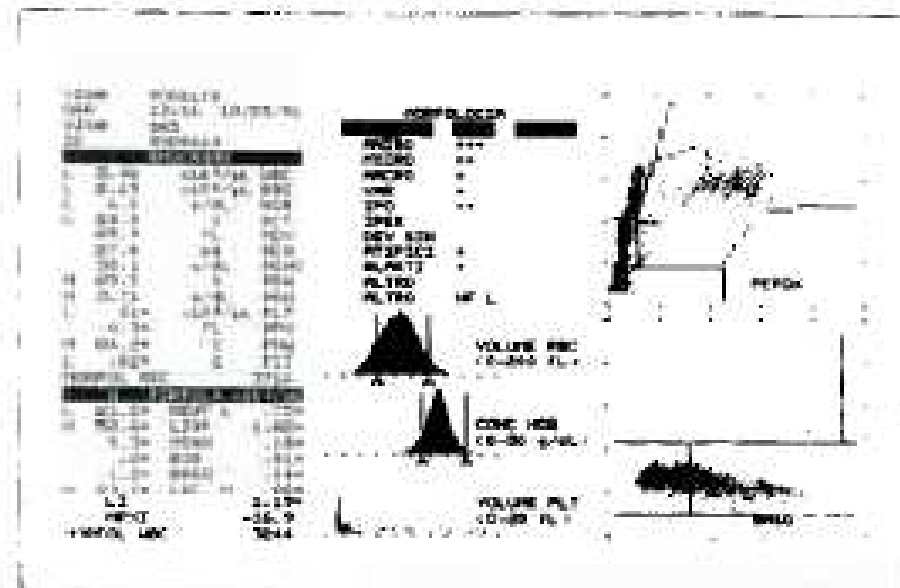
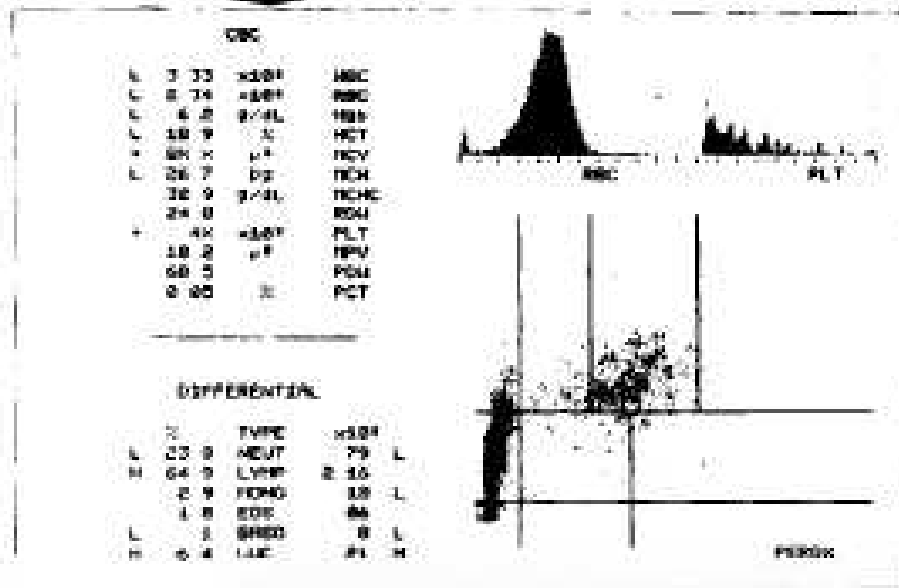


V Incontro
 del club utilizzatori
 Sistemi ematologici
 Bayer-Technicon
Atti

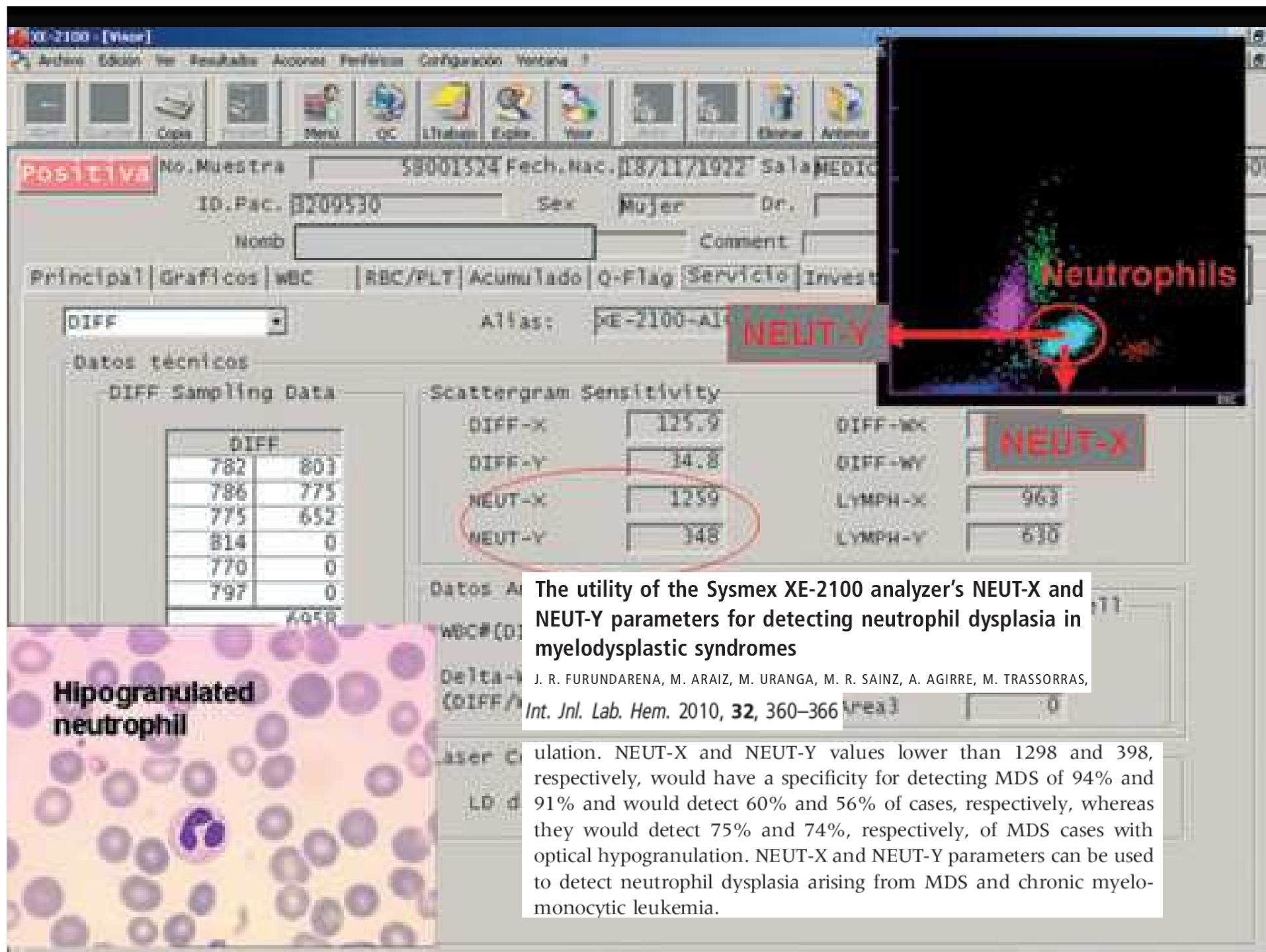
Montecatini Terme
 13-15 Giugno 1991

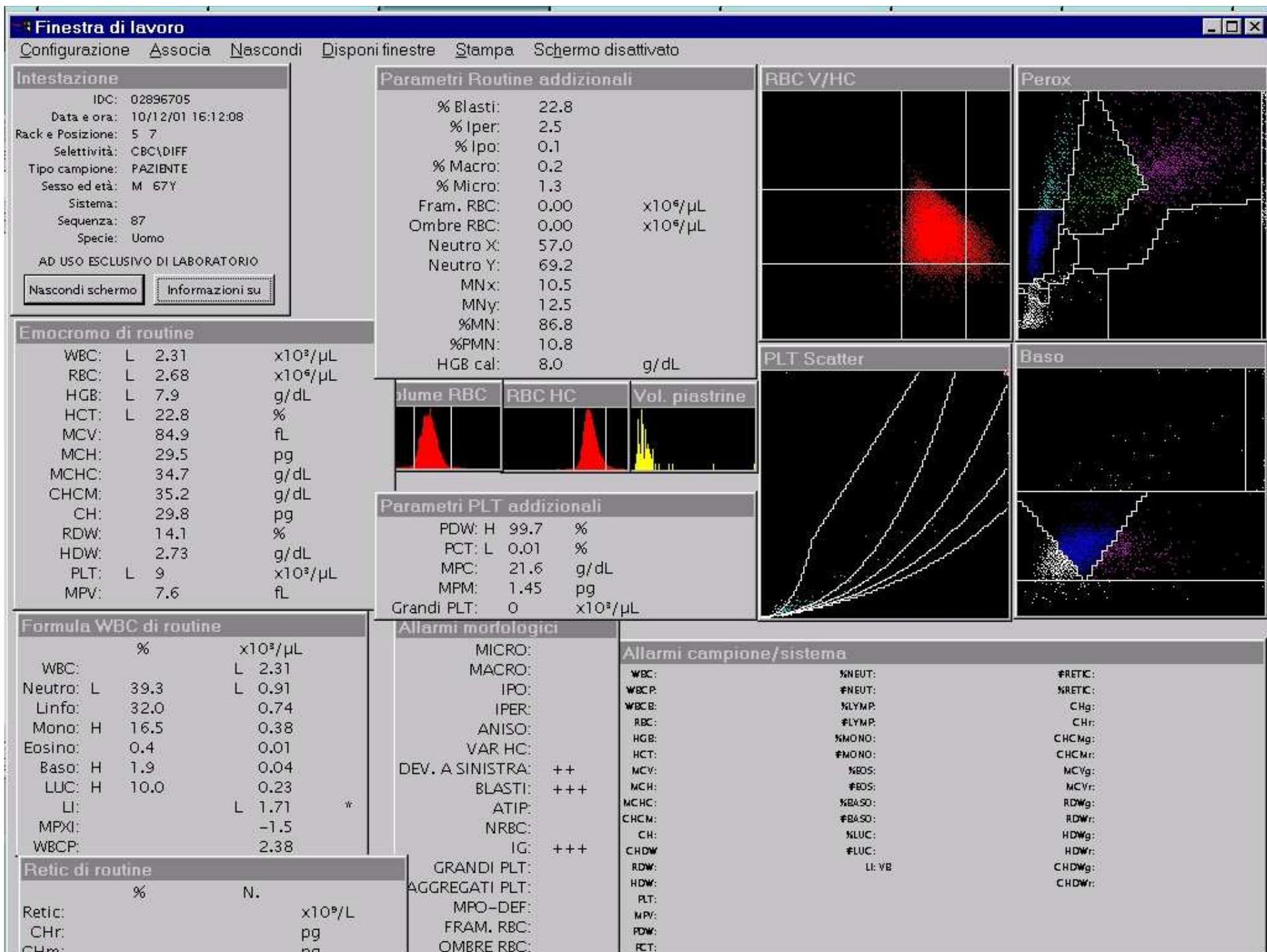
F. Lanza, A. Latorraca, S. Moretti, L. Ferrari,
 F. Rigolin*, G.L. Castoldi
 Istituto di Ematologia dell'Università, *Laboratorio Analisi,
 Arcispedale S. Anna, Ferrara

Fig. 2. L'analisi con il sistema citochimico automatizzato H*1 del sangue periferico di un paziente affetto da anemia refrattaria in fase di esordio clinico evidenzia una estrema dispersione della popolazione granulocitaria nel display x-y canale della perossidasi. L'indice MPXI è ridotto : - 14.0



**La citoematologia automatizzata
nelle sindromi mielodisplastiche**
P. Cappelletti, B. Milanesi*, D. Signori,
P. Doretto, F. Falcioni
Servizio di Chimica e Microscopia Clinica, Ospedale di
Pordenone - USL N°11 *Laboratorio di Analisi chimico-
cliniche e microbiologia - Ospedali Civili- Brescia.





Finestra di lavoro

Configurazione Associa Nascondi Disponi finestre Stampa Schermo disattivato

Intestazione

IDC: 00050537
 Data e ora: 17/08/01 18:48:06
 Rack e Posizione: 6 1
 Selettività: CBC\DIFF
 Tipo campione: PAZIENTE
 Sesso ed età: F
 Sistema: IR19270018
 Sequenza: 101
 Specie: Uomo

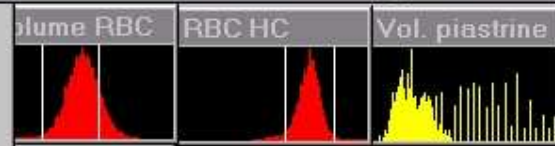
AD USO ESCLUSIVO DI LABORATORIO

Nascondi schermo

Informazioni su

Parametri Routine addizionali

% Blasti: 7.7
 % Iper: 1.2
 % Ipo: 3.5
 % Macro: 14.5
 % Micro: 1.1
 Fram. RBC: 0.01 $\times 10^6/\mu\text{L}$
 Ombre RBC: 0.00 $\times 10^6/\mu\text{L}$
 Neutro X: 65.9
 Neutro Y: 73.7
 MNx: 11.0
 MNy: 12.5
 %MN: 65.7
 %PMN: 33.3
 HGB cal: 8.2 g/dL



Parametri PLT addizionali

PDW: 64.8 * %
 PCT: 0.19 * %
 MPC: 24.4 g/dL
 MPM: 3.79 pg
 Grandi PLT: 34 $\times 10^3/\mu\text{L}$

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:

Allarmi campione/sistema

WBC: RB WC
 WBCP: NW VX NX
 WBCB:
 RBC:
 HGB:
 HCT:
 MCV:
 MCH:
 MCHC:
 CHCM:
 CH:
 CHDW:
 RDW:
 HDW:
 PLT: NW NT
 MPV: NW NT
 PDW: NW NT

%NEUT: RB VX NX
 #NEUT: RB VX NX
 %LYMP: RB VX NX
 #LYMP: RB VX NX
 %MONO: RB VX NX
 #MONO: RB VX NX
 %EOS: RB VX NX
 #EOS: RB VX NX
 %BASO:
 #BASO:
 %LUC: RB VX NX
 #LUC: RB VX NX
 LI: VB

#RETIC:
 %RETIC:
 CHg:
 CHr:
 CHCMg:
 CHCMr:
 MCVg:
 MCVr:
 RDWg:
 RDWr:
 HDWg:
 HDWr:
 CHDWg:
 CHDWr:

Emocromo di routine

WBC: 10.28 * $\times 10^3/\mu\text{L}$
 RBC: L 2.29 $\times 10^6/\mu\text{L}$
 HGB: L 7.8 g/dL
 HCT: L 23.8 %
 MCV: H 104.0 fL
 MCH: H 34.2 pg
 MCHC: L 32.9 g/dL
 CHCM: 34.4 g/dL
 CH: 35.6 pg
 RDW: H 16.7 %
 HDW: 3.19 g/dL
 PLT: L 89 * $\times 10^3/\mu\text{L}$
 MPV: H 21.7 * fL

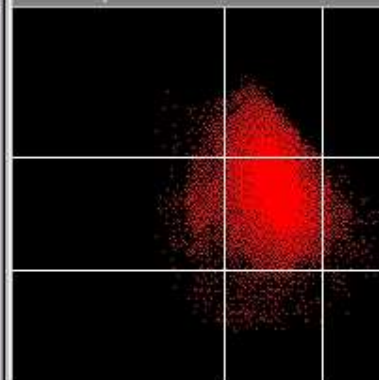
Formula WBC di routine

% $\times 10^3/\mu\text{L}$
 WBC: 10.28 *
 Neutro: 73.7 * 7.58 *
 Linfo: 17.8 * 1.83 *
 Mono: L 2.1 * 0.22 *
 Eosino: 2.7 * 0.28 *
 Baso: 0.9 0.09
 LUC: 2.8 * 0.29 *
 LI: L 1.64 *
 MPX: 6.8
 WBCP: 13.60

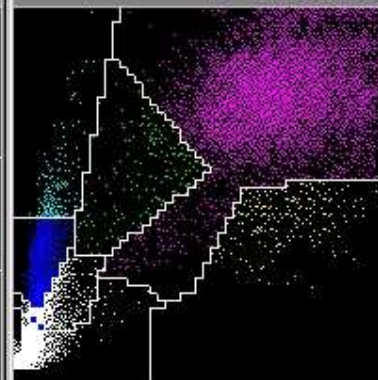
Retic di routine

% N. $\times 10^9/\text{L}$
 Retic:
 CHR: pg

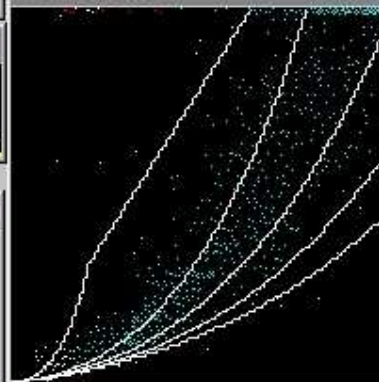
RBC V/HC



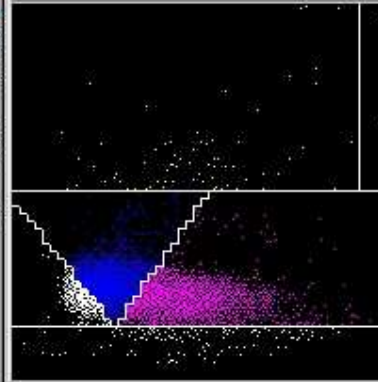
Perox

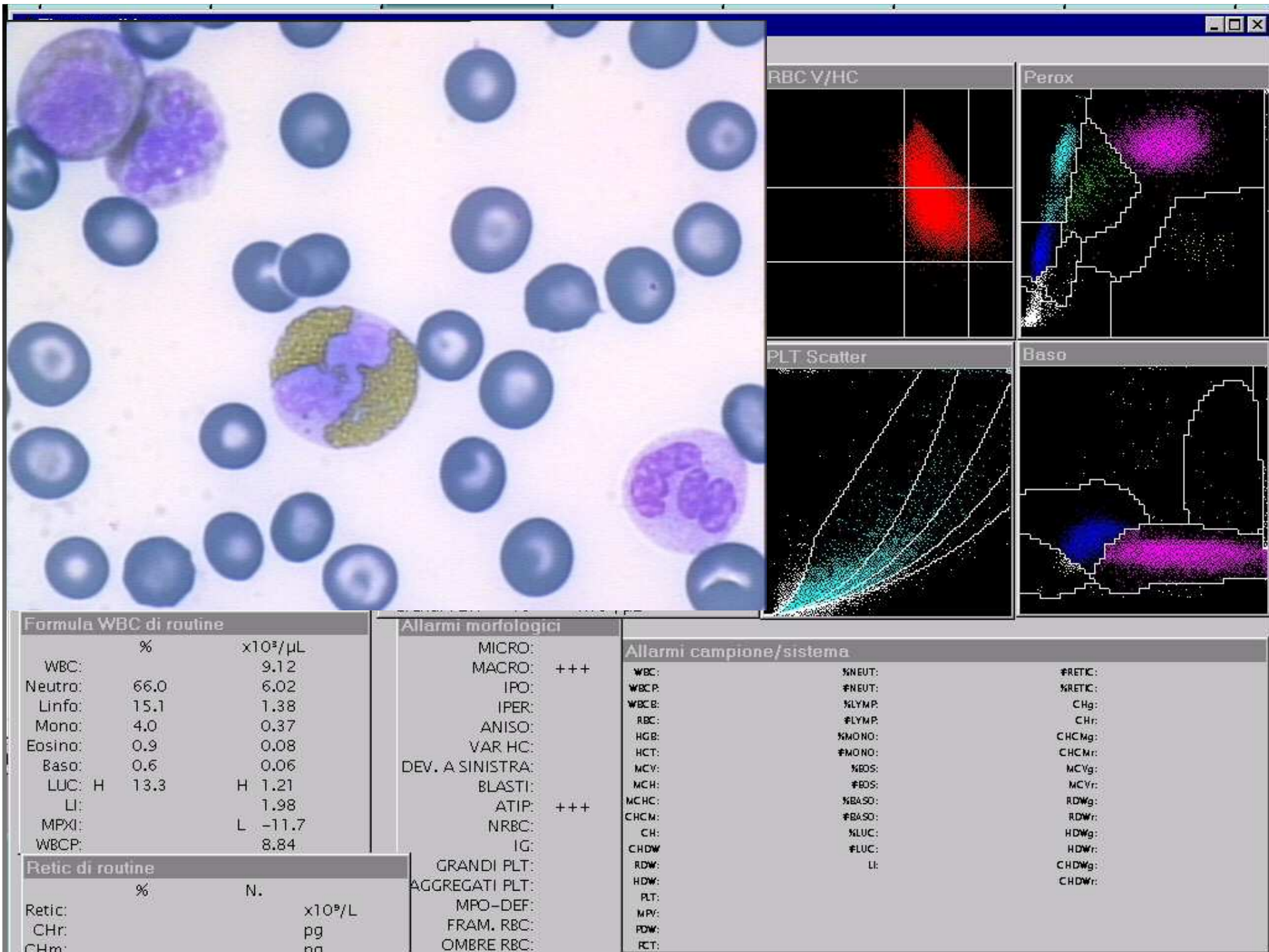


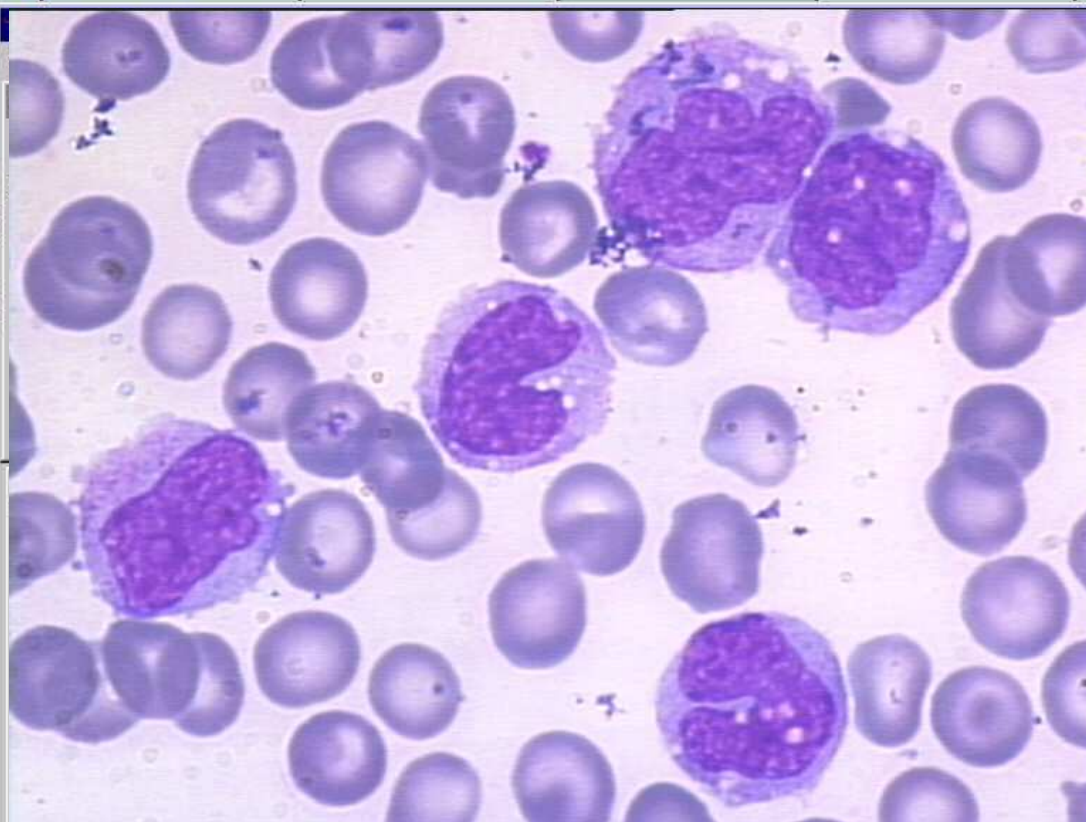
PLT Scatter



Baso







PLT: 164 $\times 10^3/\mu\text{L}$
MPV: 8.1 fL

MPC: 24.8 g/dL
MPM: 1.91 pg
Grandi PLT: 4 $\times 10^3/\mu\text{L}$

Formula WBC di routine

	%	$\times 10^3/\mu\text{L}$
WBC:		5.53
Neutro:	60.0	3.32
Linfo:	20.1	1.12
Mono: H	15.3	0.85
Eosino:	1.5	0.08
Baso:	0.7	0.04
LUC:	2.3	0.13
LI:		2.07
MPXI:		-4.3
WBCP:		5.59

Retic di routine

	%	N.	$\times 10^9/\text{L}$
Retic:			
CHR:			pg
CHM:			pg

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:

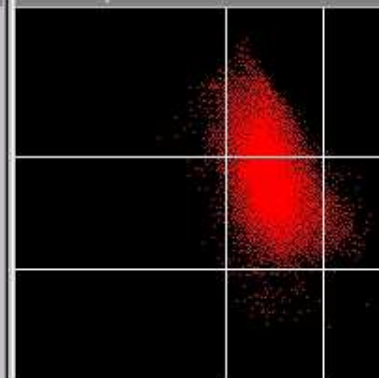
Allarmi campione/sistema

WBC:
WBCP:
WBCB:
RBC:
HGB:
HCT:
MCV:
MCH:
MCHC:
CHCM:
CH:
CHDW:
RDW:
HDW:
PLT:
MPV:
PDW:
PCT:

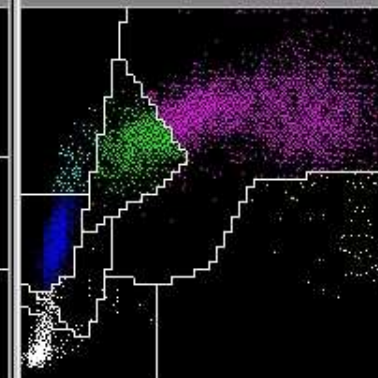
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#NEUT:
%LYMP:
#LYMP:
%MONO:
#MONO:
%EOS:
#EOS:
%BASO:
#BASO:
%LUC:
#LUC:
LI: VB

#RETIC:
%RETIC:
CHg:
CHr:
CHCMg:
CHCMr:
MCVg:
MCVr:
RDWg:
RDWr:
HDWg:
HDWr:
CHDWg:
CHDWr:

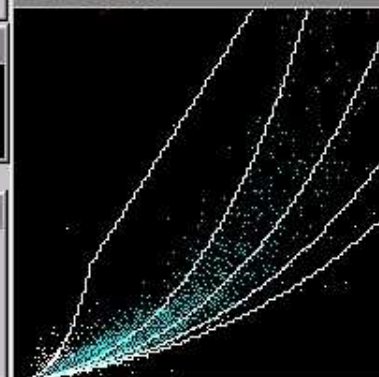
RBC V/HC



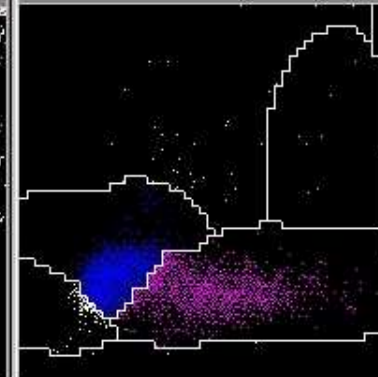
Perox



PLT Scatter



Baso



The WHO Classification of Myeloid Neoplasms
(Vardiman JW, Lee Harris N, Brunning RD.
Blood, 2002; 100:2292)

“Cytochemical studies (myeloperoxidase, nonspecific esterase) and/or immunophenotype must provide evidence that the neoplastic cells belong to one or more of the myeloid lineages.”

Specificity of the ADVIA 120 for the Diagnosis and Classification of Leukemia

- Using automated cytochemistry (PA+ND analysis) and the morphology of leukemic cell distribution, leukocyte differential count is transformed from a basic first level screening test (CBC) to a second level test (pre-microscope and pre-immunophenotype classification)
- Morphology: single cells
- Automated cytochemistry: cell populations

The PANDA Method

Peroxidase
Activity
Nuclear
Density
Analysis



Morphology of the Blood

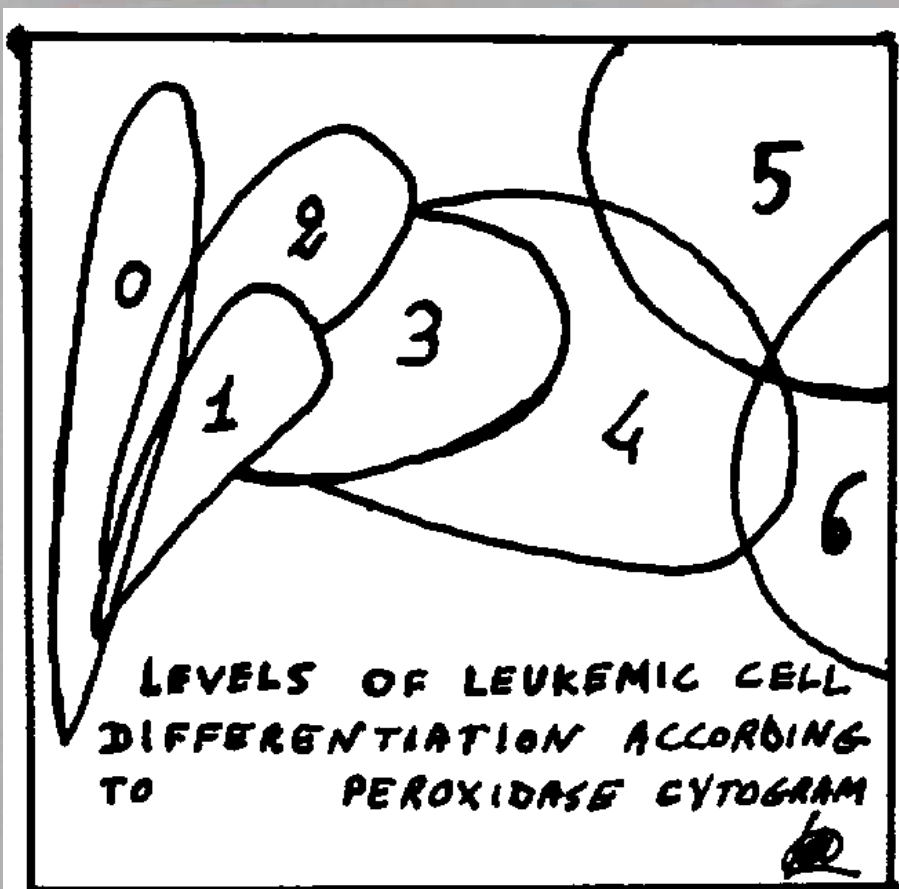
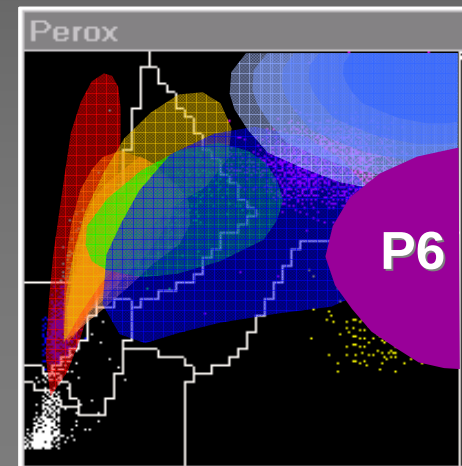
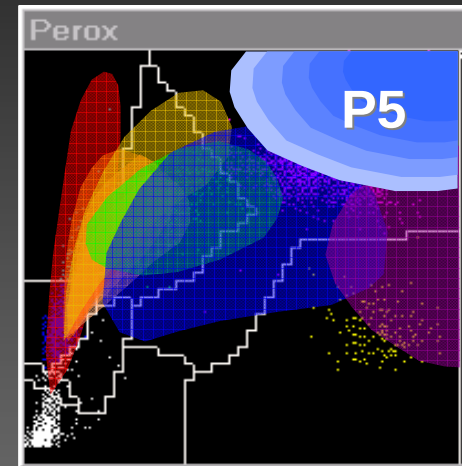
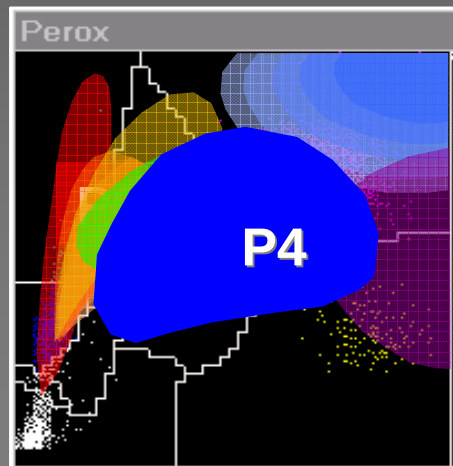
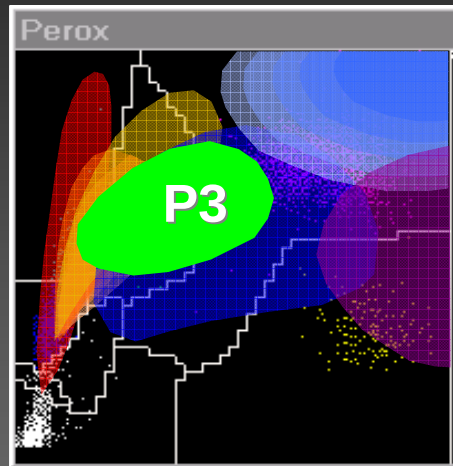
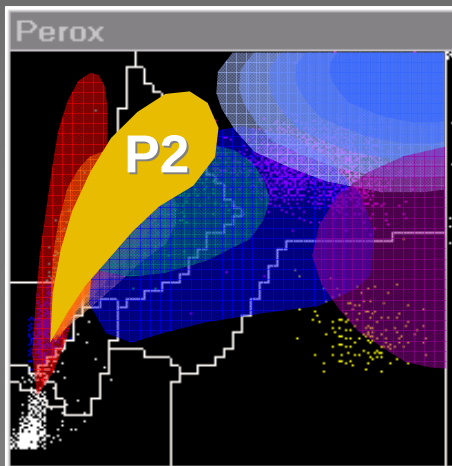
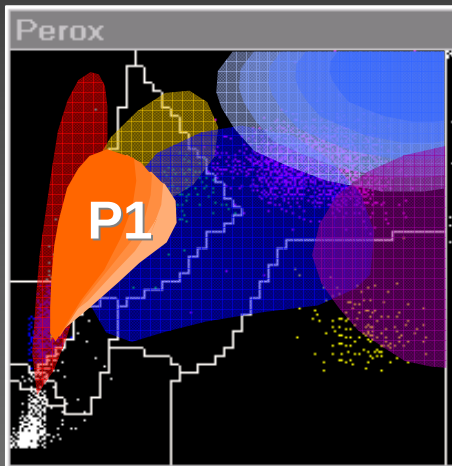
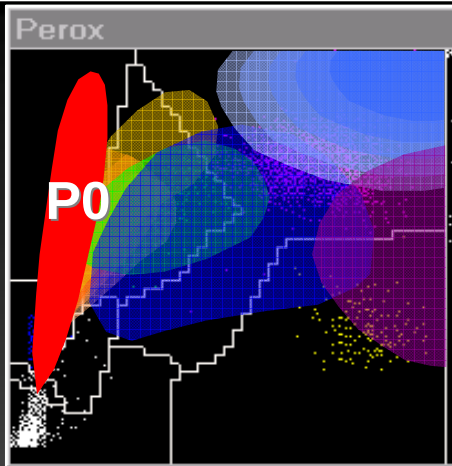


Table 2.2 Categories of peroxidase activity evaluated from the morphology of leukemic cells in the peroxidase channel of the H*1 cytogram³⁶.

Class	Characteristics of leukemic cells	Morphology of peroxidase cytogram	Diseases in which the abnormality may be observed*	Examples of printouts
P0	Absence of peroxidase activity No myeloid differentiation	A large fusiform cluster with a pointed upper end; vertical or slightly inclined to the right. The extension upwards indicates the size of the cells (and coincidence). The right hand edge of the cluster is distinct	ALL L1-L3 CLL, PLL, HCL NHL, IM AML M0 and M7 AML M5a and M6	6.1 7.1 5.1
P1	A small number of cells with weak peroxidase activity Early or partial myeloid differentiation	A fairly thick, short LUC cluster, inclined to the right, often with a rounded tip. The right hand margin is not distinct as there is spread of signals towards the center of the cytoqram	AML M1 AML M5a and M5b AML M2	5.2
P2	Homogeneous moderately strong peroxidase activity Intermediate level of myeloid differentiation	A more or less homogeneous cluster of cells separate from the LUC cluster, spreading across the monocyte area and beginning to spread into the neutrophil area	AML M1 AML M2 AML M4 AML M5a	5.3
P3	Moderate to strong peroxidase activity Marked myeloid differentiation, which is either homogeneous or heterogeneous	The cells form a large oval (M2, M4) or triangular (CML) cluster which extends from the monocyte area well into the neutrophil area	AML M2 AML M4 CML IMF	5.4 5.6 4.1
P4	Very strong peroxidase activity, very large cells Extensive but atypical granulocytic differentiation	The cells form a cluster, usually dispersed, at the upper right hand corner of the cytoqram	AML M2 (rare) Atypical CML AIDS, MDS AML M3 (rare)	3.15
P5	Extremely high peroxidase activity Promyelocytic differentiation	Homogeneous cluster which emerges from the right hand border of the cytoqram. The strong staining reduces the light scattering signal and therefore the apparent size of the cells	AML M3	5.5
PM	Multiple populations with different peroxidase activities (P0/P1, P0/P2, P0/P3, etc.)	Variable combinations of the above features. The commonest image is in the form of a V, uniting a fusiform LUC cluster with a cluster of peroxidase positive cells	AML M4 AML M5b AML M2 BC of CML IMF	5.8

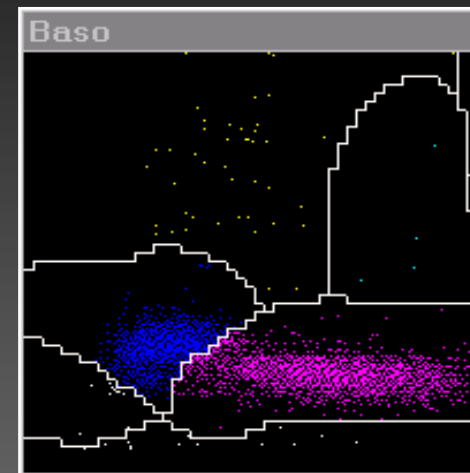
Peroxidase Patterns



Nuclear Density Patterns

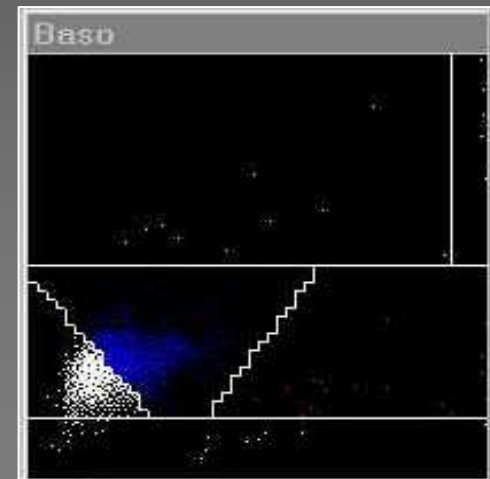
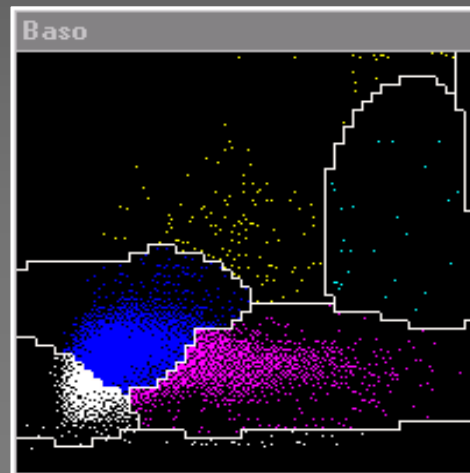
D0

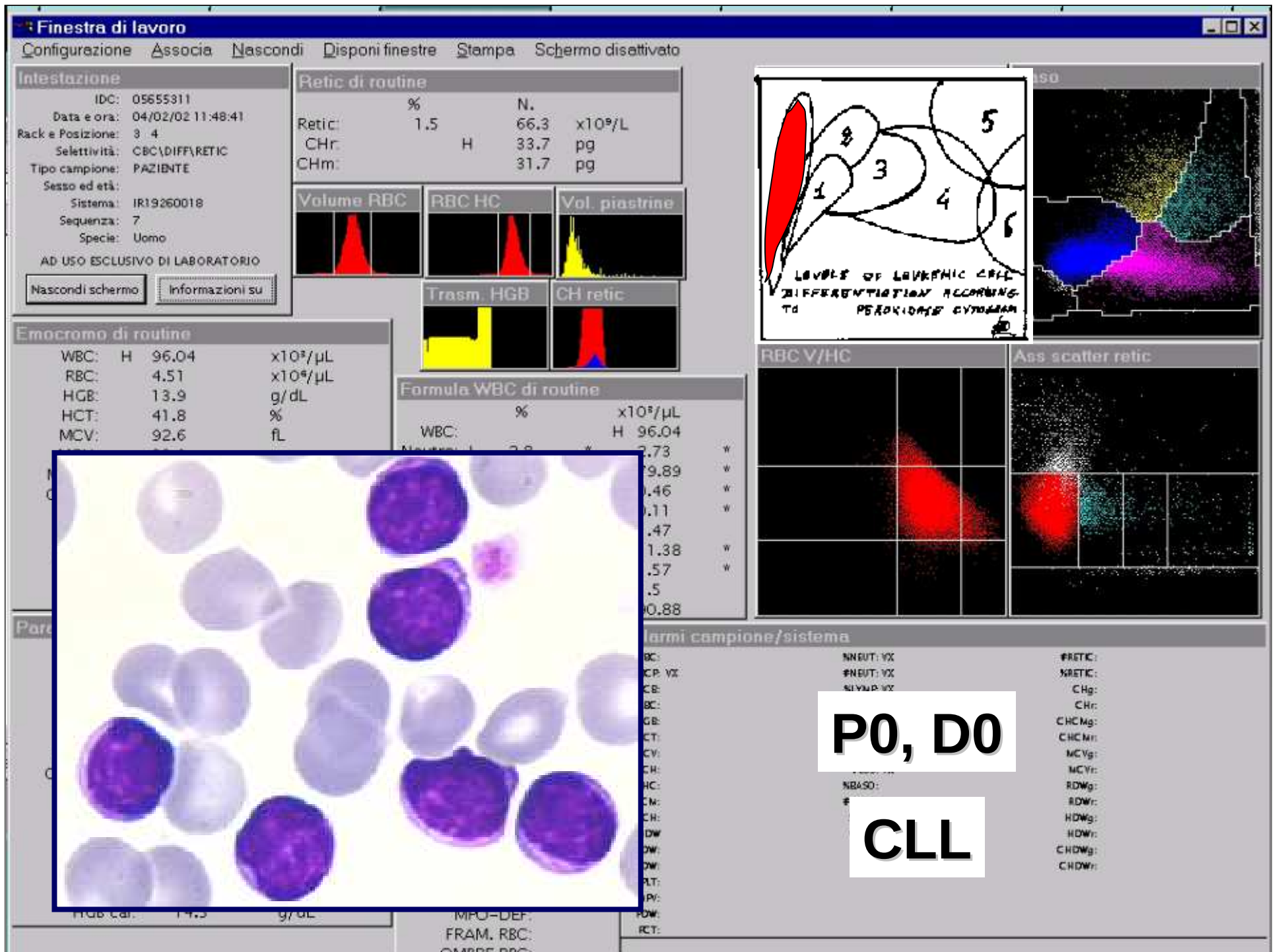
Normal rounded MNC cluster shape and no blast flag.

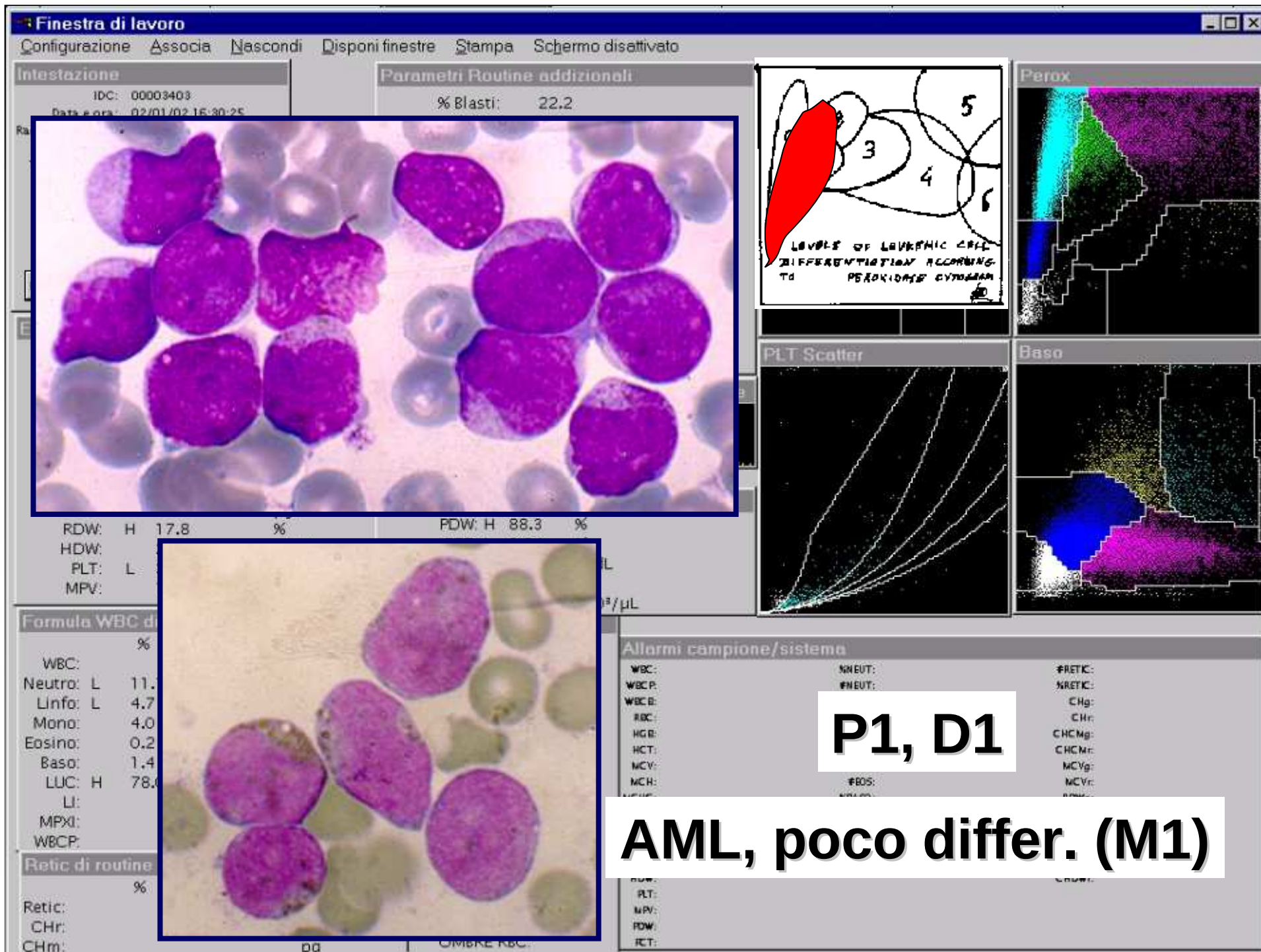


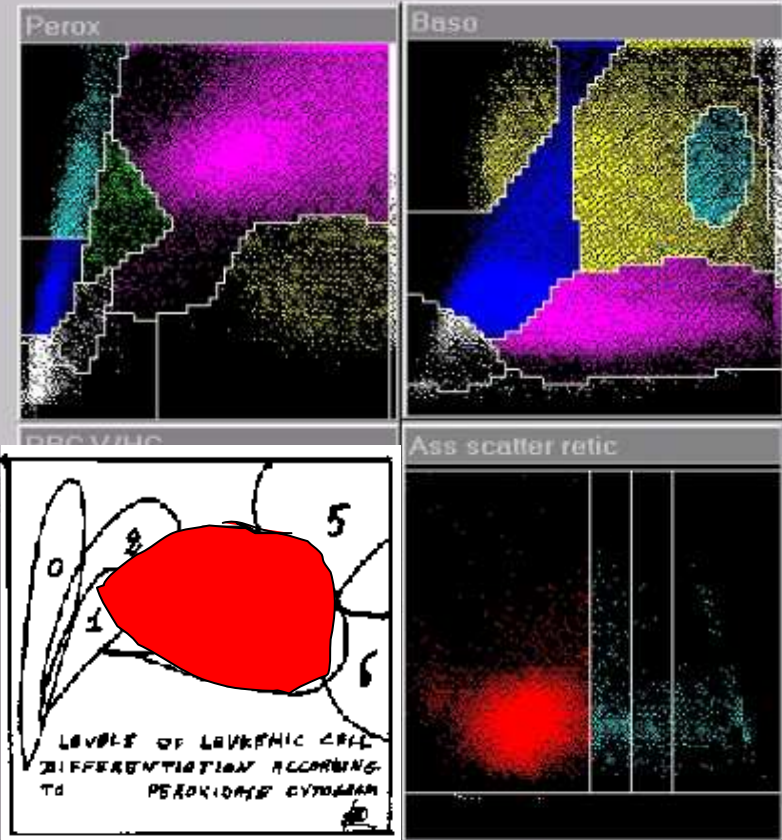
D1

The MNC Cluster is shifted down and left to blast area (usually with flag)









P4, D0

CGL

Parametri Routine

% Blast:
% Iper:
% Ipo:
% Macro:
% Micro:
Fram. RBC:
Ombre RBC:
Neutro X:
Neutro Y:
MNx:
MNY:
%MN:
%PMN:
HGB cal:

Allarmi campione/sistema

WBC: WC
WBC P: XS
WBC R:
RBC: CC
HGB: CC
HCT: CC
MCV: CC
MCH: CC
MCHC: CC
CHCM: CC
CH: CC
CHDW: CC
RDW: CC
RDW: CC
PLT:
MPV:
PDW:
PCT:

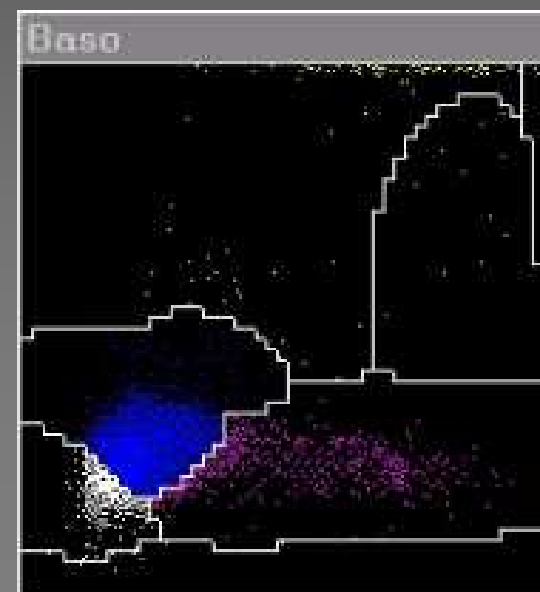
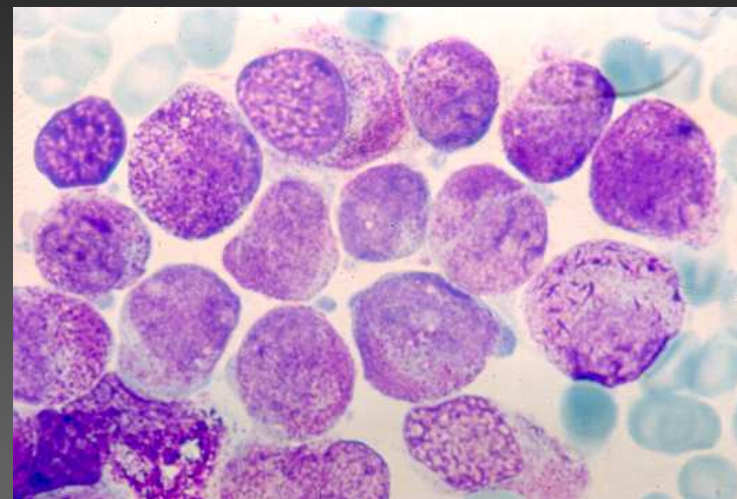
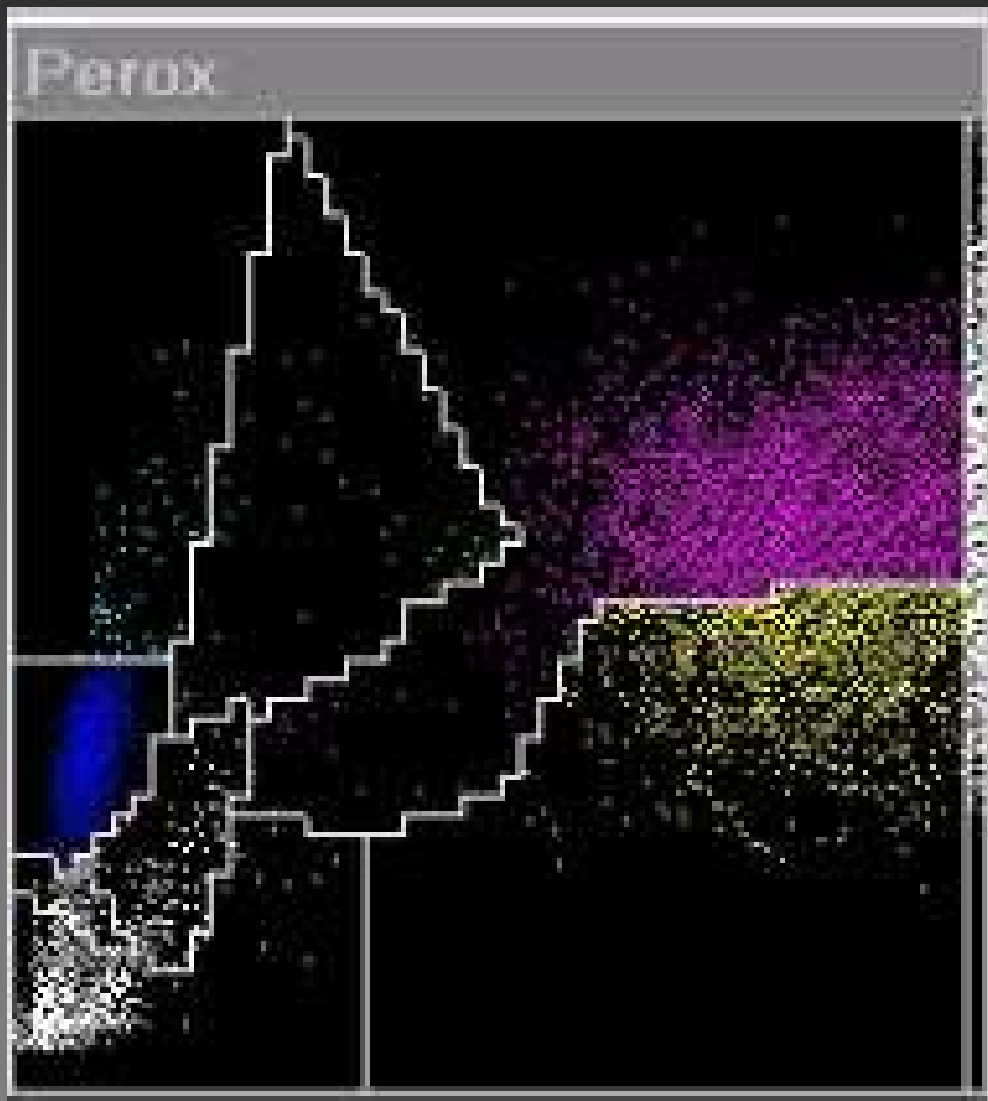
P4

#RETC:CA SE
 NRETC:CA SE
 CHg:CA SE
 CHr:CA SE
 CHCMg:CA SE
 CHCMr:CA SE
 MCVg:CA SE
 MCVr:CA SE
 RDWg:CA SE
 RDWr:CA SE
 HDWg:
 HDWr:
 CHDWg:CA SE
 CHDWr:CA SE

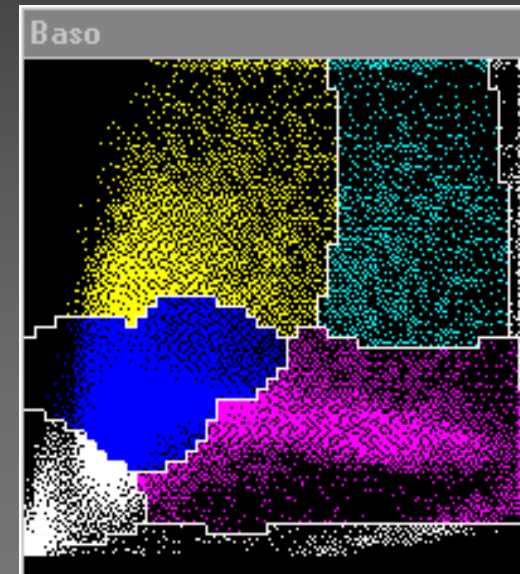
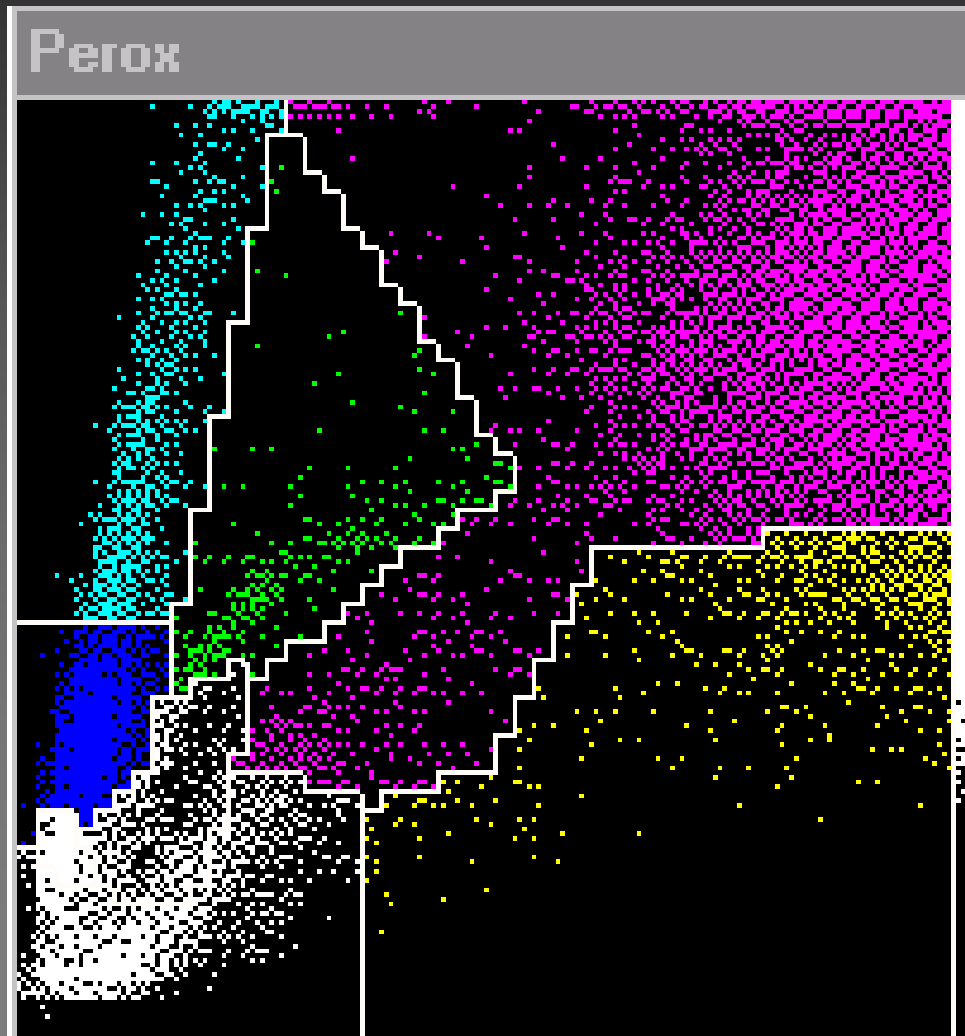
WHO Classification of Myeloid Neoplasm: Specific Cytogenetic Types

- Acute Myeloid Leukemias (AML) with recurrent cytogenetic translocations
 - AML with t(8;21)(q22;q22), AML1 (CBF α)/ETO
 - Acute promyelocytic leukemia: AML with t(15;17)(q22;q11-12) and variants, PML/RAR α
 - AML with abnormal bone marrow eosinophils with inv(16)(p13q22) or t(16;16)(p13;q11), CBF β /MYH11X)
 - AML with 11q23 (MLL) abnormalities

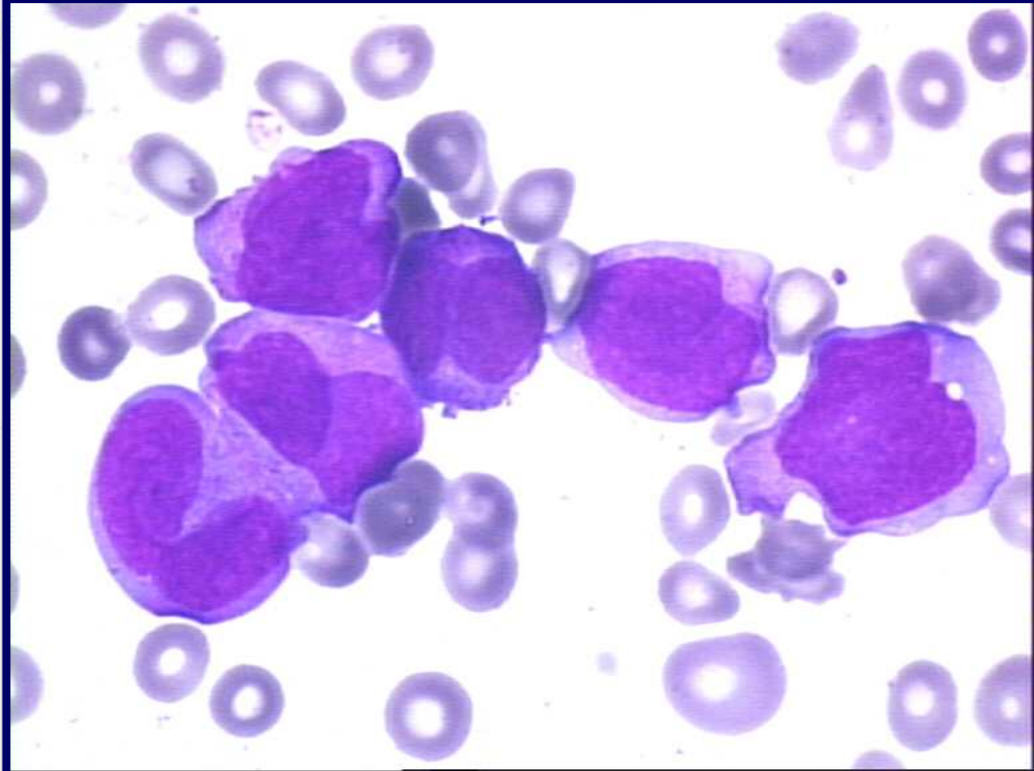
AML-M3 t(15;17) is always P6-D1



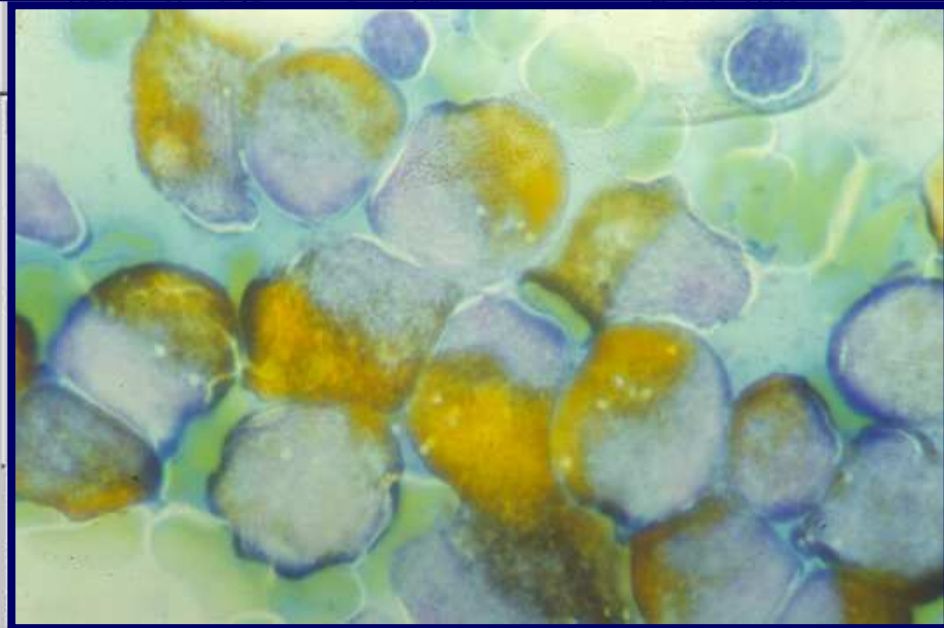
AML-M3 t(15;17) is always P6-D1: even in bone marrow



Finestra di lavoro

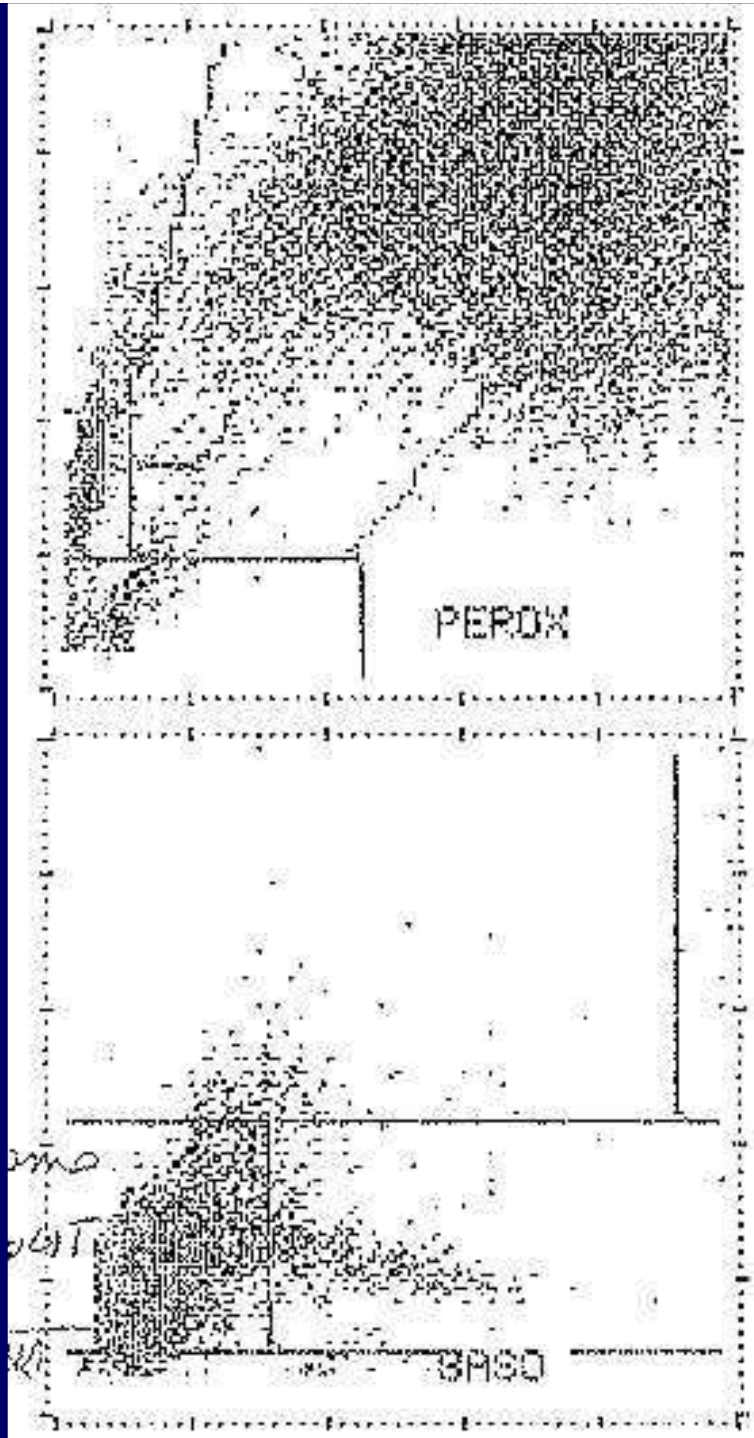


strine



armi campione

- XC: WC
- CP: XS
- CB:
- CC: CC
- GB: CC
- CT: CC
- CV: CC
- HC: CC
- CI: CC
- MI: CC
- HI: CC
- DW: CC
- W: CC
- W: CC
- CT:
- W:
- CT:



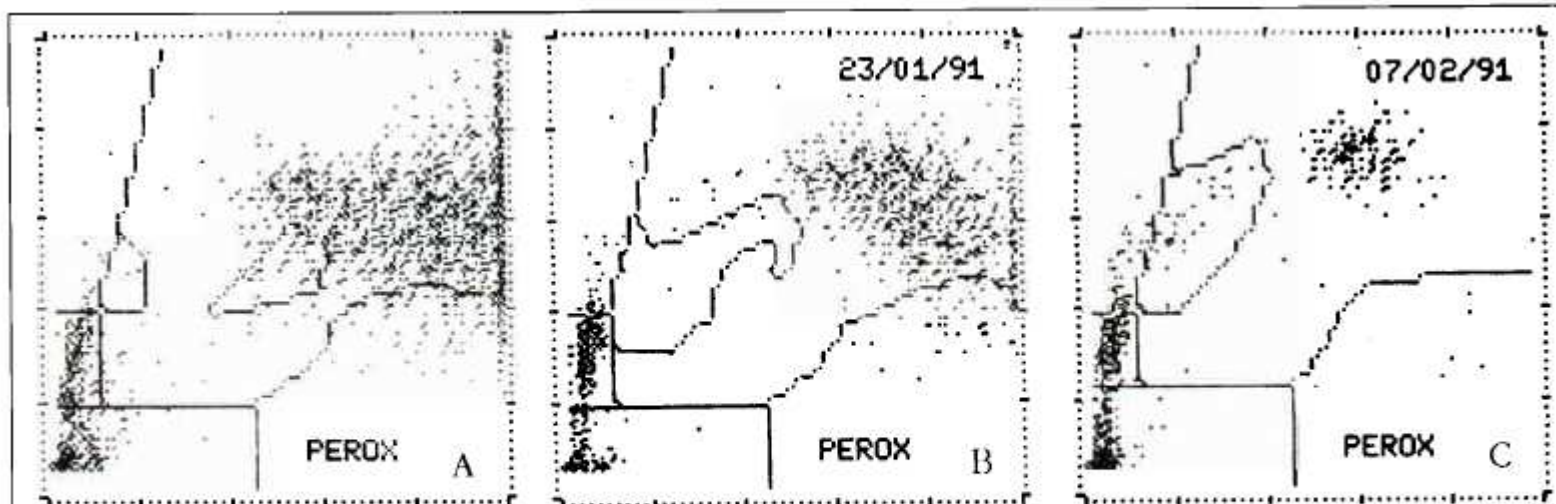


Fig. 4. Modificazioni del citogramma del canale della perossidasi del sistema H*1 durante trattamento con all-TRA in un caso di leucemia a promielociti. A: alla diagnosi si osserva il caratteristico raggruppamento dei promielociti leucemici che deborda dal lato destro del citogramma, in rapporto all'elevatissima attività perossidasi di queste cellule. B: dopo 20 giorni di trattamento con all-TRA la popolazione leucemica appare nettamente spostata verso sinistra, fino a fondersi con i neutrofili normali ricomparsi nel sangue periferico (riduzione dell'attività perossidasi). C: dopo 35 giorni di trattamento le cellule patologiche sono scomparse e si osserva solo il cluster dei neutrofili normali.

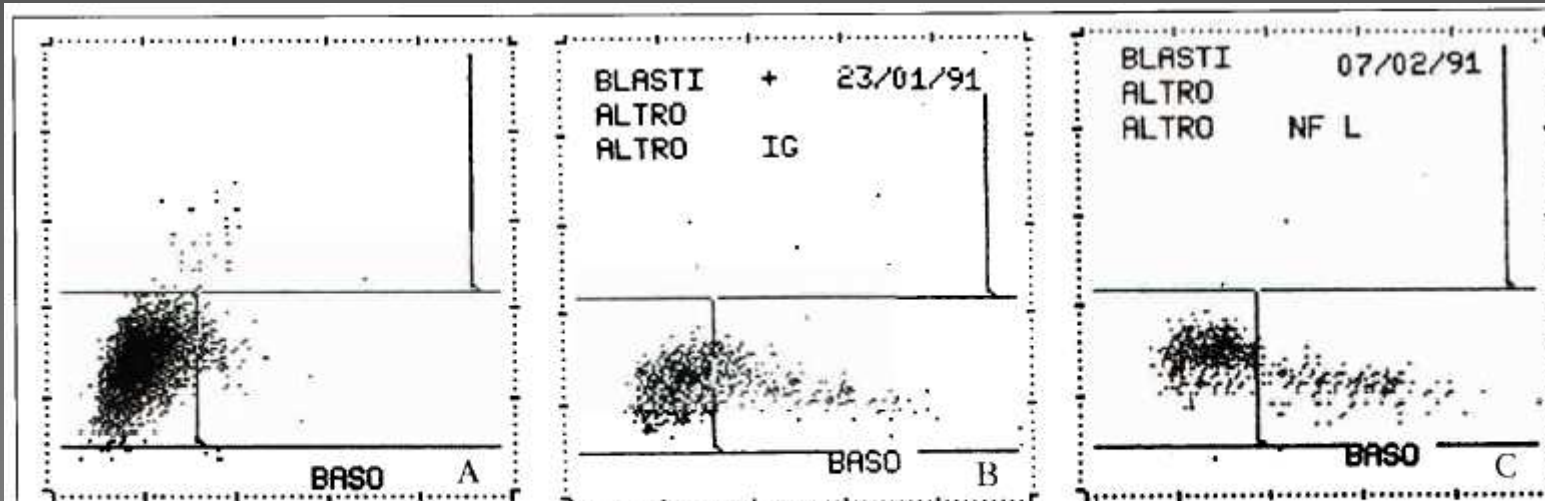
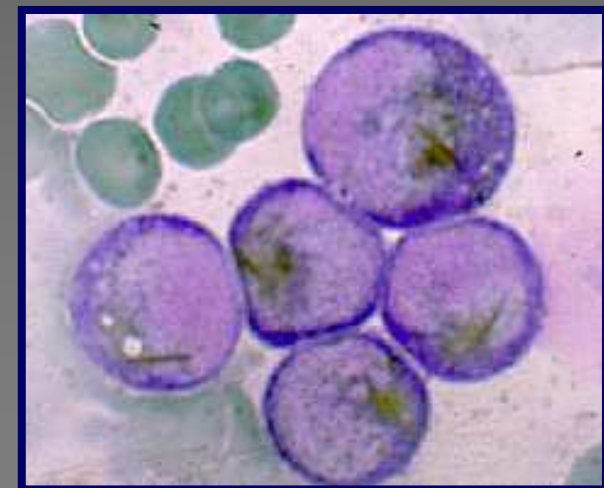
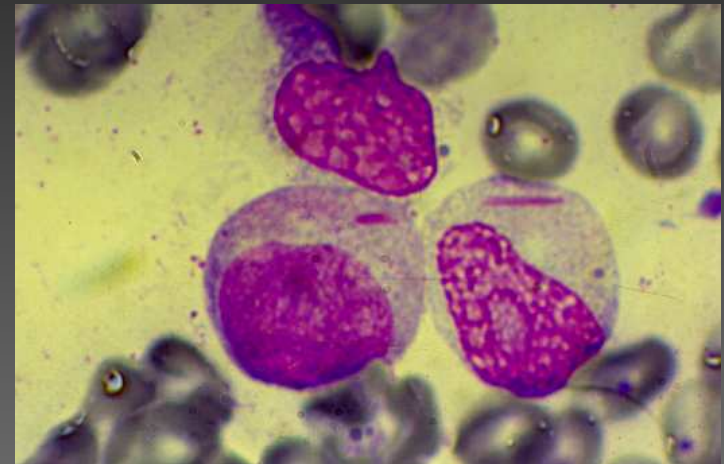
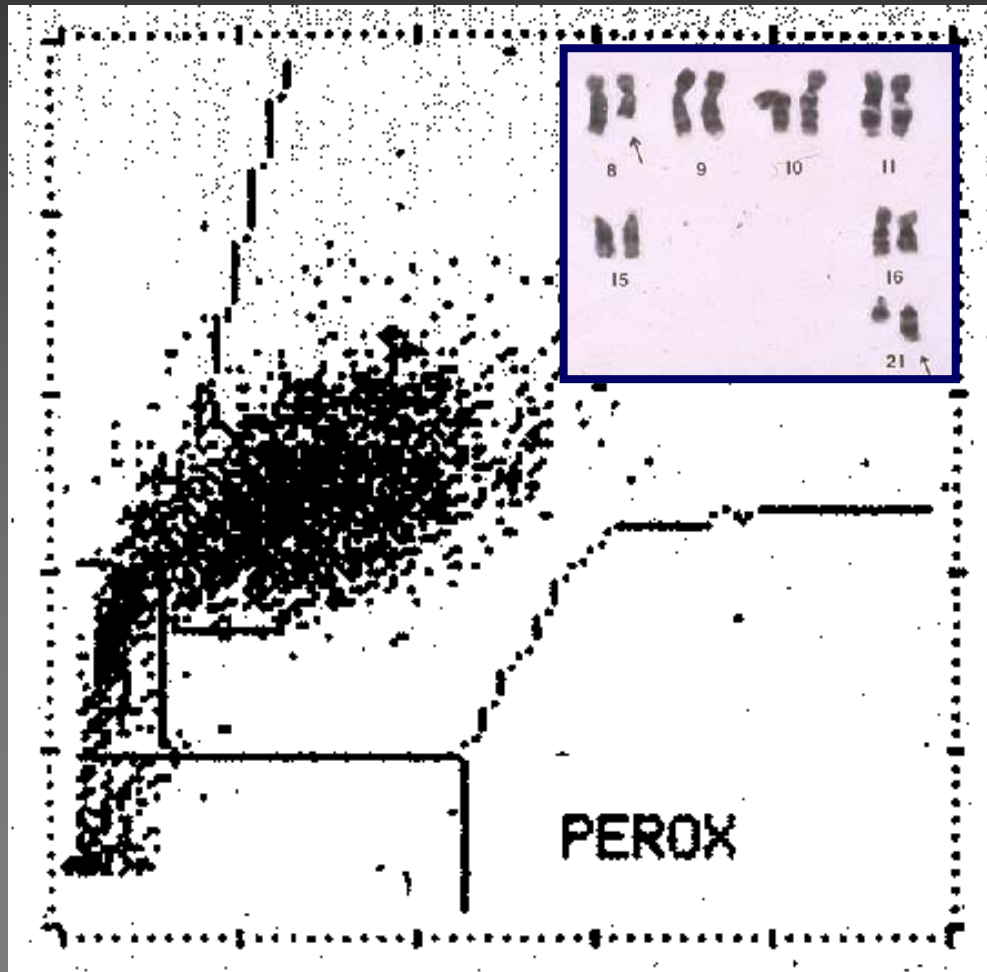
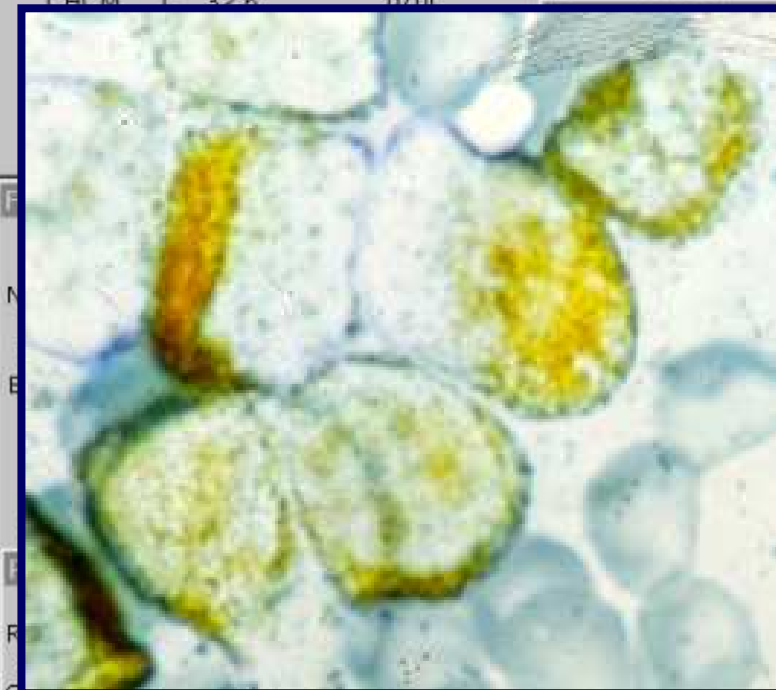
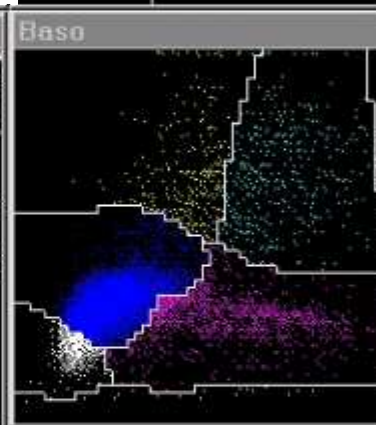
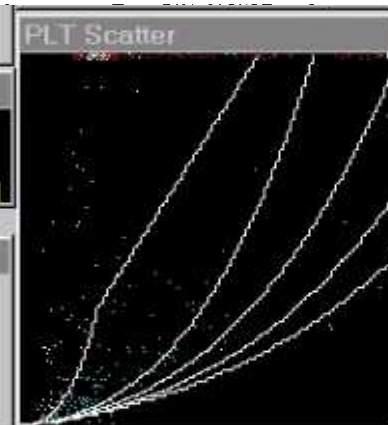
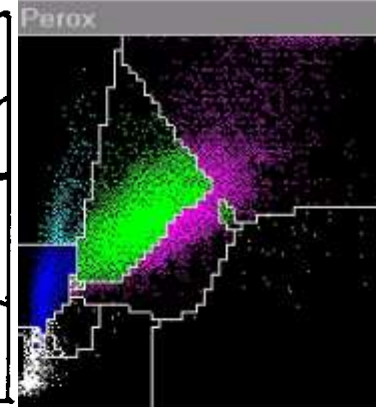
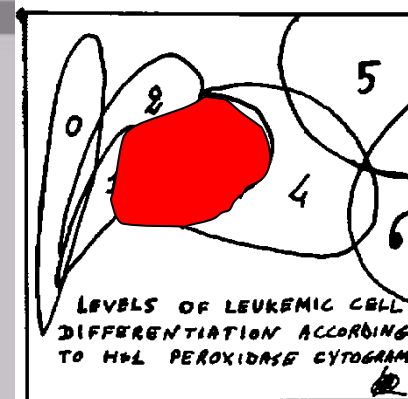
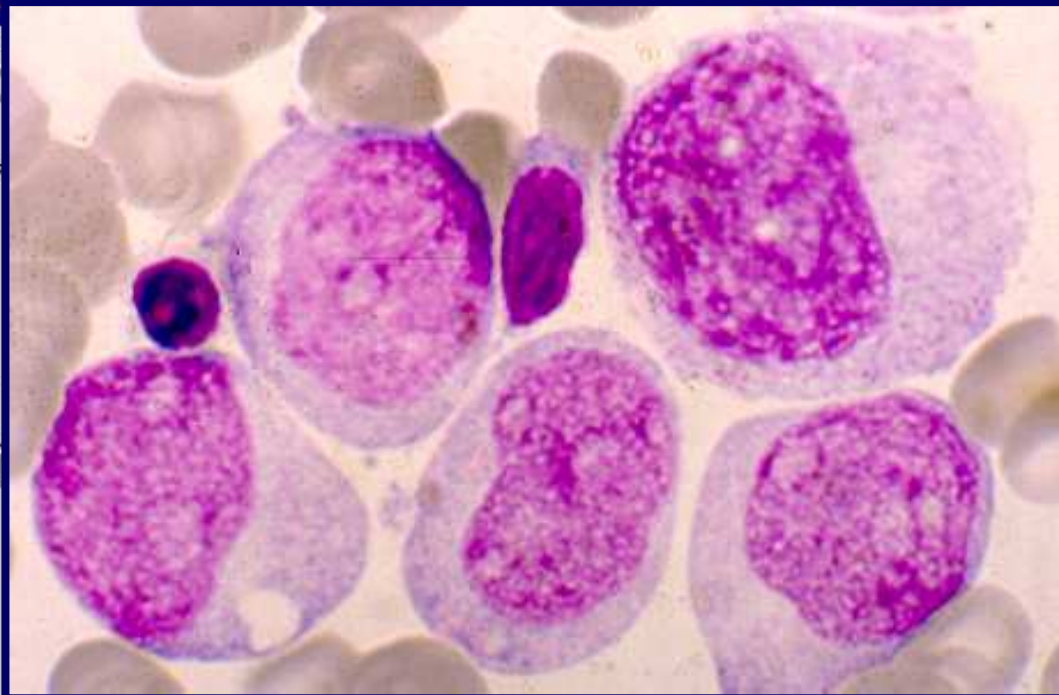


Fig. 5. Modificazioni del citogramma del canale dei basofili del sistema H*1 durante trattamento con all-TRA in un caso di leucemia a promielociti. A: alla diagnosi i promielociti leucemici si raccolgono in un cluster che deforma e sposta verso il basso e verso sinistra la "testa" dei mononucleati, in rapporto alla bassa densità della loro cromatina nucleare. È presente l'allarme Blasti. B: dopo 20 giorni di trattamento con all-TRA l'aspetto "blastico" permane, ma è meno evidente (aumento della densità nucleare); è comparsa una piccola "coda" di polimorfonucleati. C: dopo 35 giorni l'aspetto del citogramma è praticamente normale, ad eccezione della neutropenia. L'allarme Blasti è scomparso.

AML-M2 t(8;21) is almost constantly P3-D1





izionali
 * %
 * %
 g/dL
 pg
 x10³/μL

ici
 +
 +
 +
 +++
 +++
 WBC:
 WBCP:
 WBCB:
 RBC:
 HGB:
 HCT:
 MCV:
 MCH:
 MCHC:
 CHCM:
 CH:
 CHDW:
 RDW:
 HDW:
 PLT: NT
 MPV: NT
 PDW: NT
 PCT: NT

Allarmi campione/sistema
 NLEUT:
 NLEUT:
 #RETIC:
 NRETIC:
 CHg:
 CHr:
 CHCMg:
 CHCMr:
 MCVg:
 MCVr:
 RDWg:
 RDWr:
 HDWg:
 HDWr:
 CHDWg:
 CHDWr:

P3, D1

AML, M2

Predictive Value of Hematological and Phenotypical Parameters on Postchemotherapy Leukocyte Recovery

**A. Bononi,^{1*} F. Lanza,² L. Ferrari,² M. Gusella,¹ G. Gilli,³ V. Abbasciano,⁴
D. Campioni,² A. Russo,² D. Menon,¹ F. Albertini,¹ L. Stievano,¹⁻⁵ C. Barile,¹
G. Crepaldi,¹ S. Toso,¹ E. Ferrazzi,¹ and F. Pasini¹**

Results: In the nadir phase, the increase of MPXI above the upper limit of normality (>10 ; median 27.7), characterized a slow hematological recovery. MPXI levels were directly related to the cycle number and inversely related to the absolute number of LUCs and $CD34^+/CD45^+$ cells. A faster hematological recovery was associated with a higher LUC increase per day (0.56% vs. 0.25%), higher blast (median 36.7/ μ L vs. 19.5/ μ L) and $CD34^+/CD45^+$ cell (median 2.2/ μ L vs. 0.82/ μ L) counts.

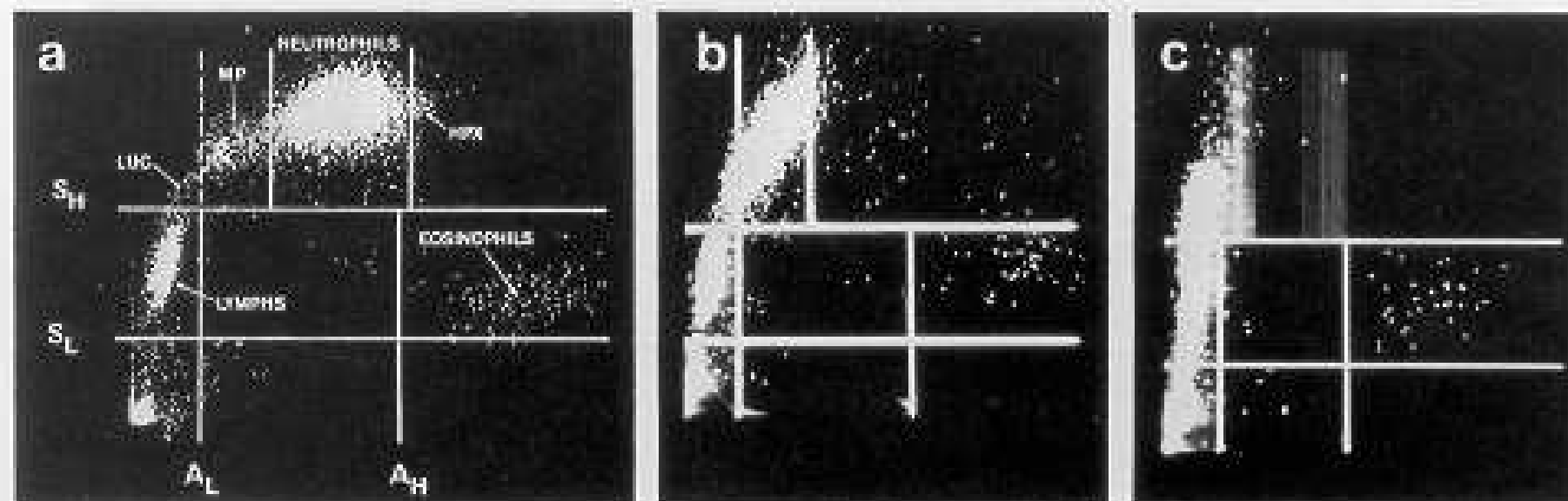


Fig. 1. **a** Normal peroxidase x-y display. Note the neutrophil cluster in the upper central box, laterally delimited by the upper segments of the two absorbance thresholds (A_L and A_H) which are automatically adjusted for each blood sample, according to neutrophil MPO activity. The LUC are large and unstained cells (usually large lymphocytes or peroxidase-negative blasts). **b** Partial MPO deficiency. The neutrophil cluster and the two automatic thresholds are clearly shifted toward the left hand. **c** Total MPO deficiency in a homozygous subject. Neutrophils completely devoid of MPO appear in the upper left hand box, directly in line with the major axis of the lymphocyte population. Note eosinophils retaining their strong MPO-positivity

Acquired Neutrophil Myeloperoxidase Deficiency: An Indicator of Subclinical Activation of Blood Coagulation?

Giuseppe d'ONOFRIO, Rosalba MANCINI, Roberto VALLONE,
Generoso ALFANO, Alfonso CANDIDO, Marina PALLA,
and Giorgio MANGO

ABSTRACT. Using an automated cytochemical analyzer used for routine differential counts, we have been able to demonstrate acquired myeloperoxidase deficiency in 102 patients at our institution. Clinical and laboratory data on these patients showed a high incidence of diabetes mellitus (25.5%) and thrombotic diseases (24.5%), as well as a strikingly constant hyperfibrinogenemia (mean = 635 mg/100 ml; range = 360–1015 mg/100 ml). In 4 additional acute leukemia patients in complete remission, a close time correlation was noted between acquired MPO deficiency, diffuse intravascular coagulation

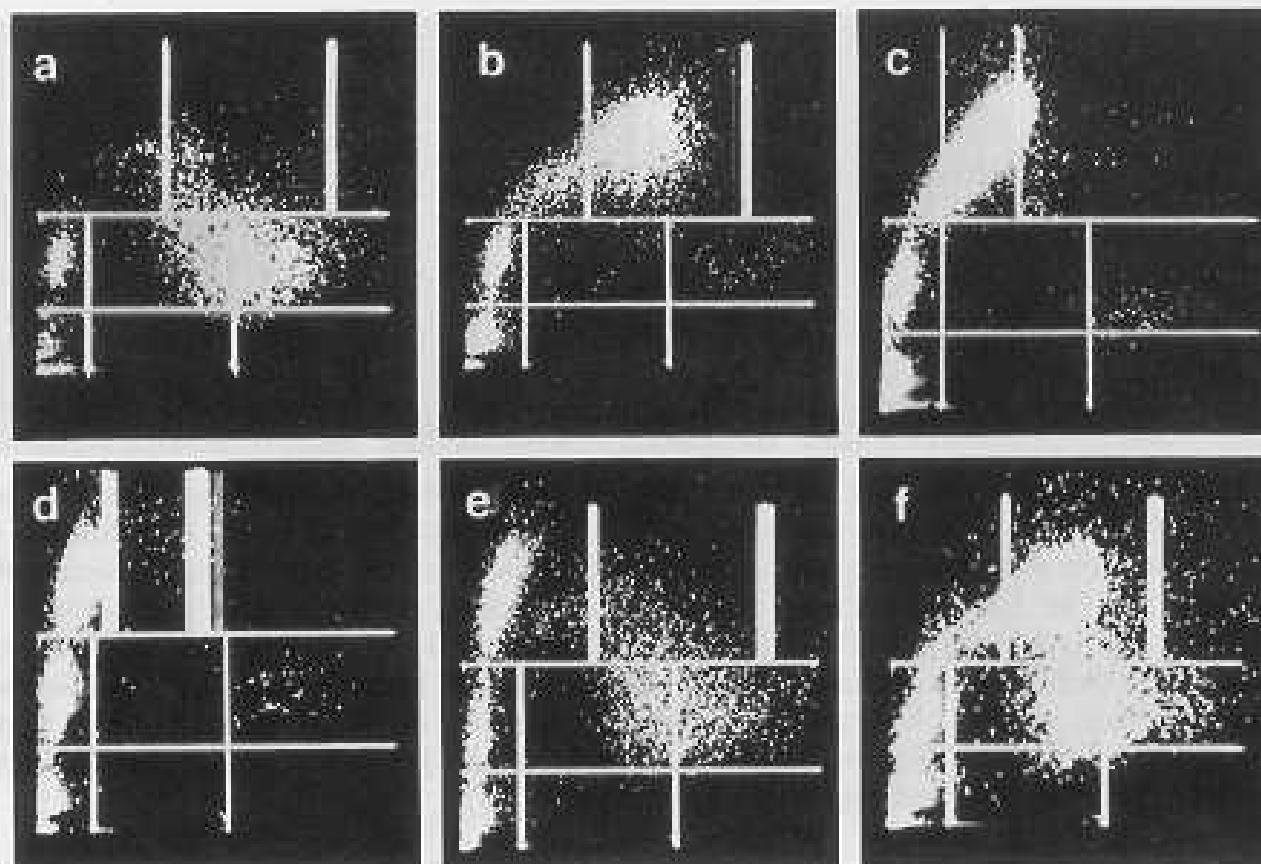
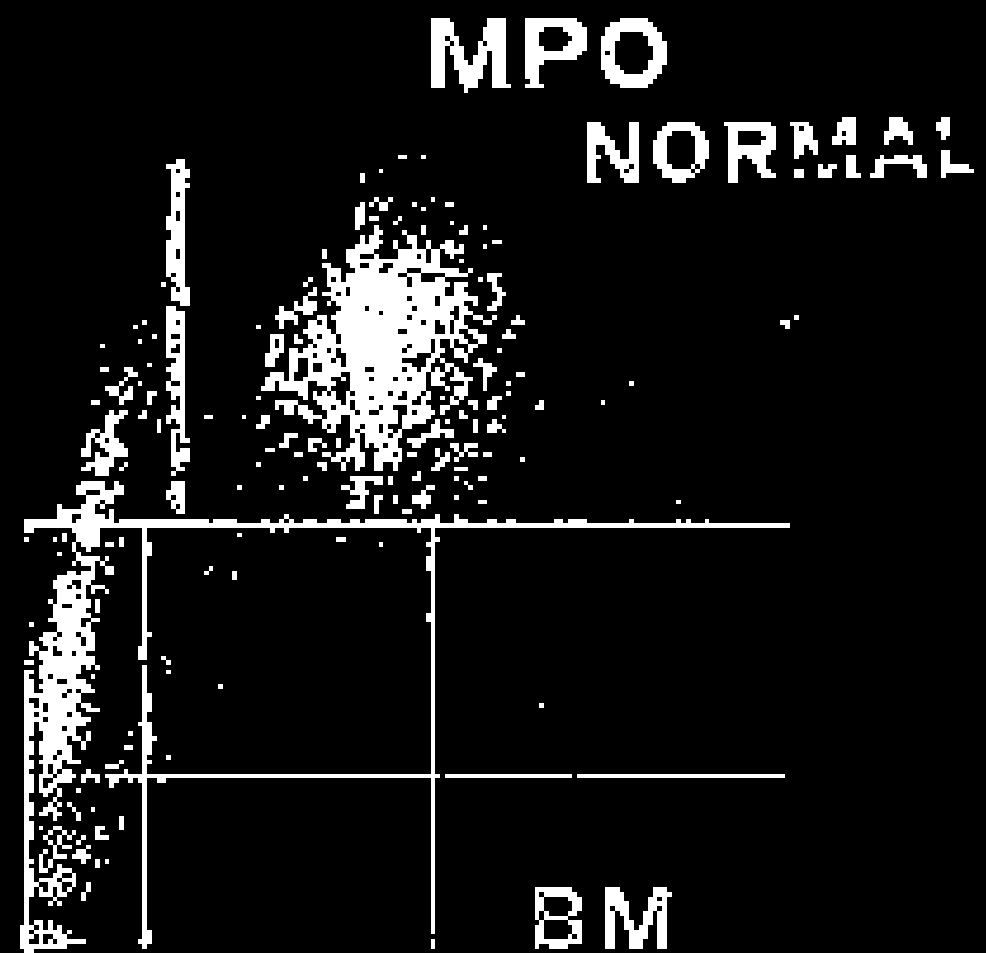
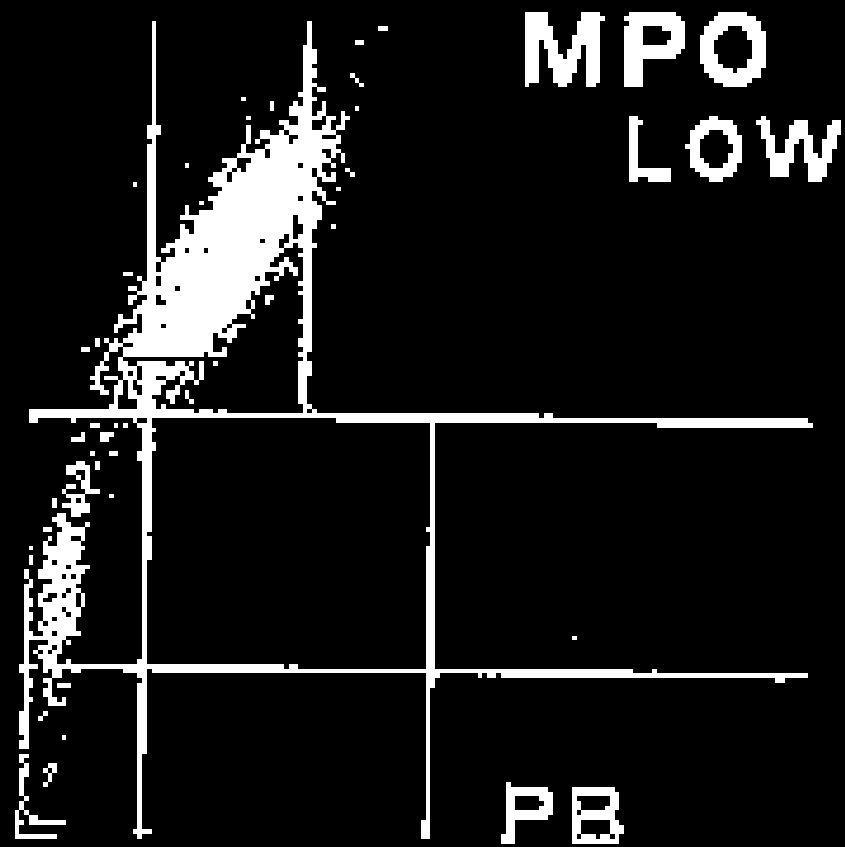
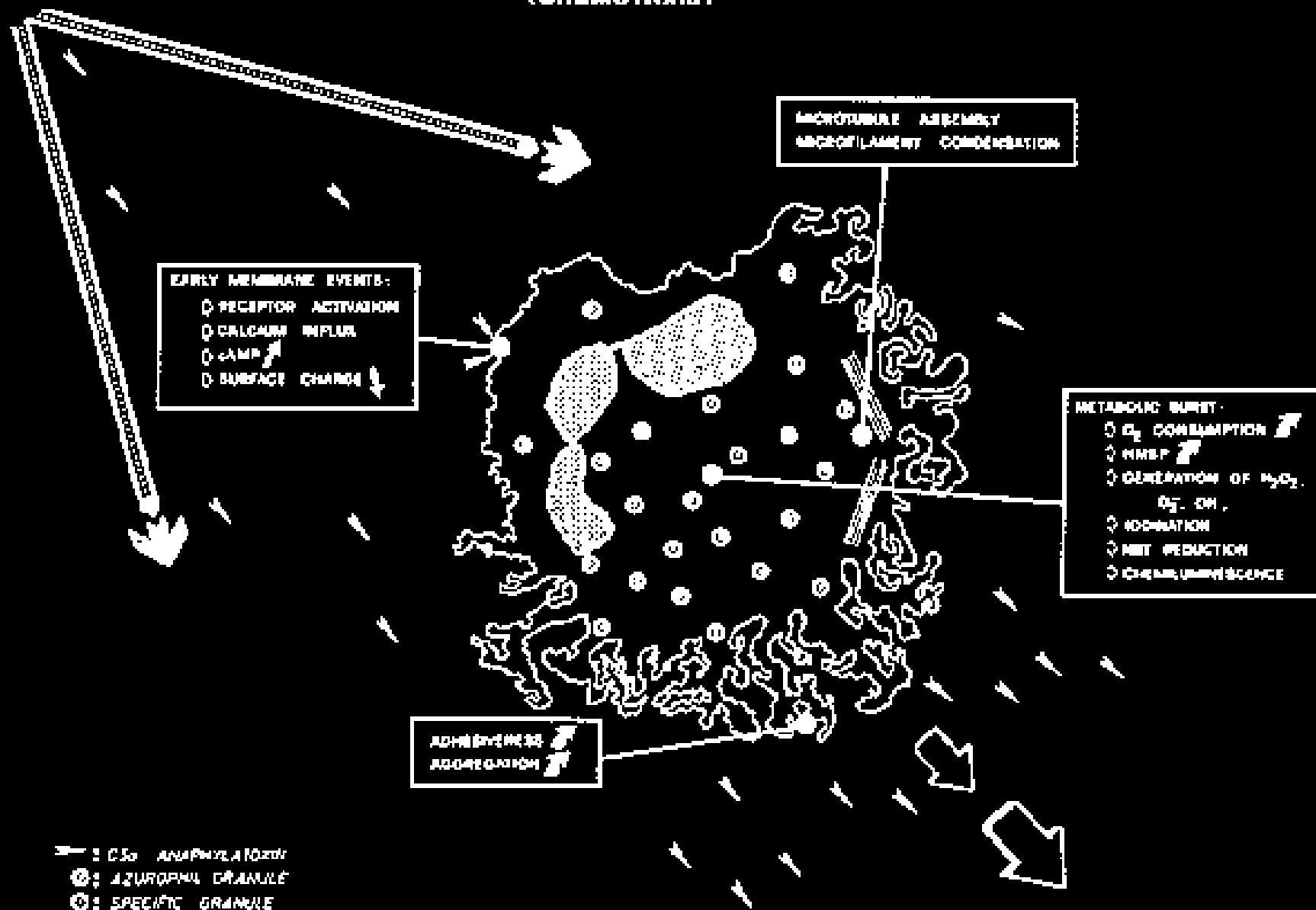


Fig. 2. **a** Typical cluster of leukemic promyelocytes in the middle central box, at diagnosis of acute promyelocytic leukemia. **b** Reversal to a normal x-y display picture after complete remission. **c** Shift to the left hand of the neutrophil population, indicating the development of a partial MPO deficiency during complete remission. **d** Several days after, neutrophils become totally MPO-deficient, and appear clustered in the LUC box. **e** The neutrophils are still devoid of MPO, but a cluster of heavily stained promyelocytes is appearing in the middle central box. **f** Two populations of MPO-positive cells are finally evident: the upper cluster is formed again by normal MPO-positive neutrophils, while the lower one is made by the leukemic promyelocytes (see text)

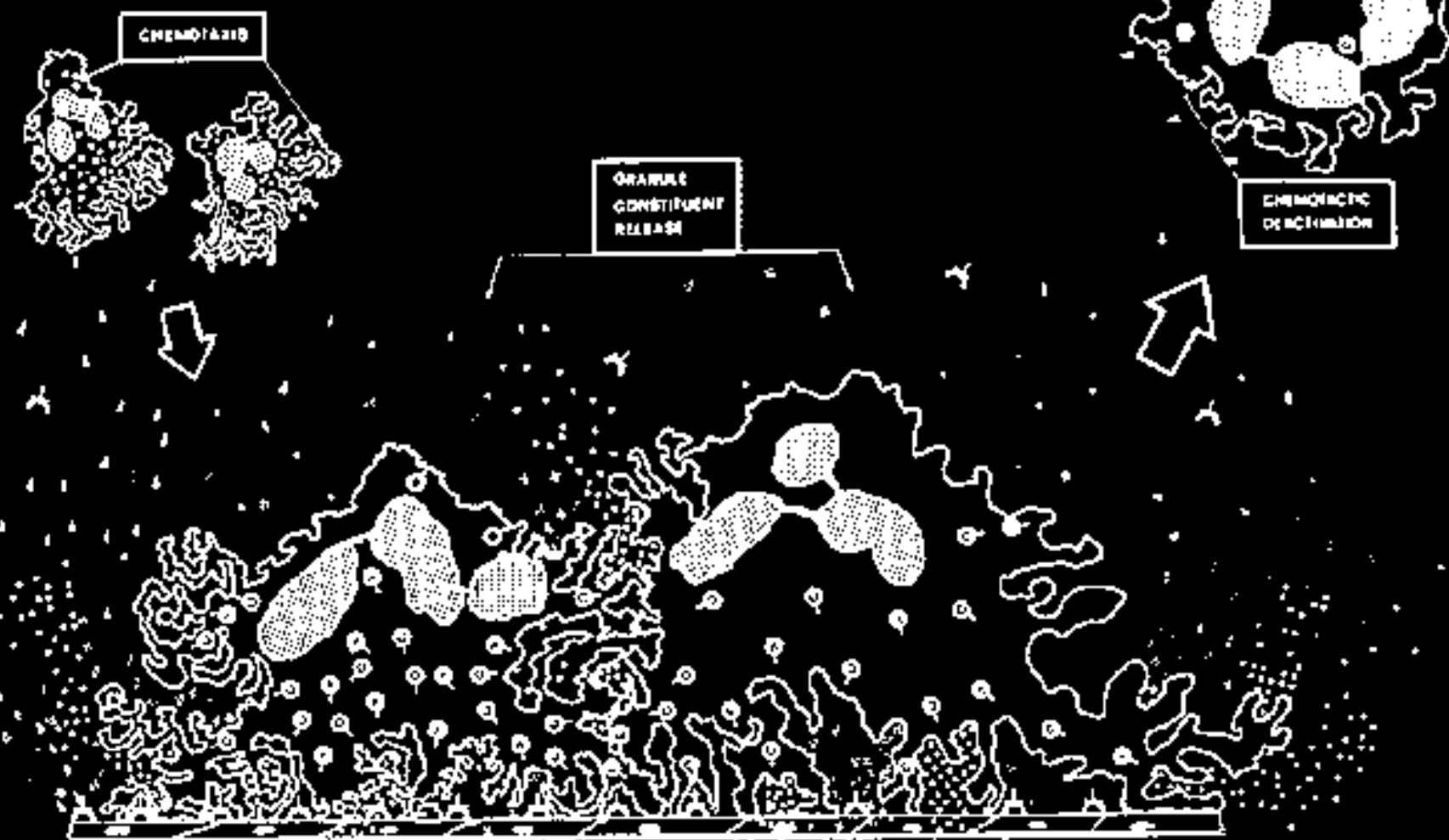


NEUTROPHIL ACTIVATION IN LIQUID PHASE (CHEMOTAXIS)



NEUTROPHIL SURFACE ACTIVATION

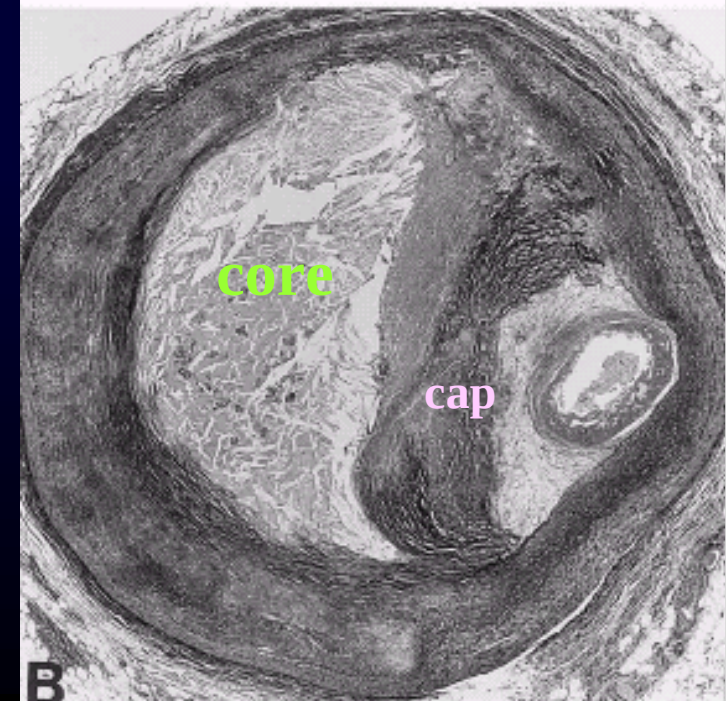
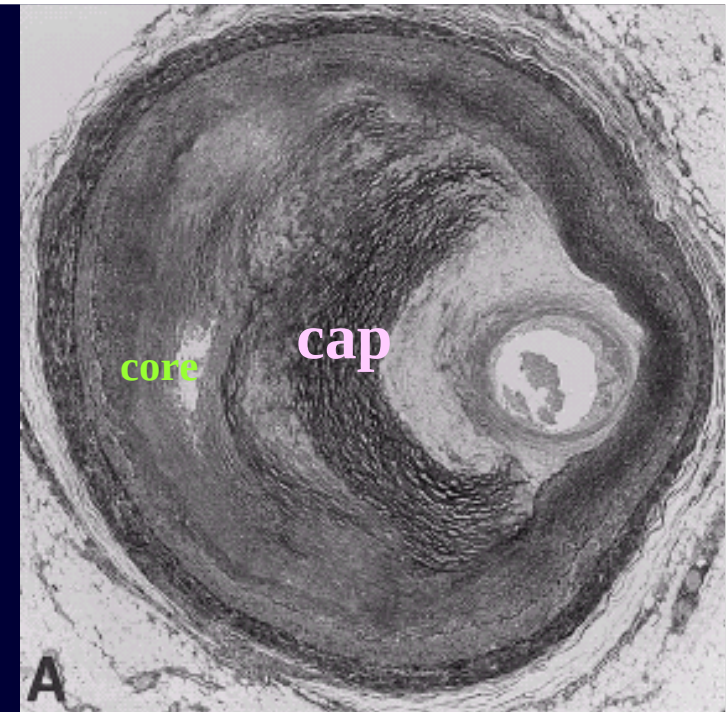
(«REVERSE ENDOCYTOSIS»)



- ⊙ : AZUROPHIL GRANULE
- ⊙ : SPECIFIC GRANULE
- : C5a
- ⊙ : IMMUNE COMPLEX

Acute Coronary Syndromes

- 1) atherosclerotic plaque
 - soft core (gruel)
 - collagen cap
- 2) plaque disruption or fissuring
 - hemodynamic triggers
 - inflammation
- 3) plaque thrombosis
 - thrombogenic substrate
 - local flow disturbances
 - systemic thrombotic propensity:
 - increased platelet reactivity
 - increased procoagulant activity
 - decreased endogenous fibrinolysis



IL-1
IL-6
TNF- α
IFN- γ

ICAM-1
ICAM-2
VCAM-1
Selectins

neutrophils
monocytes
platelets

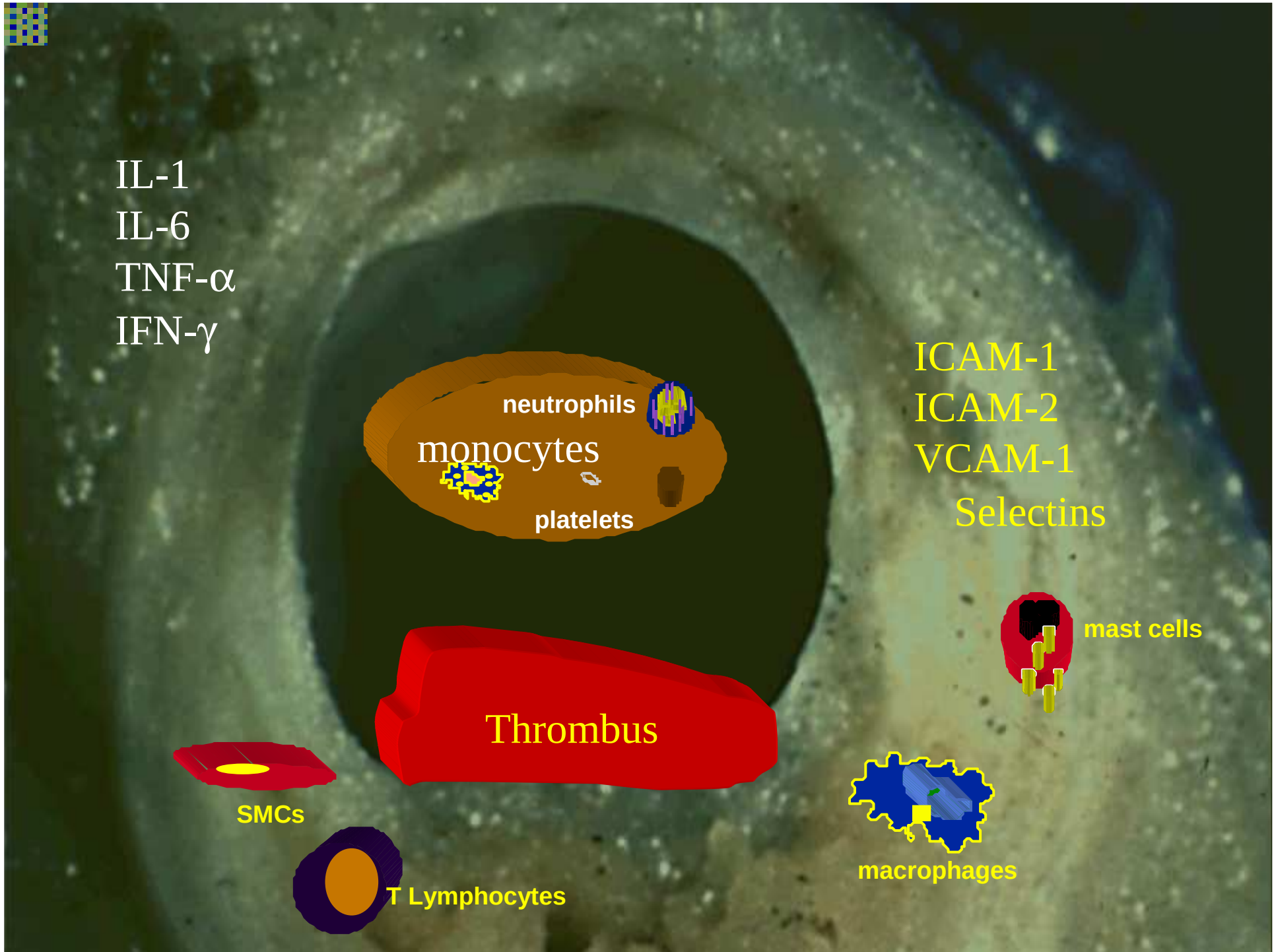
Thrombus

mast cells

SMCs

T Lymphocytes

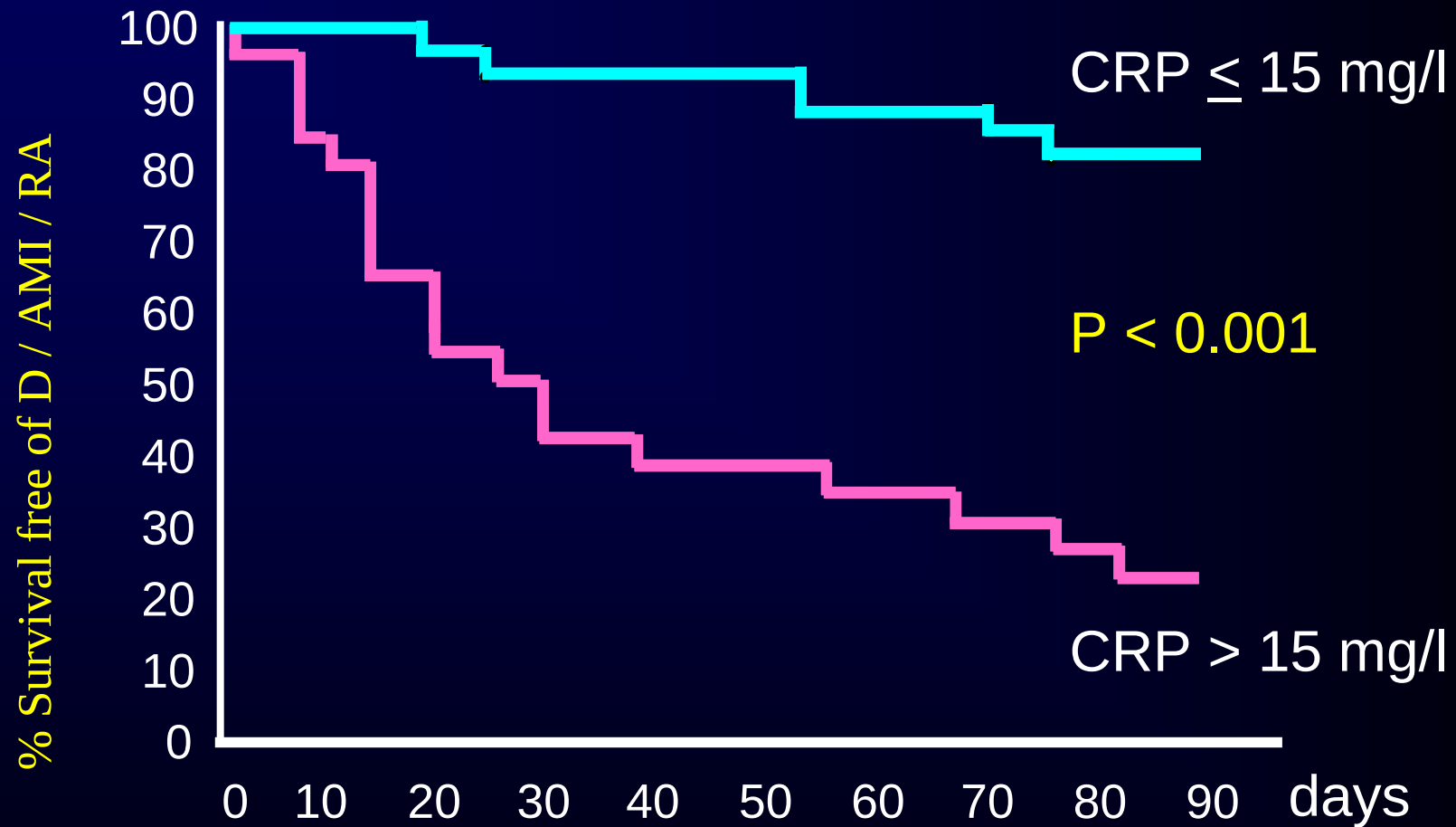
macrophages



Systemic markers of inflammation in ACS

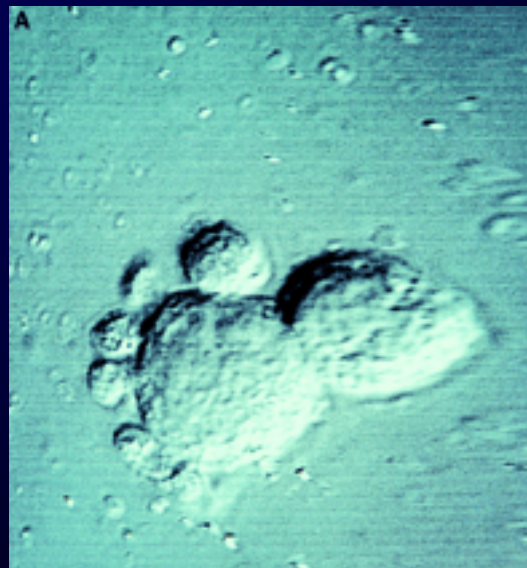
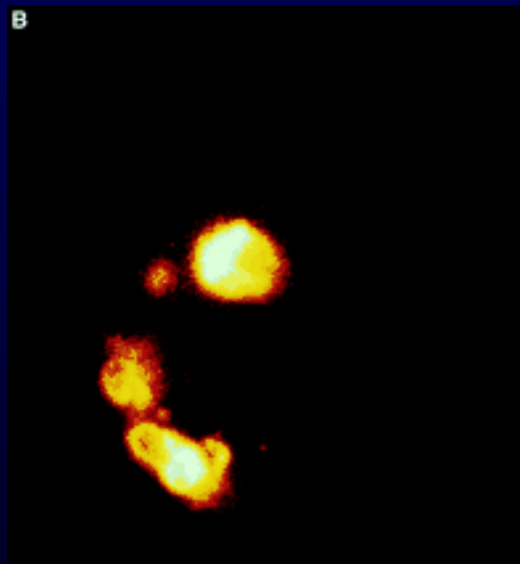
- Soluble mediators (acute phase reactants):
 - elevated plasma levels of CRP, SAA, IL-6.....
- Cellular activation:
 - in the plaque
 - in the coronary vessels
 - in the systemic circulation

CRP and prognosis: event free survival according to CRP levels at discharge in UA (AMI, Recurrent Ang.)



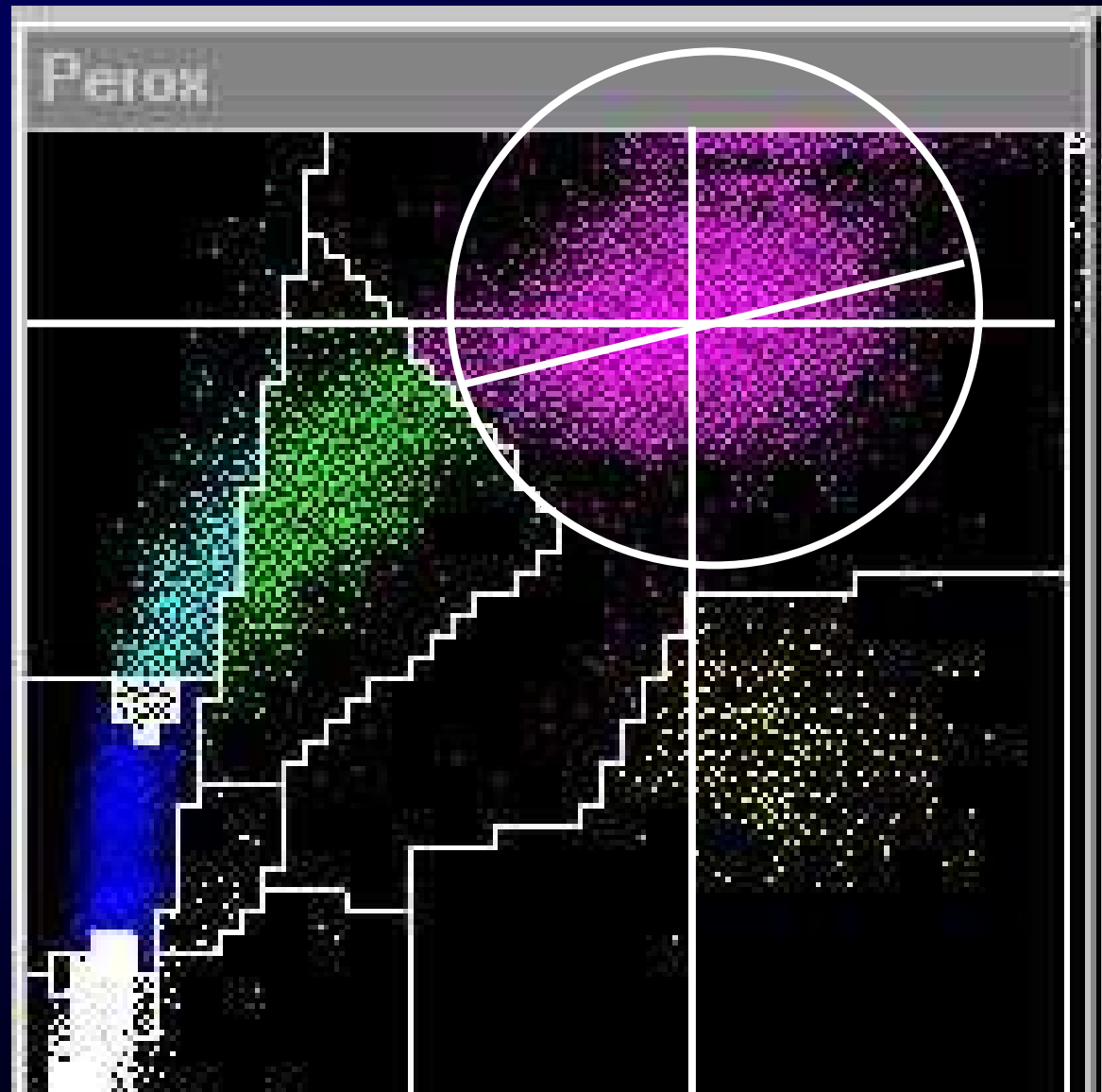
Ferreiros ER, Circulation 1999; 100:1958

Neutrophil activation and platelet adhesion in patients with UA (Ott et al.)



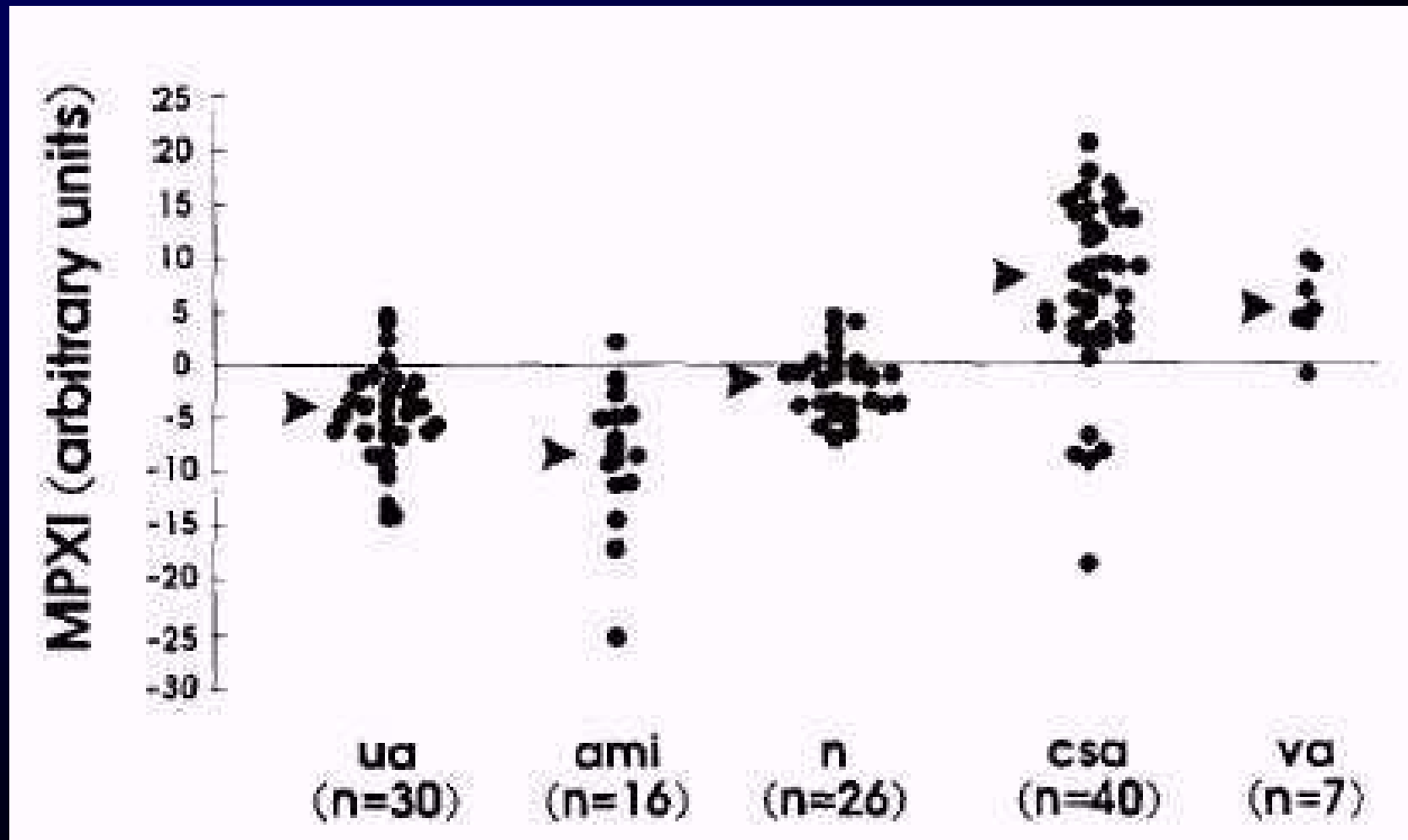
- increased neutrophil-platelet adhesion (flow-cytometry with anti-gp IIb/IIIa))

(Circulation, 1996)



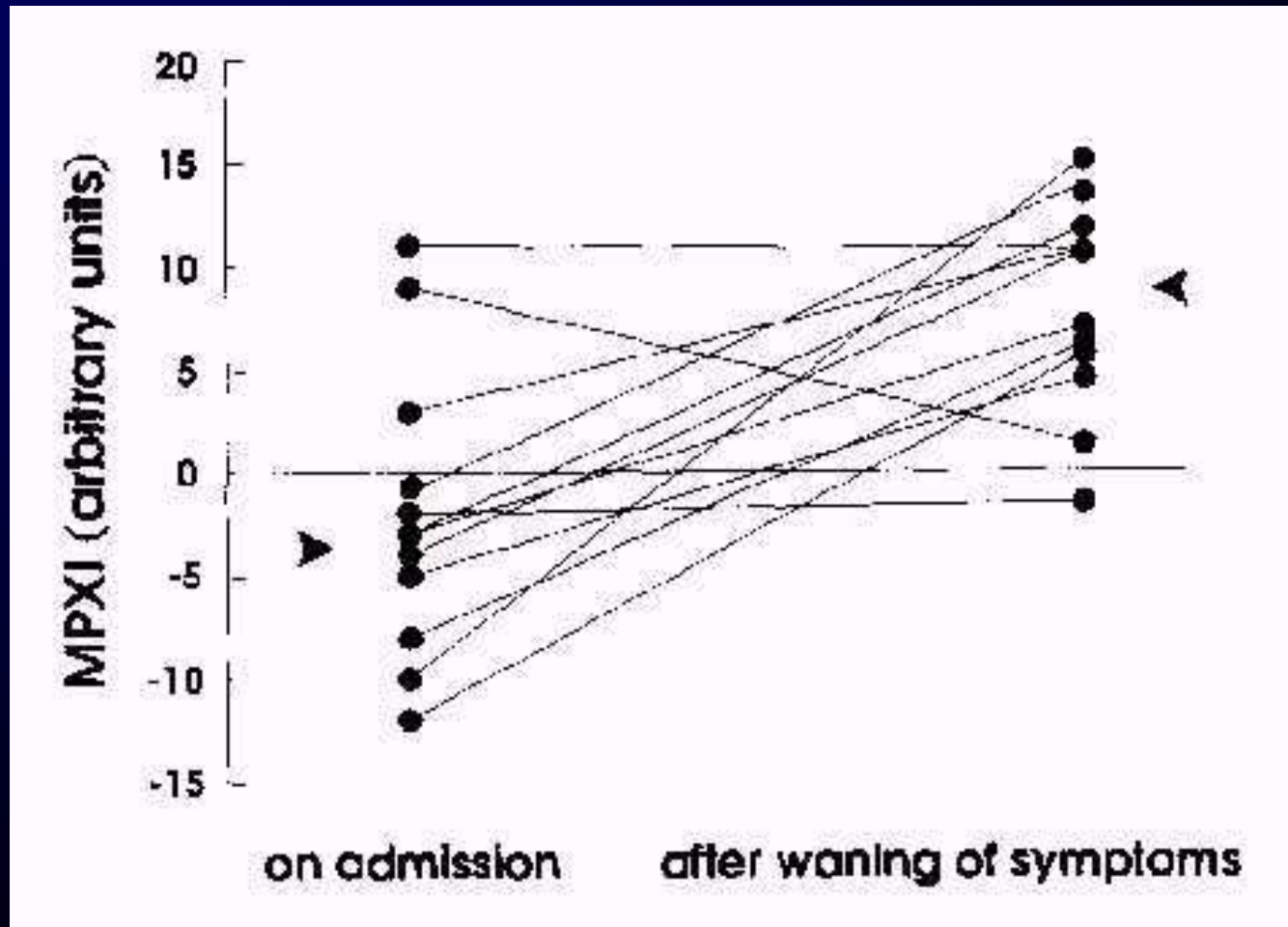
MPXI: arbitrary number, based on the position of the neutrophil cluster along the X and Y axes: standardized to be 0 ± 10 in normal subjects

Reduction of intracellular neutrophil myeloperoxidase as a marker of activation in UA and AMI



(Biasucci, d'Onofrio et al, JACC 1996)

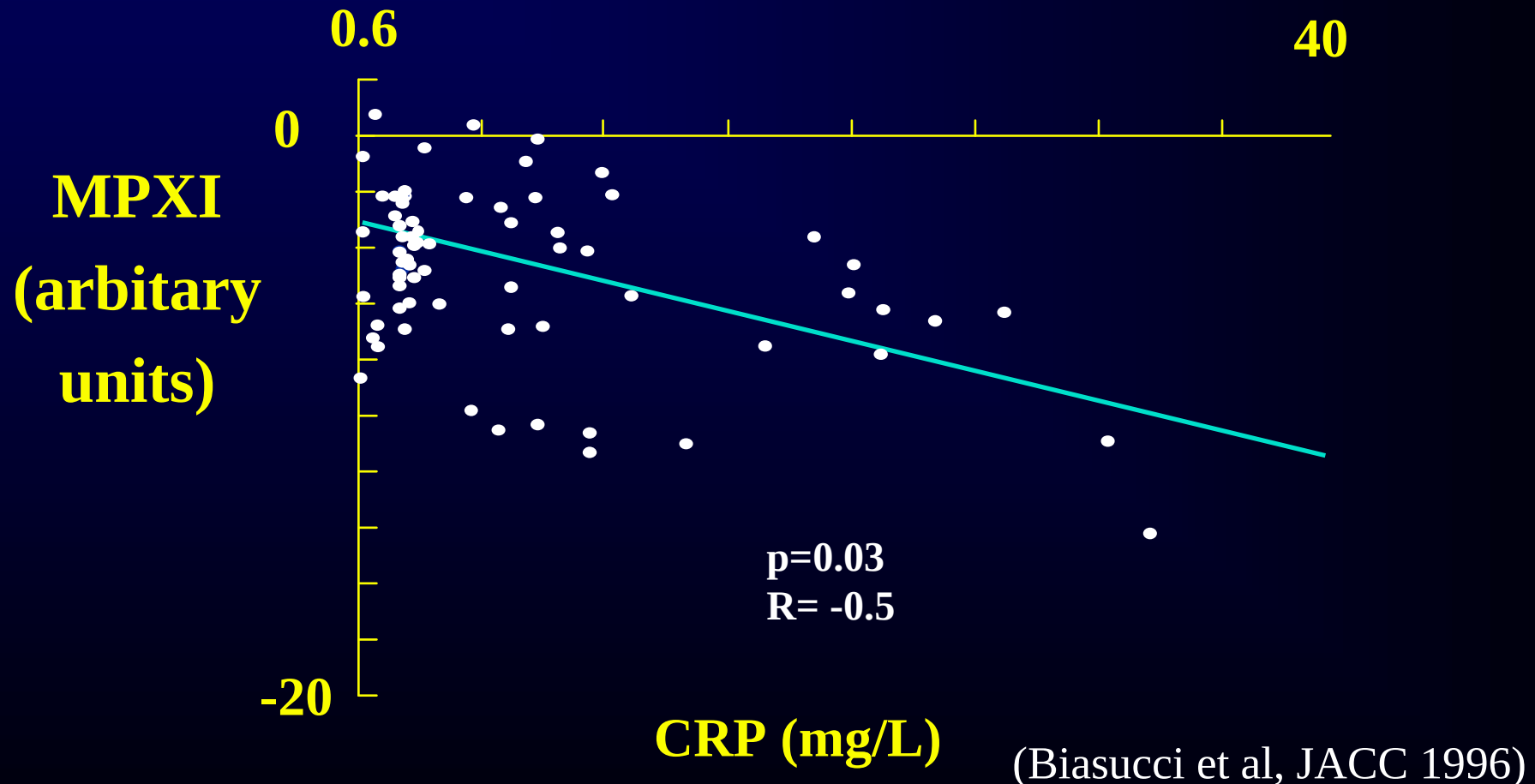
Neutrophil myeloperoxidase in patients with UA on admission and after waning of symptoms



(Biasucci, d'Onofrio et al, JACC 1996)

CORRELATION BETWEEN MPXI AND CRP

(Spearman's rank correlation)



The New England Journal of Medicine

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VOLUME 347

JULY 4, 2002

NUMBER 1

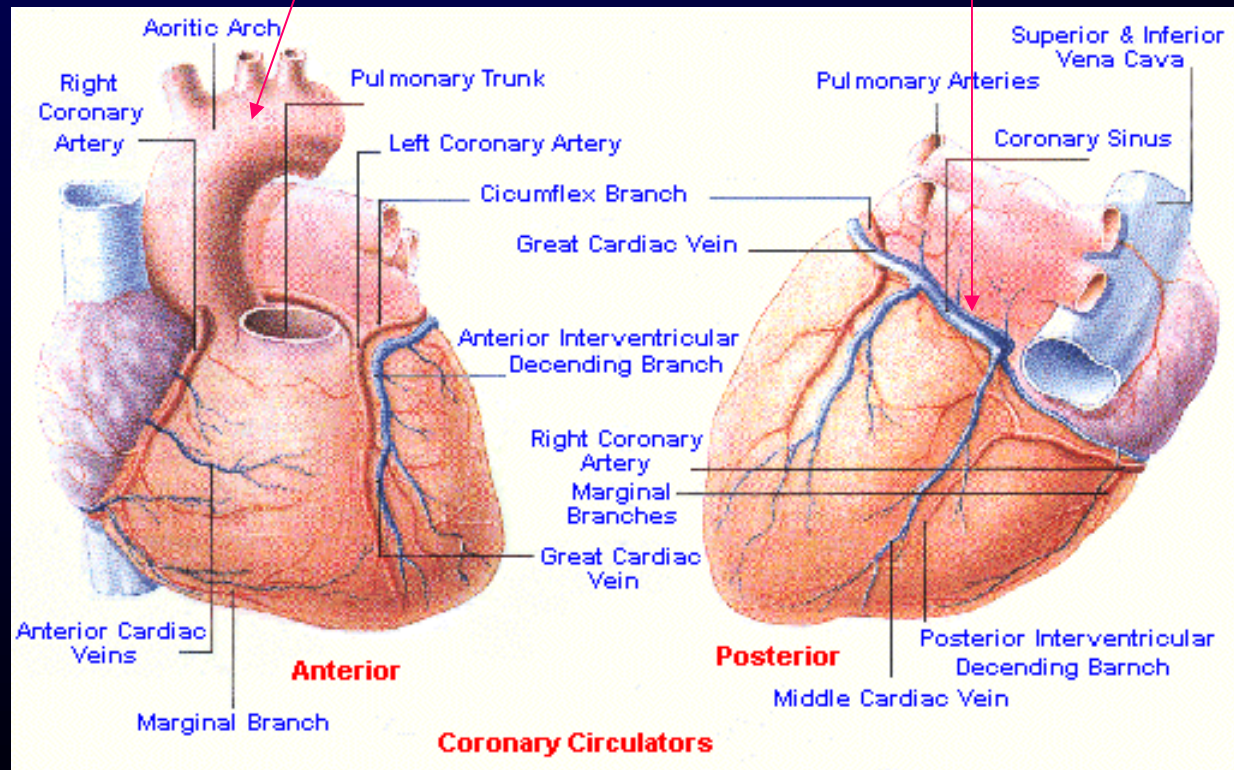


WIDESPREAD CORONARY INFLAMMATION IN UNSTABLE ANGINA

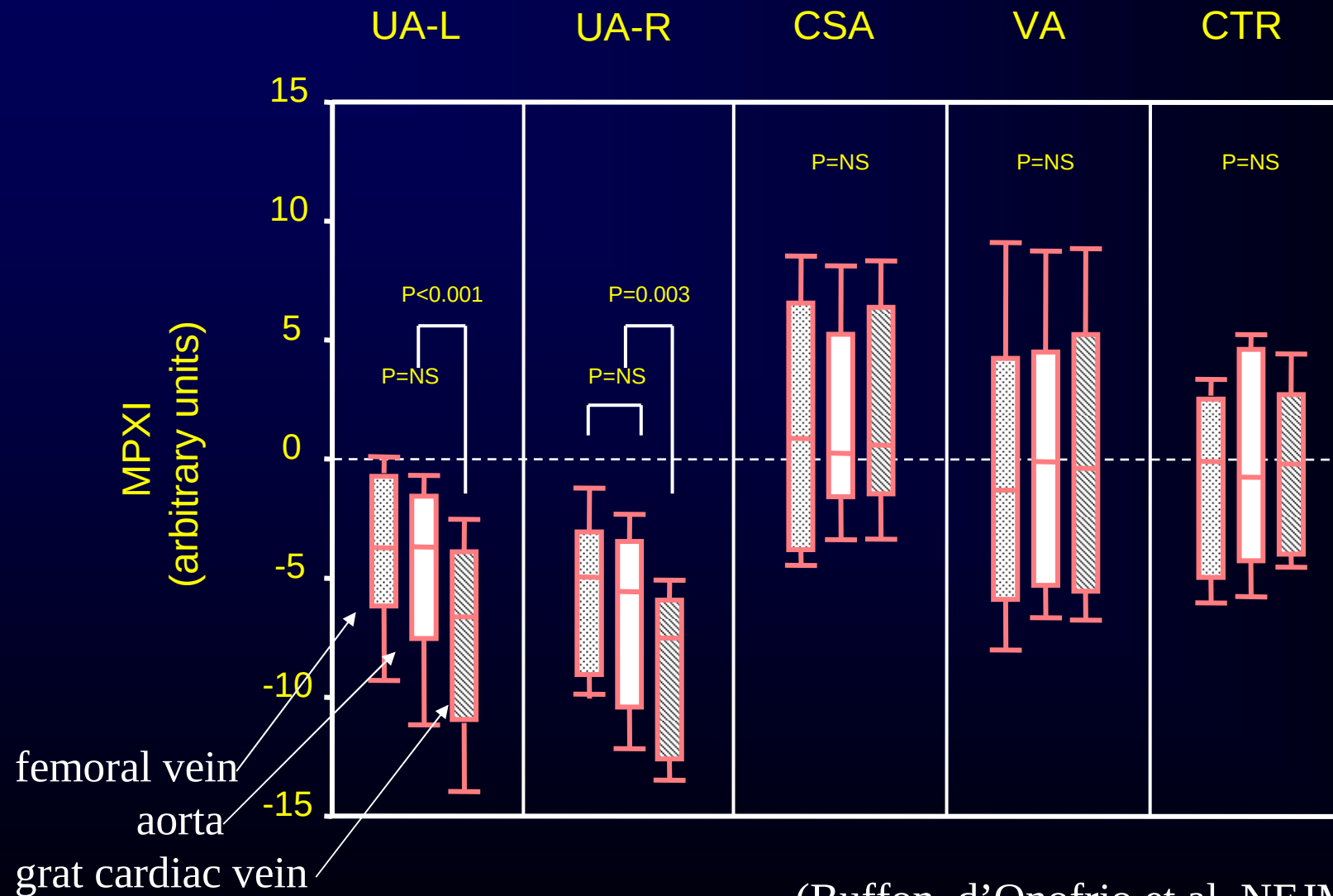
ANTONINO BUFFON, M.D., LUIGI M. BIASUCCI, M.D., GIOVANNA LIUZZO, M.D., GIUSEPPE D'ONOFRIO, M.D.,
FILIPPO CREA, M.D., AND ATTILIO MASERI, M.D.

Methods We measured the content neutrophil myeloperoxidase content in the cardiac and femoral circulations in five groups of patients: two groups with unstable angina and stenosis in either the left anterior descending coronary artery (24 patients) or the right coronary artery (9 patients); 13 with chronic stable angina; 13 with variant angina and recurrent ischemia; and 6 controls. Blood samples were taken from the aorta, the femoral vein, and the great cardiac vein, which selectively drains blood from the left but not the right coronary artery.

- blood from aorta: systemic circulation
- blood from the great cardiac vein has circulated across the left, but not the right coronary artery



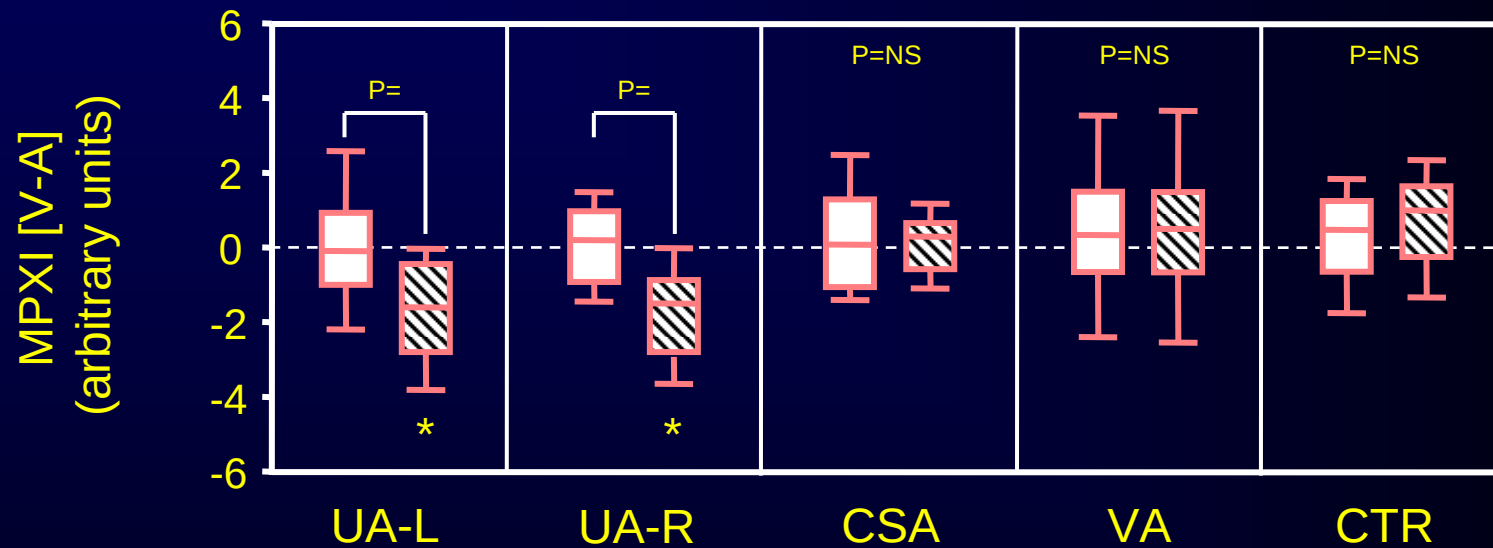
Reduction of neutrophil myeloperoxidase takes place through the coronary tree, but not only in the culprit lesion



(Buffon, d'Onofrio et al, NEJM 2001)

Venous-arterial difference in MPXI across the coronary circulation:

- 1) is present only in patients with UA,
- 2) but is not limited to the culprit lesion



(Buffon, d'Onofrio et al, NEJM 2001)

Serum myeloperoxidase levels and platelet activation parameters as diagnostic and prognostic markers in the course of coronary disease

Int. Jnl. Lab. Hem. 2010, **32**, 320–328

J. PAWLUS*, M. HOŁUB*, M. KOŻUCH†, M. DĄBROWSKA*, S. DOBRZYCKI†

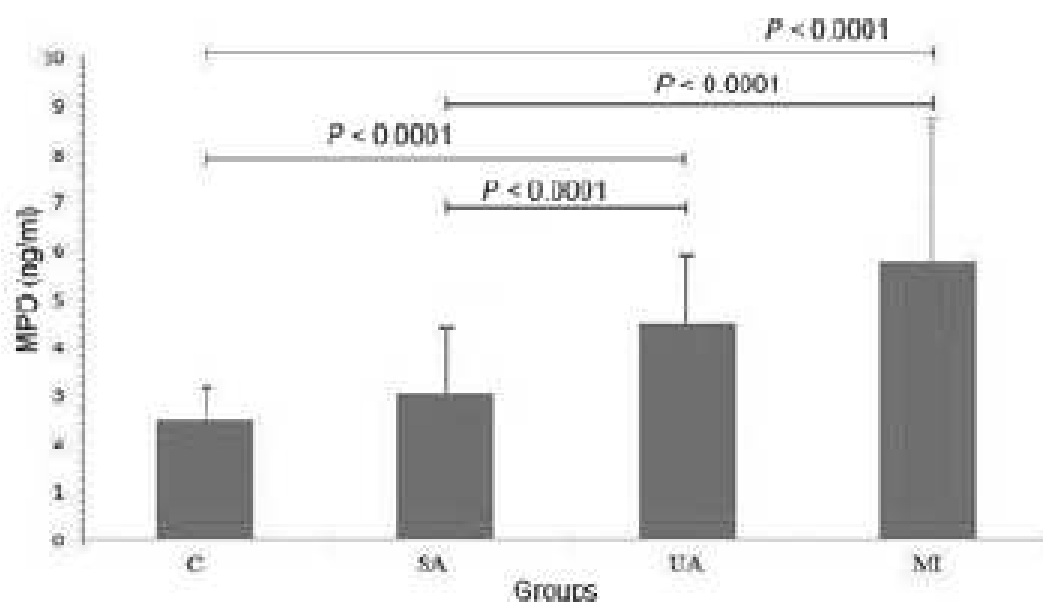


Figure 2. Serum myeloperoxidase (MPO) concentration.

Review Article

Myeloperoxidase: A New Biomarker of Inflammation in Ischemic Heart Disease and Acute Coronary Syndromes

Mediators of Inflammation

Volume 2008, Article ID 135625, 4 pages

Valentina Loria, Ilaria Dato, Francesca Graziani, and Luigi M. Biasucci

TABLE 1: Prognostic and diagnostic value and analytical performance of inflammatory markers in CAD: myeloperoxidase (MPO), C reactive protein (CRP), pregnancy-associated plasma protein-A (PAPP-A), CD40 ligand (CD40L), and interleukins.

	Inflammatory markers in CAD		
	Prognosis	Diagnosis	Analyt. perform.
MPO	+++	++	++ (more?)
CRP	++++	+/-	++++
PAPP-A	++	+	-
CD40L	++	+/-	-
Interleukins	+++	+/-	-

Acquired Neutrophil Myeloperoxidase Deficiency: An Indicator of Subclinical Activation of Blood Coagulation?

Giuseppe d'ONOFRIO, Rosalba MANCINI, Roberto VALLONE,
Generoso ALFANO, Alfonso CANDIDO, Marina PALLA,
and Giorgio MANGO

ABSTRACT. Using an automated cytochemical analyzer used for routine differential counts, we have been able to demonstrate acquired myeloperoxidase deficiency in 102 patients at our institution. Clinical and laboratory data on these patients showed a high incidence of diabetes mellitus (25.5%) and thrombotic diseases (24.5%), as well as a strikingly constant hyperfibrinogenemia (mean = 635 mg/100 ml; range = 360–1015 mg/100 ml). In 4 additional acute leukemia patients in complete remission, a close time correlation was noted between acquired MPO deficiency, diffuse intravascular coagulation

Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase

© Med Sci Monit, 2008; 14(8): CR406-410

Jacobijne J. Wiersma^{1ABCDEF}, Marijn C. Meuwese^{2ADEF}, Joram N.I. van Miert^{2B},
Arnoud Kastelein^{2B}, Jan G.P. Tijssen^{1ACDG}, Jan J. Piek^{1ADG}, Mieke D. Trip^{1,2ADEG}

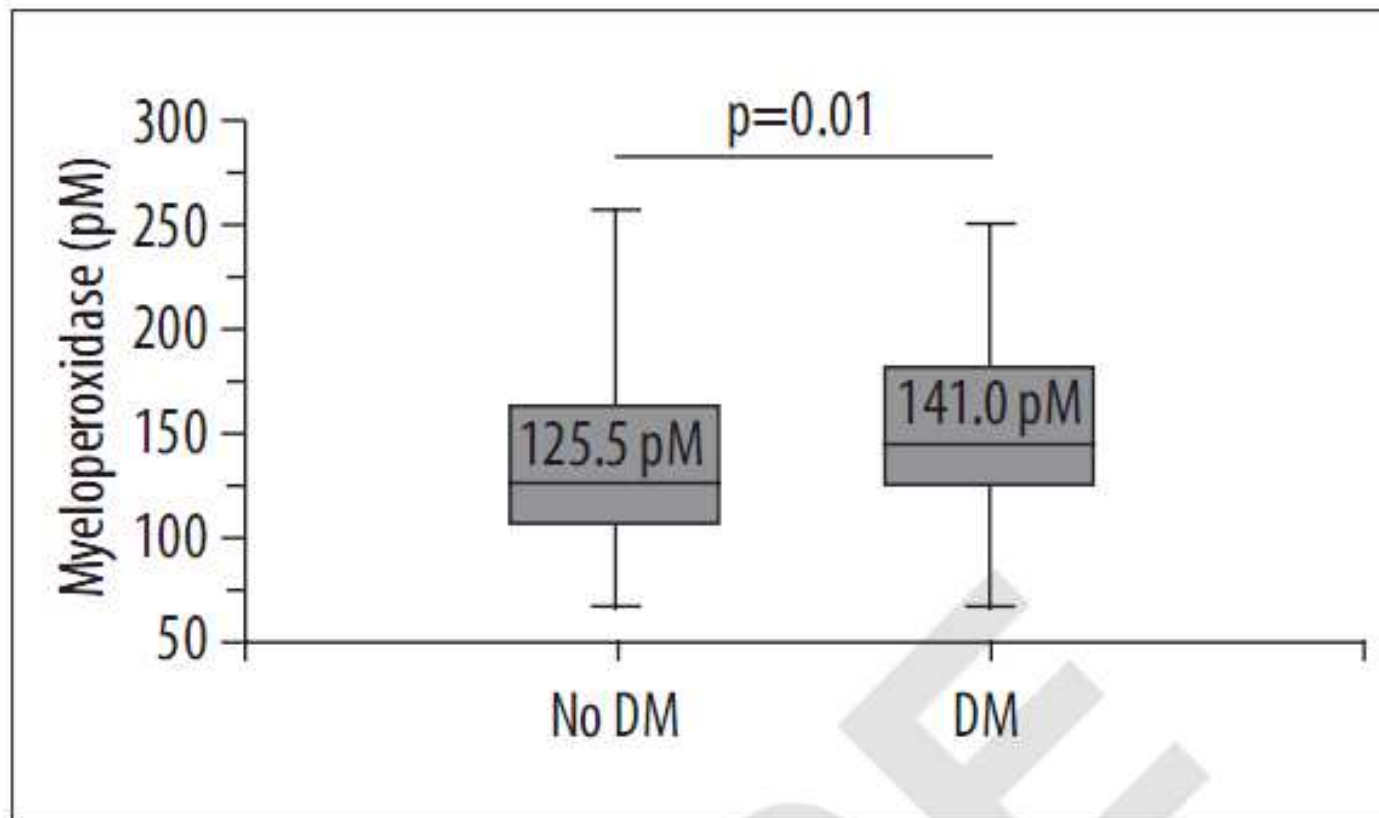


Figure 1. Whisker box-plot of MPO data in relation to the presence of diabetes mellitus type 2.

