HAEMATOLOGY

ANALYSIS OF THE BLOOD CELLS AND INTERPRETATION OF RESULTS

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19th september 2019

2nd practice

PARAMETERS OF CBC

- **WBC** (white blood cell count, Giga/l) **5-10 G/l**
  - **NEU**%, NEU# (neutrophil granulocyte) **40-70%**
  - **LY**%, LY# (lymphocyte) **20-40%**
  - **MO**%, MO# (monocyte) **4-8%**
  - **EO**%, EO# (eosinophil granulocyte) **2-4%**
  - **BAS**%, BAS# (basophil granulocyte) **0,5-1%**

Relative lymphocytosis

Vérkép automatával:

Fehérvérsejt	2,500	L	Giga/l	4,000-10,000
Neutrofil	27,4	L	%	34,0-71,1
Neutrofil (abs)	0,69	L	Giga/l	1,56-6,13
Limfocita	65,9	H	%	19,3-51,7
Limfocita (abs)	1,65		Giga/l	1,18-3,74
Monocita	4,9	U	%	4,7-12,5
Monocita (abs)	0,120	U~L	Giga/l	0,240-0,860
Eozinofil	1,3	U	%	0,0-5,8
Eozinofil (abs)	0,030		Giga/l	0,000-0,360
Bazofil	0,6		%	0,0-1,2
Bazofil (abs)	0,010		Giga/l	0,000-0,080
Vörösvértest	2,66	U~L	T/l	3,90-5,30
Hemoglobin	86	U~L	g/l	120-157
Hematokrit	24,1	U~L	%	34,1-44,9
MCV	90,6		fl	80,0-95,0
MCH	32,3		pg	26,0-33,0
MCHC	356		g/l	310-360
RDW	16,1	H	%CV	11,6-14,4
Trombocita	36,0	L	Giga/l	140,0-440,0
MPV	6,55	L	fl	9,40-12,40

PARAMETERS OF CBC

- **WBC** (white blood cell count, Giga/l) **5-10 G/l**
  - **NEU**%, NEU# (neutrophil granulocyte) **40-70%**
  - **LY**%, LY# (lymphocyte) **20-40%**
  - **MO**%, MO# (monocyte) **4-8%**
  - **EO**%, EO# (eosinophil granulocyte) **2-4%**
  - **BAS**%, BAS# (basophil granulocyte) **0,5-1%**
- **RBC** (red blood cell count, Tera/l) **4,1-5,1/4,5-5,9 T/l**
- **HGB** (haemoglobin, g/l) **123-153/140-175 g/l**
- **HCT** (haematocrit, %) **35-43/38-49 %**
- **MCV 80-95 fl**
- **MCH 26-33 pg**
- **MCHC 310-360 g/l**
- **RDW-CV 11,6-14,4%**
- **PLT** (platelet, Giga/l) **150-400 G/l**
- **MPV** (mean platelet volume)-fl

CALCULATED PARAMETERS

MCV= HTC/RBC (mean volume of red blood cells in fl)*

MCH= HGB/RBC (mean corpuscular hemoglobin in pg)

MCHC= HGB/HTC (mean corpuscular hemoglobin concentration in g/l)-the hemoglobin concentration of 1 l red cell mass

RDW –CV% (**11,6-14,4**)

RDW-SD (fl)

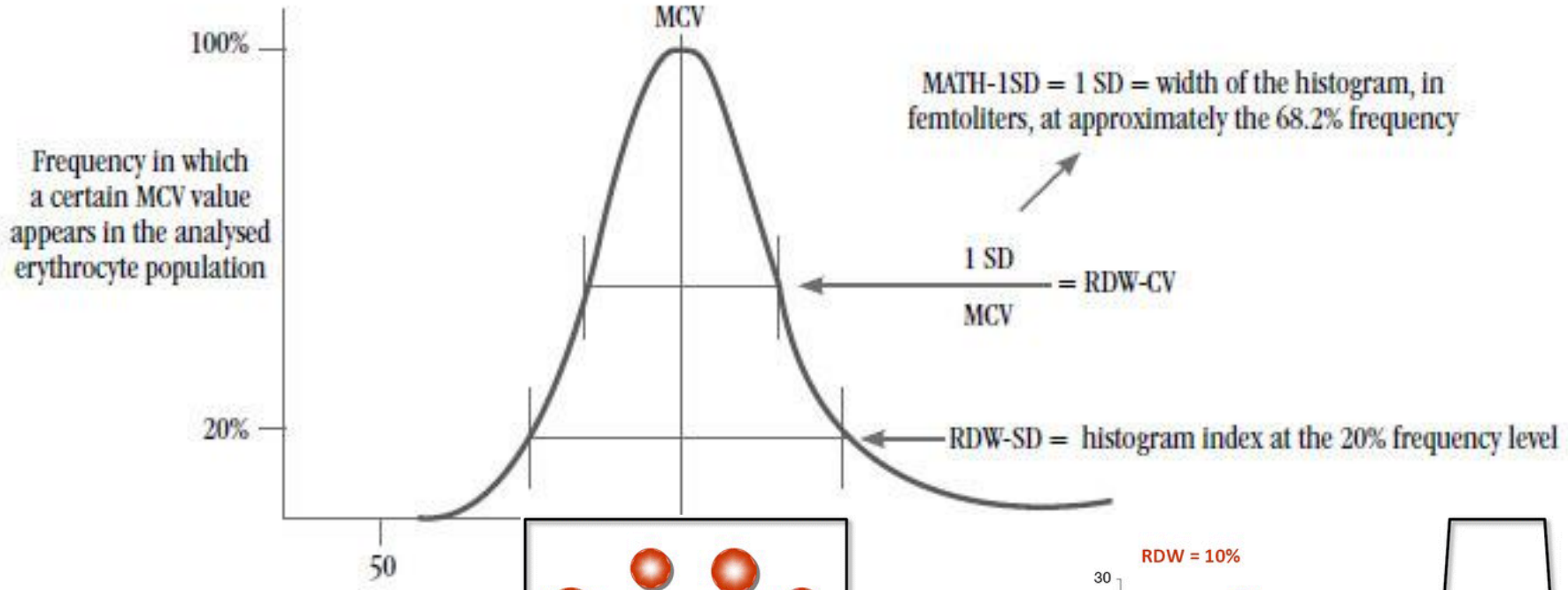
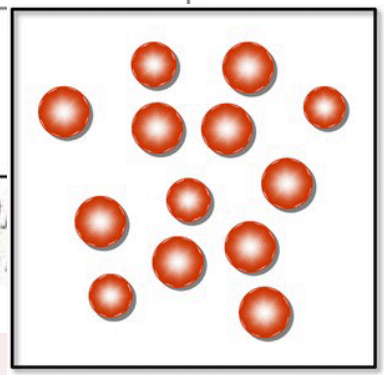
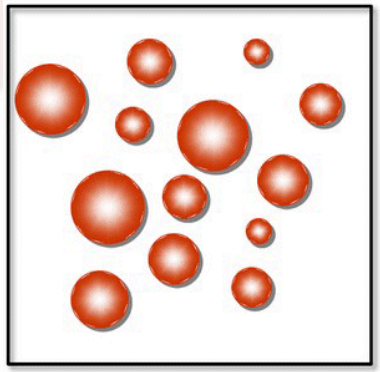
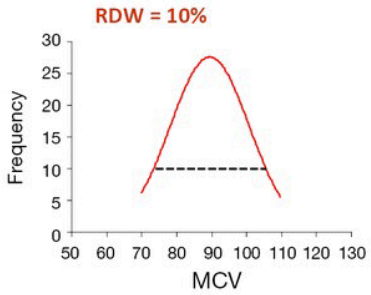


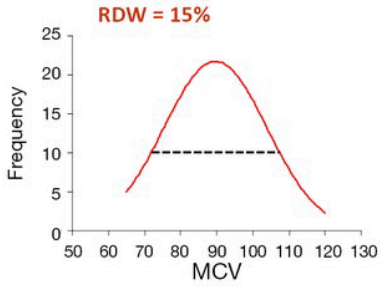
FIGURE – *Obtainment of RDW-CV, MATH-1SD and RDW-SD.*

RDW-CV: coefficient of variation of red cell distribution width; volume.





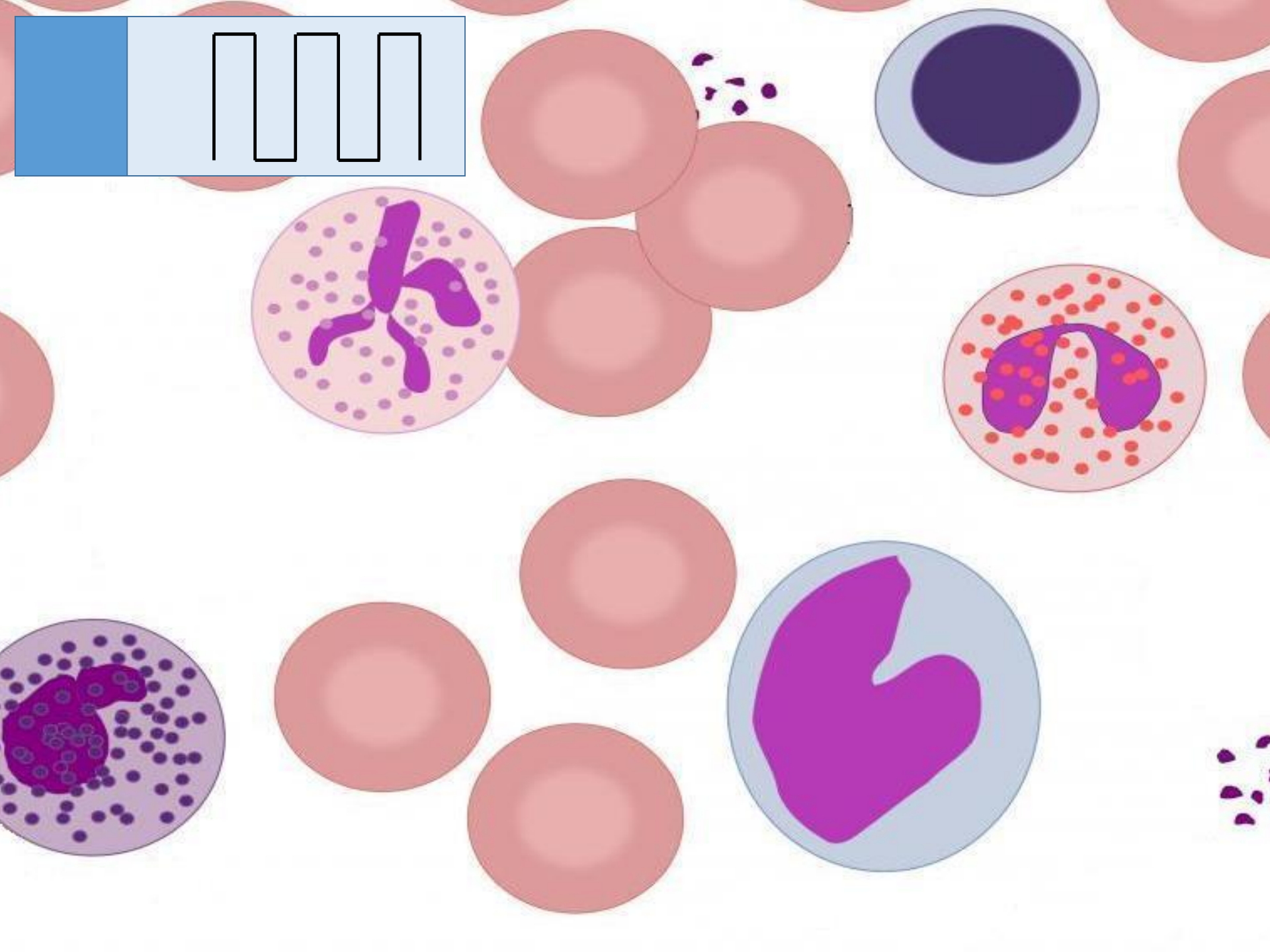




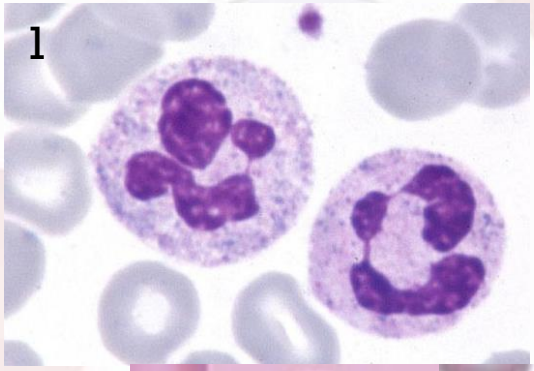
Anisocytosis

PREANALYTICS

- K3-EDTA, lavender tube, mixing well
- posture (getting up: HGB, HCT increase by 10% or more)
- must be measured within 4 hours, but as fast as possible except for: reticulocyte (within 24 hrs in room temp., 72 hrs if stored at 4°C)
- peripheral smear procedure:
  - capillary blood from fingertip, or from EDTA-tubes within 2-3 hours

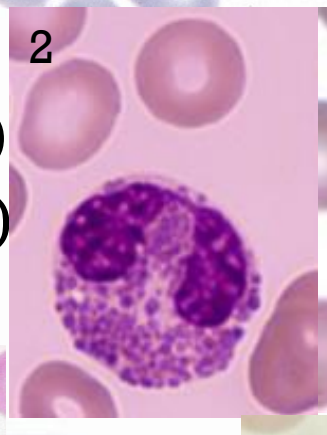


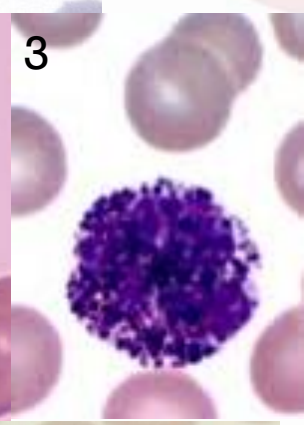
BLOOD CELLS

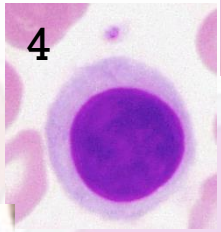


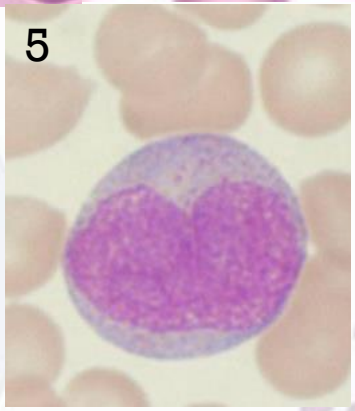
white blood cells:

1. neutrophil granulocyte (10-12 μm)
2. eosinophil granulocyte (10-12 μm)
3. basophil granulocyte (10-12 μm)
4. lymphocyte (9 μm)
5. monocyte (15-30 μm)



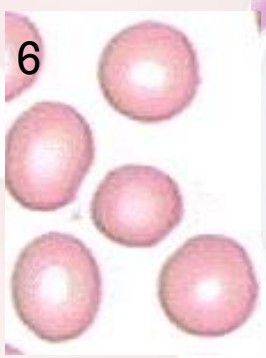






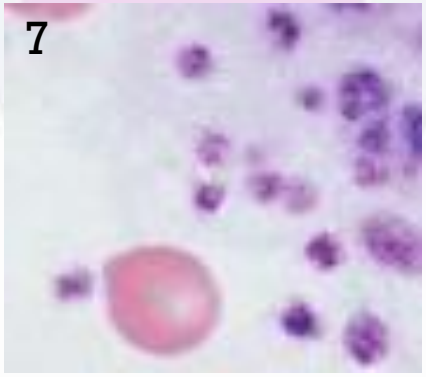
red blood cells:

- diameter: 7-8.5 μm
- thickness: 2 μm



platelets:

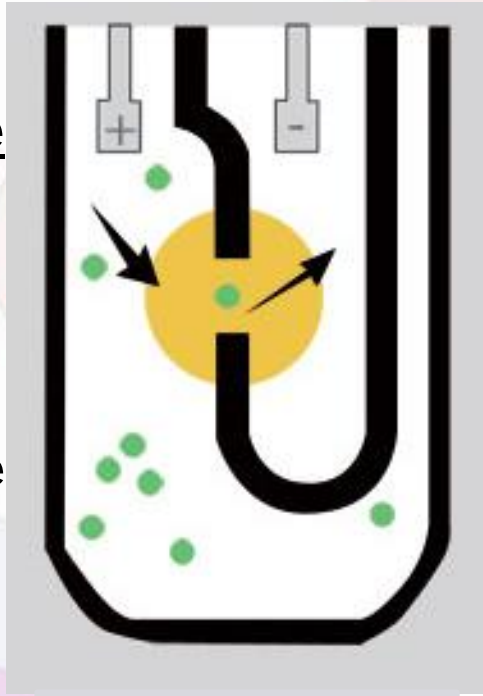
- diameter: 2-5 μm



HAEMATOLOGICAL INSTRUMENTS: PRINCIPLES 1.

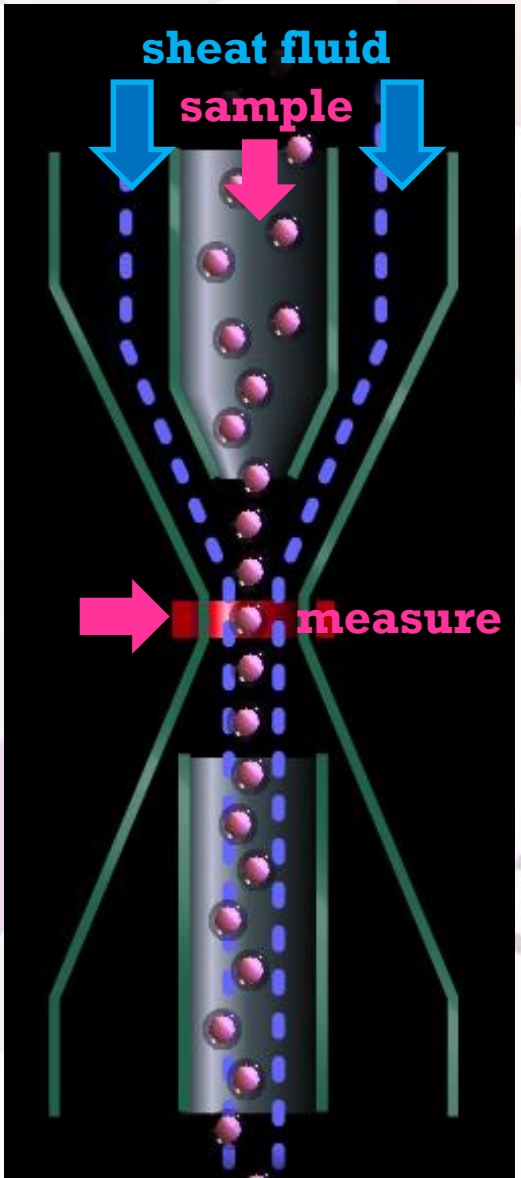
Coulter-principle (Wallace Coulter-1953)

- detection of **changes** in electric **impedance**
- orifice, between its sides DC (direct electric current)–gradient is present
- the sample is diluted with a good conductor liquid, when a cell passes through the orifice electrical resistance increases→ current intensity decreases(U=IxR)
- the size of the current change is related to the size of the cell, the number of it related to the number of the cells (volume-distribution histogram)



Sources of error:
•coincidence
•recirculation

HAEMATOLOGICAL INSTRUMENTS: PRINCIPLES 2.



hydrodynamic focusing

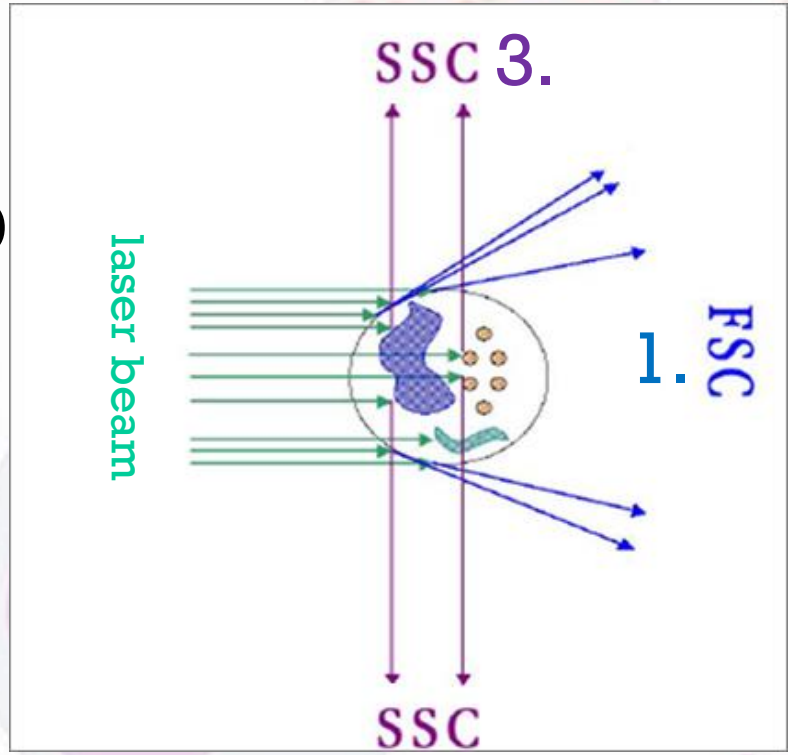
- to eliminate coincidence and recirculation
- the created laminar flow (sheat fluid) permits an unilinear pass of the cells through the site of measurement
- the constant flow decreases the chance of clog formation (proteins)

HAEMATOLOGICAL INSTRUMENTS: PRINCIPLES 3.

optical principal

- the refracitve index of cells differs from the diluent solution
- argon/helium laser beam

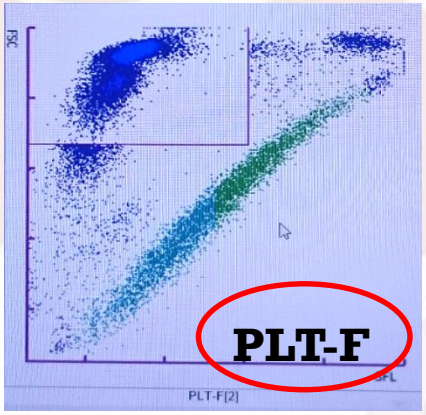
1. forward scatter detector (1-3°): size/volume of cells (scattering light)
2. low angle scatter detector (7-11°): indicator of cells' complexity
3. side scatter detector (70-110°): lobularity of the cells (refraction and reflexion of the light)

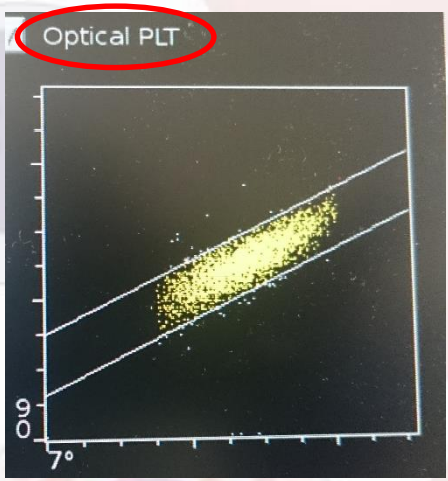


other specific prospects

- special stains (fluorescent, cytochemical)
- special characteristics of cells (selective lysis, depolarization, etc.)

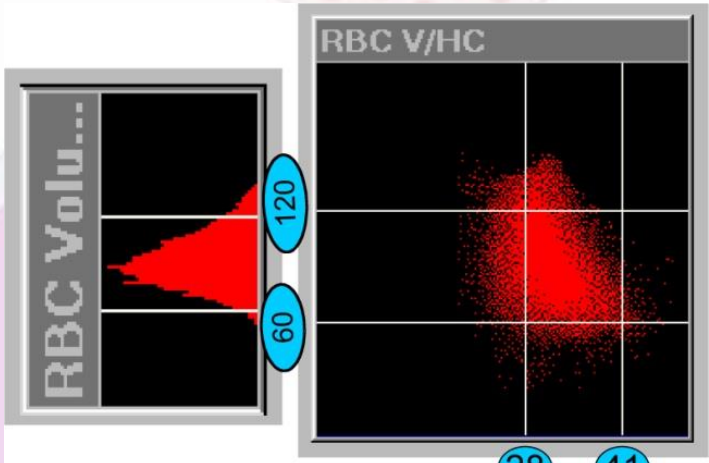
Red cell and platelet measurements



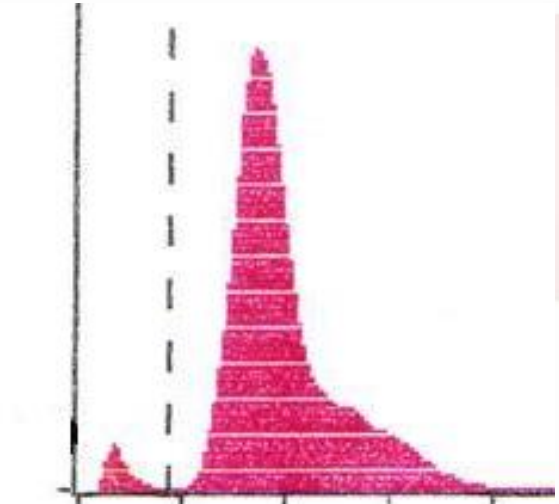


- Coulter-principle (impedance) supplemented with optical method
- FSC: volume (MCV)
- SSC: haemoglobin content

RBC volume/haemoglobin content histogram and dot-plot based on light scatter



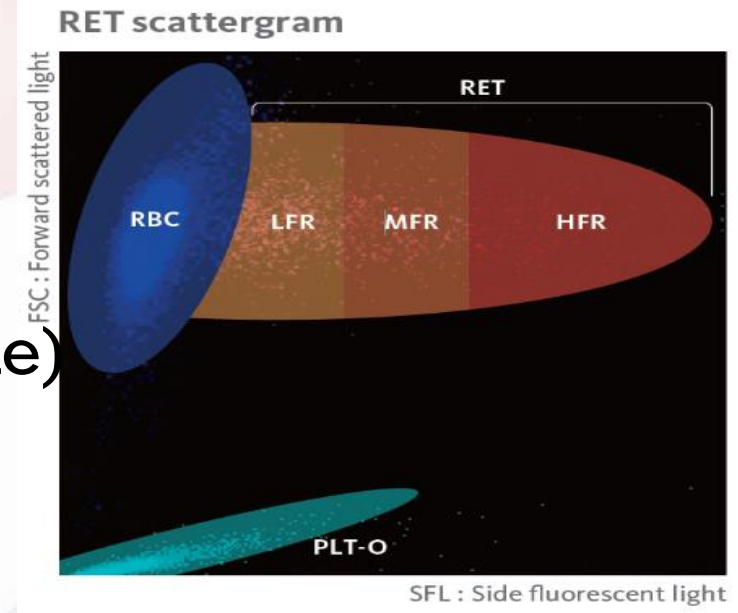
WBC-do we have to remove them for measuring RBC-s?



PLT-RBC volume distribution histogram

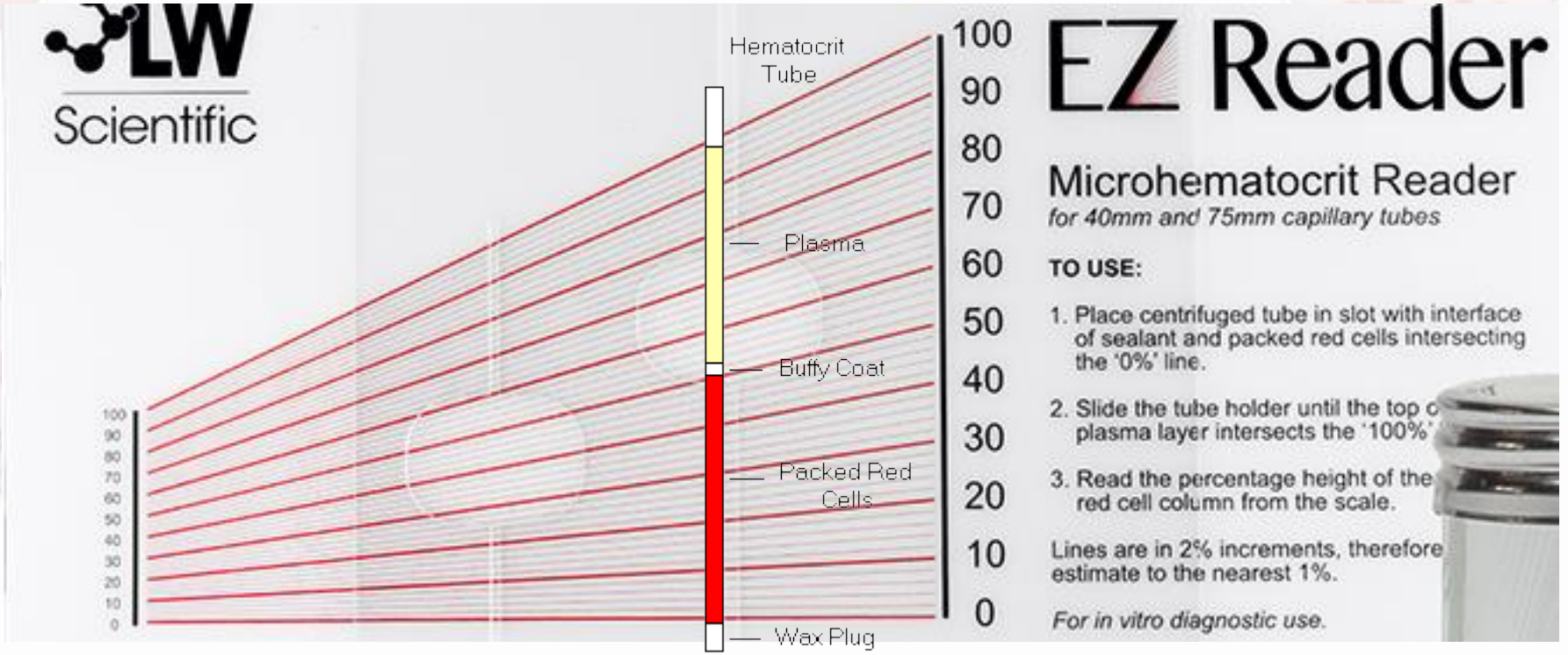
COUNTING RETICULOCYTES

- last stage of premature RBCs (no nucleus, contains RNP)
- 1-1,5 days in periphery before becoming mature RBC
- using supravital stain (brillant cresyl blue, methylene-blue)
- analyser:
  - nucleic acid stain (fluorescent)
  - IRF (immature reticulocyte fraction)
  - CHr (haemoglobin content of reticulocyte)-iron deficiency
- reticulocyte crisis (anaemia of vitamin B12, folic acid deficiency 24 hours after treatment)-IRF increases
- information about bone marrow function



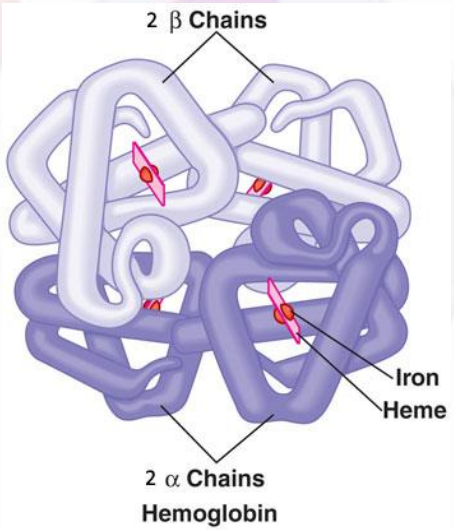
HAEMATOCRIT MEASUREMENT

- the past

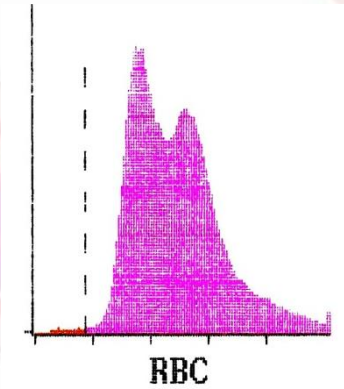


HAEMATOCRIT, HAEMOGLOBIN MEASUREMENTS

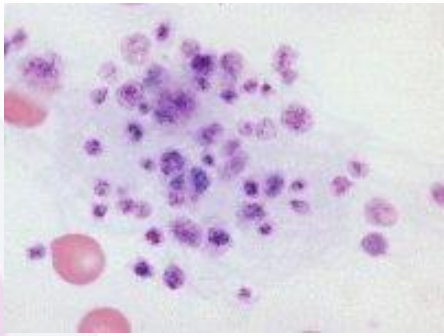
- haematocrit: the present
  - analyser: PCV=MCVxRBC
- haemoglobin:
  - photometric detection
  - stabile hemoglobin complex (SLS, (cyanid))
  - interfering factors:
    - lipaemic samples
    - increased bilirubin
    - (cold-agglutinins)
    - (hemolysis)



Transfusion, pseudothrombocytopaenia, plt aggregates, cold agglutinin

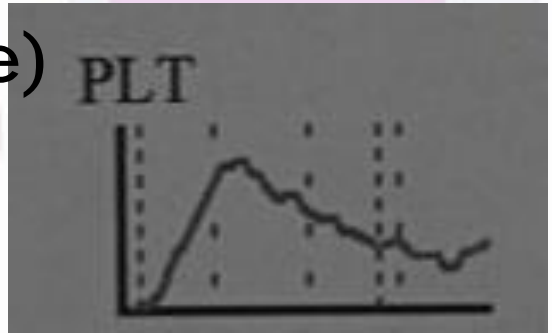


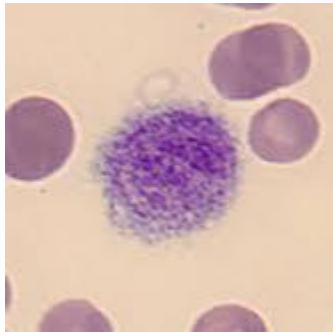
- transfusion/recovery from pernicious anaemia, or iron deficiency anaemia: two populations of rbc-s
- pseudotrombocytopenia: when plt count~0 from EDTA tube, normal from citrate tube, blood smear: tct aggregates



- giant platelets (impedance)
- cold agglutinin:

repeat measurement at 37°C

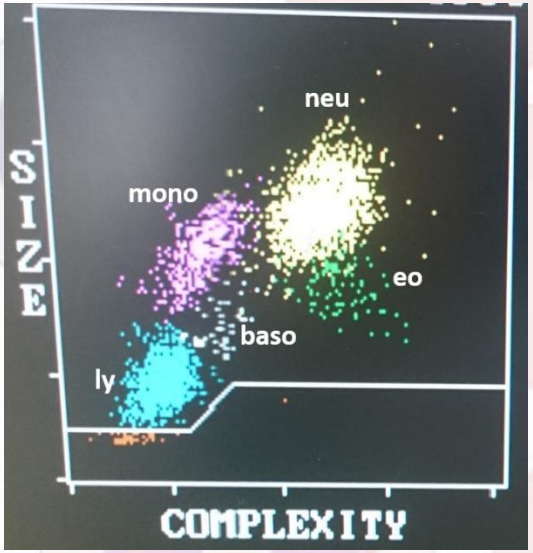


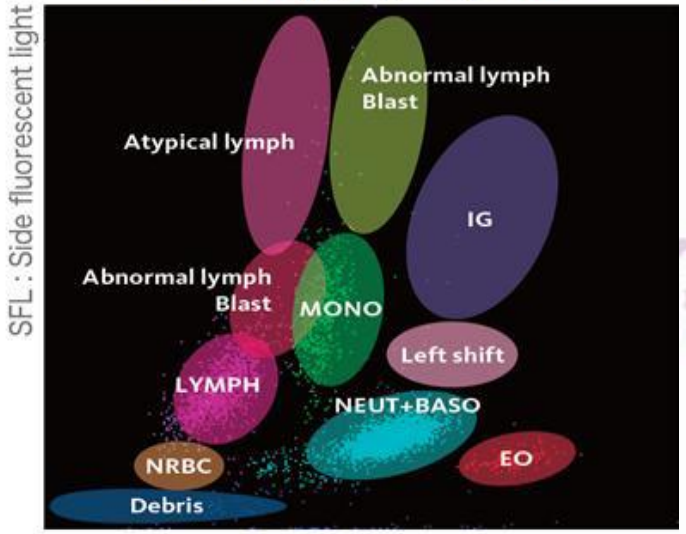


RBC-do we have to remove them for measuring WBC-s?

WBC COUNT

- site of measurement: optical flow cell
- dot-plots
- 5 part diff
- 6 part diff:
  - IG (immature granulocites)





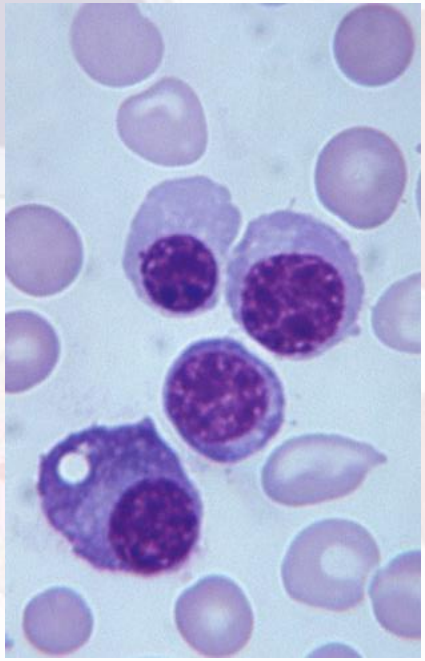
FLAGS (notifications)

- suspicious population of cells because of inadequate separation (DIFF)
- cells at abnormal site at the dot-plot (IG/BAND, BLAST/VARLYMPH)
- plt aggregates (PLTR), nucleic rbc-s (NRBC)
- smear should be examined:

suspicion of presence of malignant cells, or measuring error

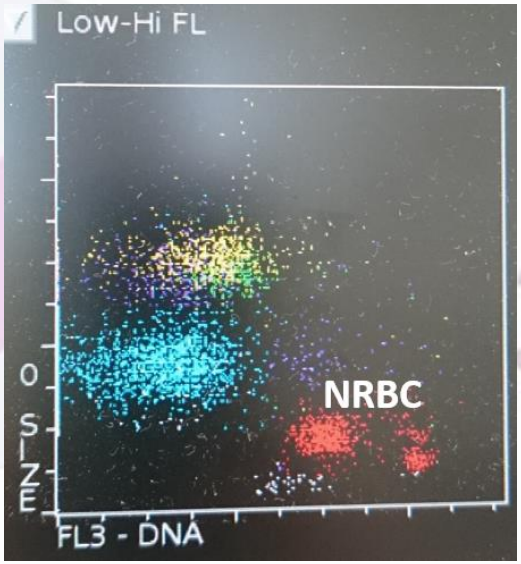
- nucleic RBC-s

Nucleic red blood cells



- normally they are in the bone marrow
- common in newborn
- in some disease it can be found in adults
- can falsely determined as lymphocites (high WBC, high ly % and abs. count)
- methods to count them:
  - nucleic acid stain

    (e.g.propidium-iodide)



ANAEMIAS

- definition: reduced red cell mass
- according to red cell morphology or MCV, MCH:
  - normochrom normocytic
  - hypochrom microcytic
  - hyperchrom macrocytic
- based on etiology:
  - decreased production (bone marrow)
  - deficiency anemias
  - shortened lifespan (hemolysis, inherited diseases, kidney failure…)
  - increased loss (occult bleeding, acute bleeding)
  - dilution (pregnancy)

Születési dátum...: 1946.05.31 Esetszám: 8885902
Anyja neve.....: Vicsik Julianna Telj. AZ: 8166940
Lakcím.....: 7960 Drávaszt ára, Kossuth L. utca 91. TAJ.....: 103-573-391
Vizsgálatkér ő.int.: B103 024222801 22801 I.Belkl. Haematológia I.
Vizsgálatkér ő.orv.:
46696 Szomor Árpád Dr.
Iránydiagnózis....: D4690
Minta visszaigazolás: 2014.09.03 11:21

Megnevezés	Érték	Abn	Egység	Referencia tart.
Vérkép automatával:				
Fehérvérsejt	4,100		Giga/l	4,000-10,000
Neutrofil	55,1		%	34,0-67,9
Neutrofil (abs)	2,26		Giga/l	1,78-5,38
Limfocita	31,5		%	21,8-53,1
Limfocita (abs)	1,29	L	Giga/l	1,32-3,57
Monocita	7,1		%	5,3-12,2
Monocita (abs)	0,292	L	Giga/l	0,300-0,820
Eozinofil	5,1		%	0,0-7,0
Eozinofil (abs)	0,208		Giga/l	0,000-0,540
Bazofil	1,2		%	0,0-1,2
Bazofil (abs)	0,047		Giga/l	0,000-0,080
RBC	2,22	L	T/l	4,50-6,00
HGB	90	L	g/l	137-175
Haematocrit	25,7	L	%	40,1-51,0
MCV	116,0	H	fl	80,0-95,0
MCH	40,7	H	pg	26,0-33,0
MCHC	352		g/l	310-360
RDW	14,8	H	%CV	11,6-14,4
Trombocita	359,0		Giga/l	140,0-440,0
MPV	5,74	L	fl	9,40-12,40
Megjegyzések a vérképhez	:	X		
RBC morphology f				

Vérkép automatával:

Fehérvérsejt	5,840		Giga/l	4,000-10,000
Neutrofil	65,2		%	34,0-71,1
Neutrofil (abs)	3,81		Giga/l	1,56-6,13
Limfocita	26,0		%	19,3-51,7
Limfocita (abs)	1,52		Giga/l	1,18-3,74
Monocita	5,7		%	4,7-12,5
Monocita (abs)	0,330		Giga/l	0,240-0,860
Eozinofil	1,0		%	0,0-5,8
Eozinofil (abs)	0,060		Giga/l	0,000-0,360
Bazofil	0,4		%	0,0-1,2
Bazofil (abs)	0,020		Giga/l	0,000-0,080
Gép által nem azonosítható FV3	1,700		%	<5,000
RBC	5,77	H	T/l	3,90-5,30
HGB	124		g/l	120-157
Haematocrit	40,0		%	34,1-44,9
MCV	69,3	L	fl	80,0-95,0
MCH	21,5	L	pg	26,0-33,0
MCHC	310		g/l	310-360
RDW	20,6	H	%CV	11,6-14,4
Trombocita	206,0		Giga/l	140,0-440,0
MPV	6,90	L	fl	9,40-12,40

Születési dátum...: 1938.06.22
Anyja neve.....: Czigler Mária
Lakcím.....: 7623 Pécs, Károly u. 5.
Vizsgálatkérő.int.: B1FV 02421A921 I. Belgy.Kl., Geriátria
Vizsgálatkérő.orv.:
40706 KUN LÁSZLÓ dr.
Iránydiagnózis....: D6490
Minta visszaigazolás: 2014.09.03 08:50

Esetszám: 8867958
Telj. AZ: 8164597
TAJ.....: 008-391-676

Megnevezés	Érték	Abn	Egység	Referencia tart.
Protrombin INR	1,78	H	.	0,90-1,15
Vérkép automatával:				
Fehérvérsejt	5,800		Giga/l	4,000-10,000
Neutrofil	73,3	H	%	34,0-67,9
#				
Neutrofil (abs)	4,25		Giga/l	1,78-5,38
#				
Limfocita	12,0	L	%	21,8-53,1
#				
Limfocita (abs)	0,70	L	Gi	#
#				RDW
Monocita	9,6		%	#
#				Trombocita
Monocita (abs)	0,560		Gi	#
#				MPV
Eozinofil	1,7		%	#
#				
Eozinofil (abs)	0,100		Giga/l	0,000-0,540
#				
Bazofil	0,6		%	0,0-1,2
#				
Bazofil (abs)	0,030		Giga/l	0,000-0,080
#				
Gép által nem azonosítható FVS	2,800		%	<5,000
#				
RBC	4,92		T/l	4,50-6,00
HGB	88	L	g/l	137-175
Haematocrit	34,9	L	%	40,1-51,0
#				
MCV	71,1	L	fl	80,0-95,0
#				
MCH	17,9	L	pg	26,0-33,0
#				
MCHC	252	L	g/l	310-360

	25,5	H	%CV	11,6-14,4
	403,0		Giga/l	140,0-440,0
	9,50		fl	9,40-12,40

DIAGNOSING ANAEMIAS

- CBC (RBC, HGB, HTC, MCV, MCH, MCHC, RDW)
- reticulocyte
- iron parameters (iron, ferritin, transferrin, transferrin saturation, soluble transferrin receptor, bone marrow iron storage-Prussian-blue)
- CRP
- blood smear
- deficiencies: serum vit. B12, folic acid
- in the case of haemolysis: LDH, indirect bilirubin, urine UBG, haptoglobine
- special investigations: haemoglobin elpho, genetical investigation, Coombs-test

THROMBOCYTE DISORDERS

- thrombocytopenia:
  - under 50 Giga/l risk for bleeding
  - decreased production: inherited (Fanconi anaemia, May-Hegglin anomaly)/acquired (drugs, tumours, hematological malignancies, radiotherapy)
  - increased destruction: ITP, TTP, HUS
  - hypersplenia (increased storage)
- thrombocytosis:
  - ET, MDS
  - after splenectomy transiently, chronic inflammation, tumor, iron deficiency, hemolytic anaemia
- qualitative disorders:
  - acquired (ASA, uraemia, liver disease)/inherited (Bernard-Soulier syndrome, Gray platelet syndrome)

Ultraszenzitiv CRP	18,72	H	mg/l	<5,00
Ultraszenzitiv CRP	8,27	H	mg/l	<5,00
Ultraszenzitiv CRP #	4,99		mg/l	<5,00

Vérkép automatával:

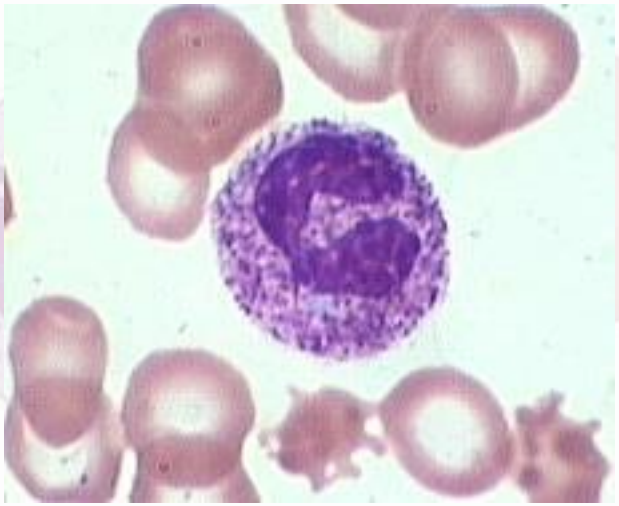
Fehérvérsejt	3,530	L	Giga/l	4,000-10,000
Neutrofil	35,2		%	34,0-67,9
Neutrofil (abs)	1,24	L	Giga/l	1,78-5,38
Limfocita	46,9		%	21,8-53,1
Limfocita (abs)	1,65		Giga/l	1,32-3,57
Monocita	13,6	H	%	5,3-12,2
Monocita (abs)	0,479		Giga/l	0,300-0,820
Eozinofil	3,9		%	0,0-7,0
Eozinofil (abs)	0,138		Giga/l	0,000-0,540
Bazofil	0,4		%	0,0-1,2
Bazofil (abs)	0,016		Giga/l	0,000-0,080
Vörösvértest	4,84		T/l	4,50-6,00
Hemoglobin	140		g/l	137-175
Hematokrit	41,2		%	40,1-51,0
MCV	85,1		fl	80,0-95,0
MCH	28,9		pg	26,0-33,0
MCHC	340		g/l	310-360
RDW	16,2	H	%CV	11,6-14,4
Trombocita	127,0	U~L	Giga/l	140,0-440,0
MPV	6,96	L	fl	9,40-12,40

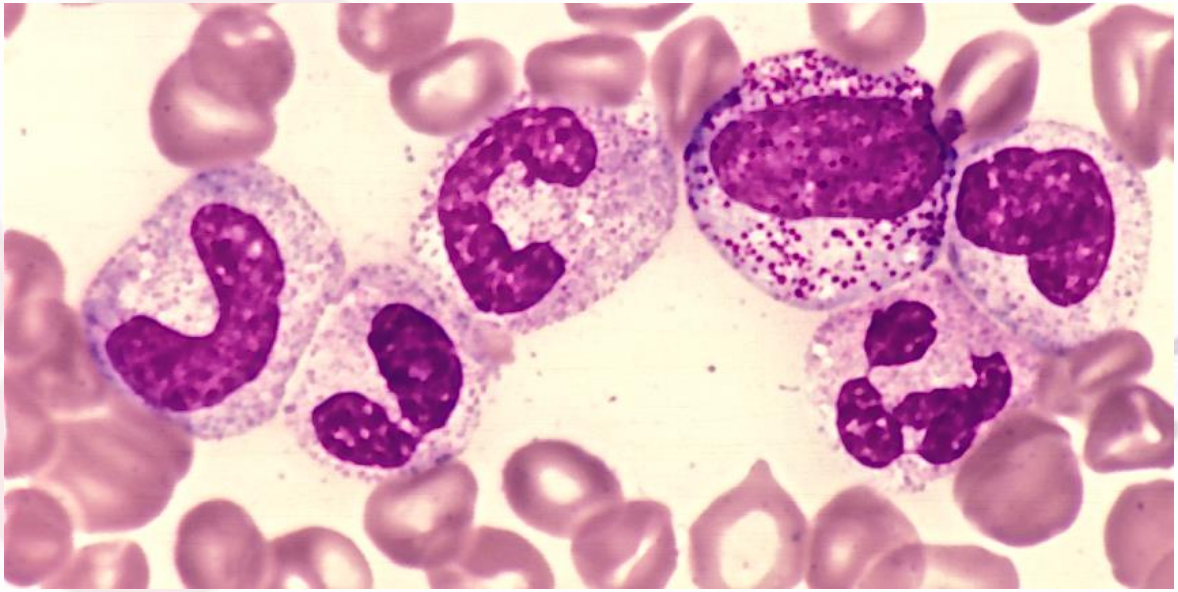
Retikulocita HG tartalom #	25,3	L	pg	28,0-35,0

NEUTROPHILIA, LEUKAEMOID REACTION

- neutrophilia: stress, adrenaline, corticosteroids, pregnancy, infection, inflammation
- leukaemoid reaction: present in inflammation, important to differentiate from CML (granulocyte alkaline phosphatase=GAPA)

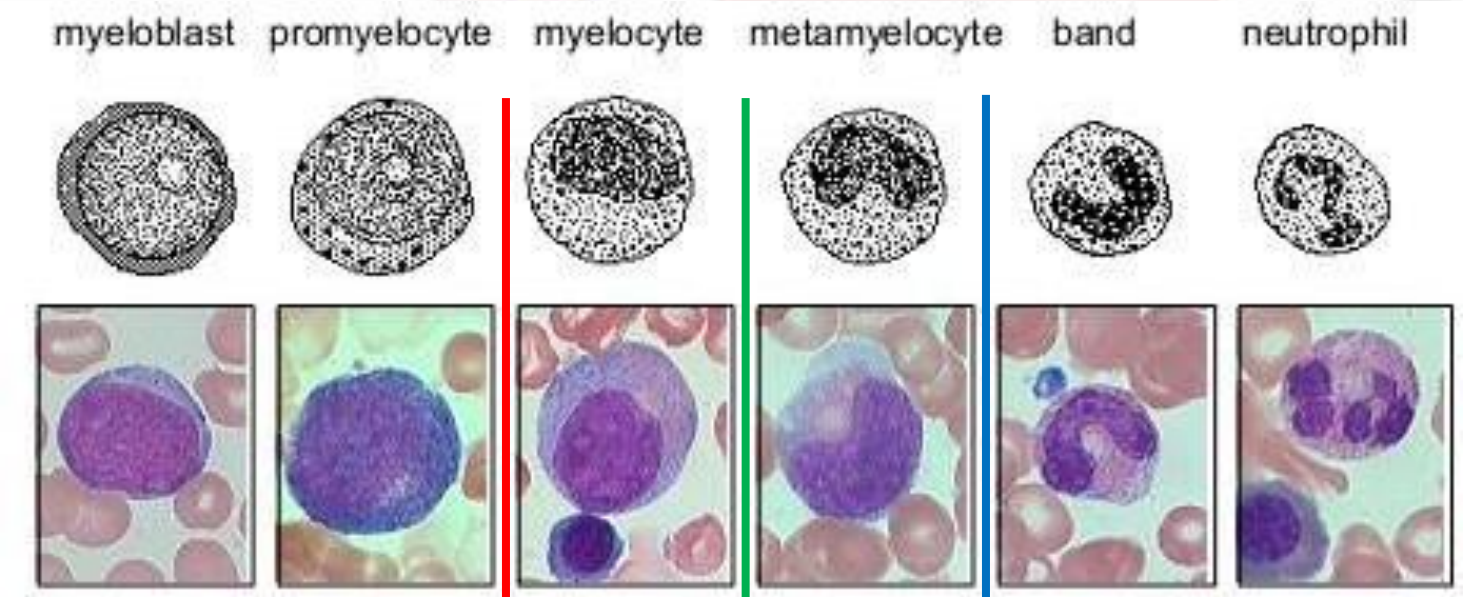
toxic granules





left shift, younger stages of maturation appear in periphery

Maturation of granulocytes

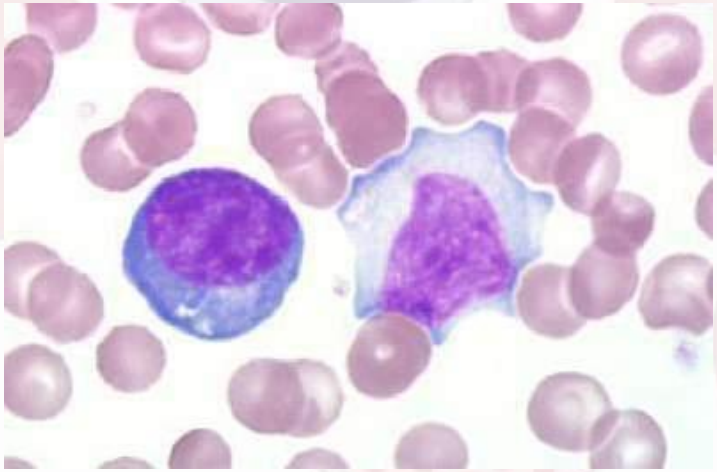


neutrophilic-eosinophil-basophilic-myelocyte

division

maturation

MONONUCLEOSIS INFECTIOSA



- caused by EBV
- fever
- adenomegaly (usually in the neck),
- hepatosplenomegaly
- elevation of liver enzymes, because of hepatitis
- presence of atypical mononuclear cells (usually lymphocytosis, maybe monocytosis, when measured with the analyser)

LEUKAEMIAS

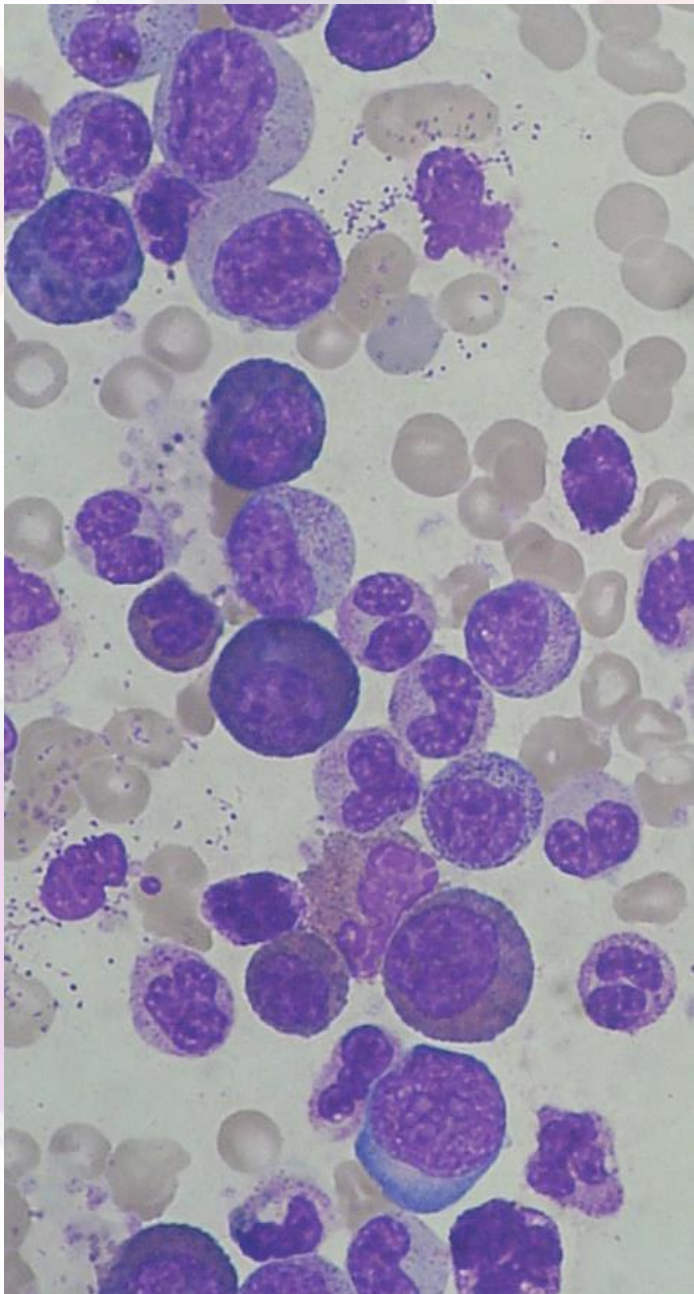
- =higher numbers of abnormal WBCs
- blast cells (stem cells present normally in the bone marrow, less than 5%)
- the clonal expansion of the cells results from mutations in the mother cell's DNA
- depending on origin it can be lymphoid or myeloid
- depending on the number of blasts it can be acute (more than 20%) or chronic

CHRONIC MYELOID LEUKAEMIA (CML)

- high WBC

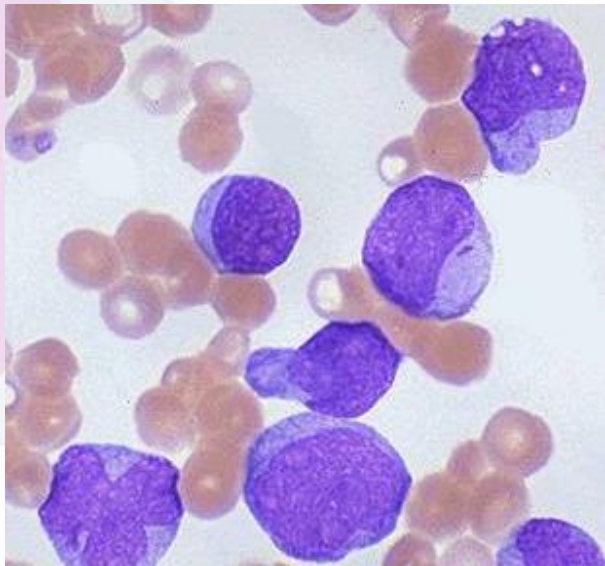
(more than 50 Giga/l)

- in the periphery all the maturation stages present
- decreased GAPA activity, Philadelphia chromosome (BCR-ABL fusion gene)
- can transform to acute leukaemia

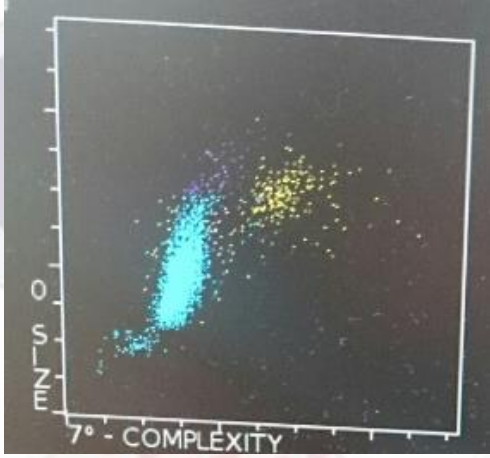


ACUTE MYELOID LEUKAEMIA (AML)

- in the bone marrow or in the periphery the rate of blast cells increase up to more than 20%
- hiatus leukaemicus
- in the blasts Auer-rods can be present
- usually positive with MPO stains (except for the completely immature blast cells)
- exact diagnosis: flow cytometry



CHRONIC LYMPHOID LEUKAEMIA (CLL)

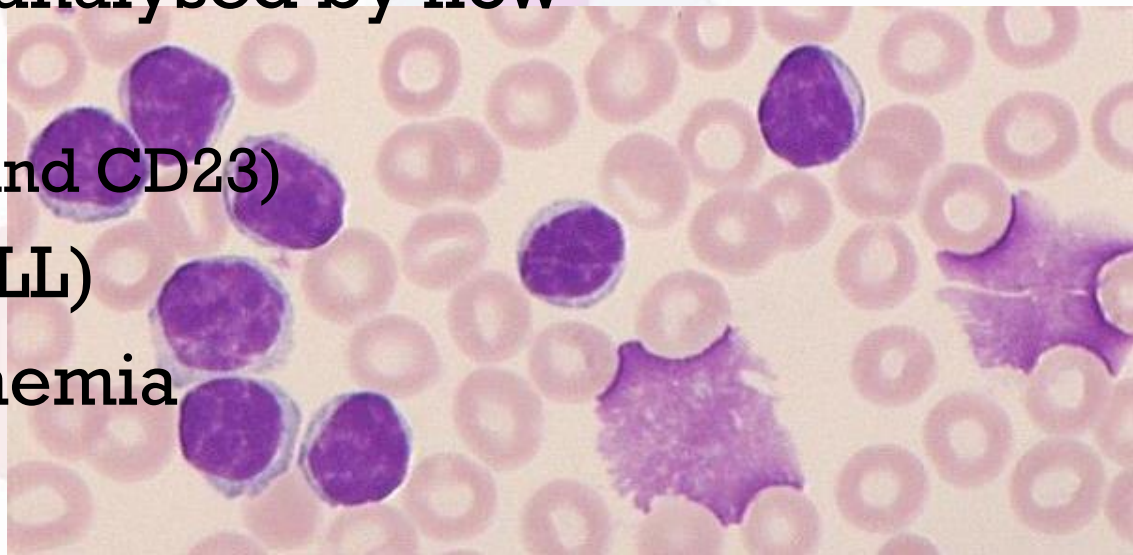


- the appearance of small mature lymphocytes in the peripherial blood (having heterochromatic nucleus)
- cells are fragile, when spreading the smear
- typical phenotype analysed by flow cytometry

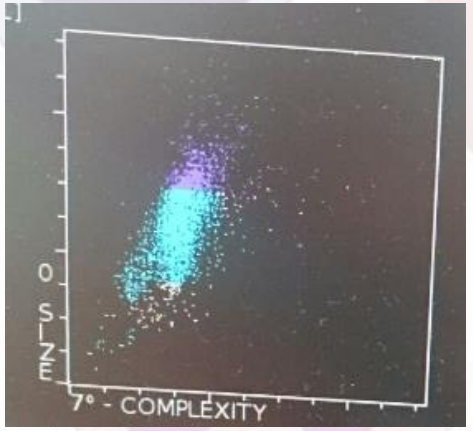
(positive with both CD5 and CD23)

- can transform to (PLL)

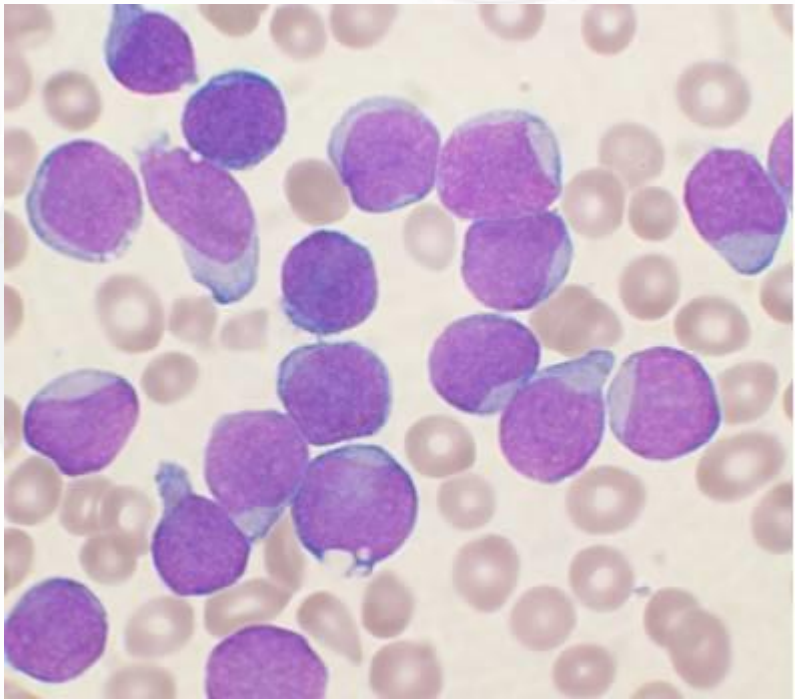
prolymphocytic leukaemia



ACUTE LYMPHOID LEUKAEMIA (ALL)



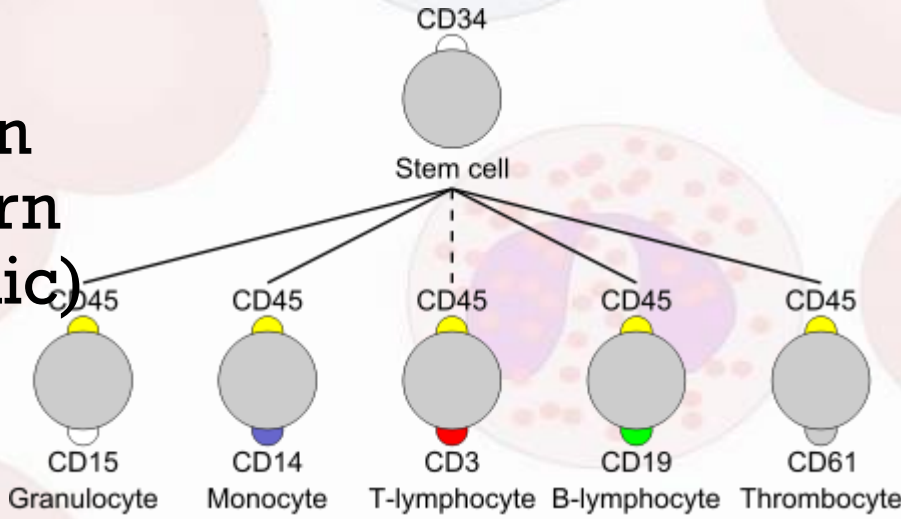
- increase in number of lymphoblastic cells (from bone marrow or extramedullar organs)
- most common type of cancer in children
- classified based on immunophenotyping and genotyping (low risk/high risk)

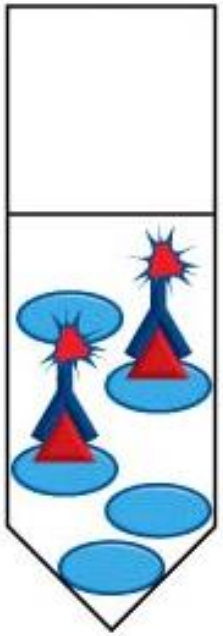


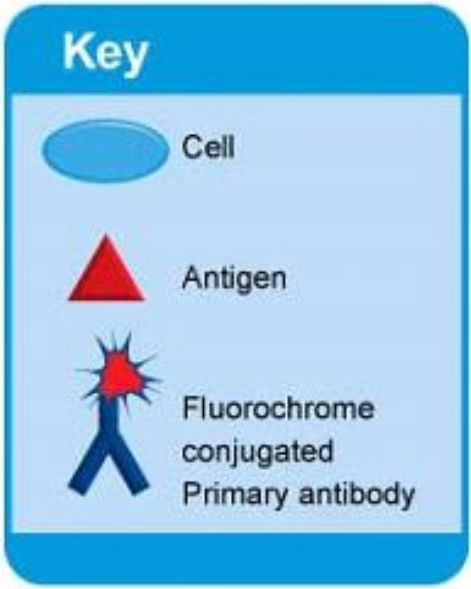
Flow cytometry 1.

BASICS:

- every type of cell has its own individual CD-marker pattern (surface and intracytoplasmic)
- adding the sample different monoclonal antibodies conjugated with different coloured fluorescent dyes result in specific binding of antibodies by the cells
- then the fluorophores are excited by a laser beam, after it the intensity of emission will be measured

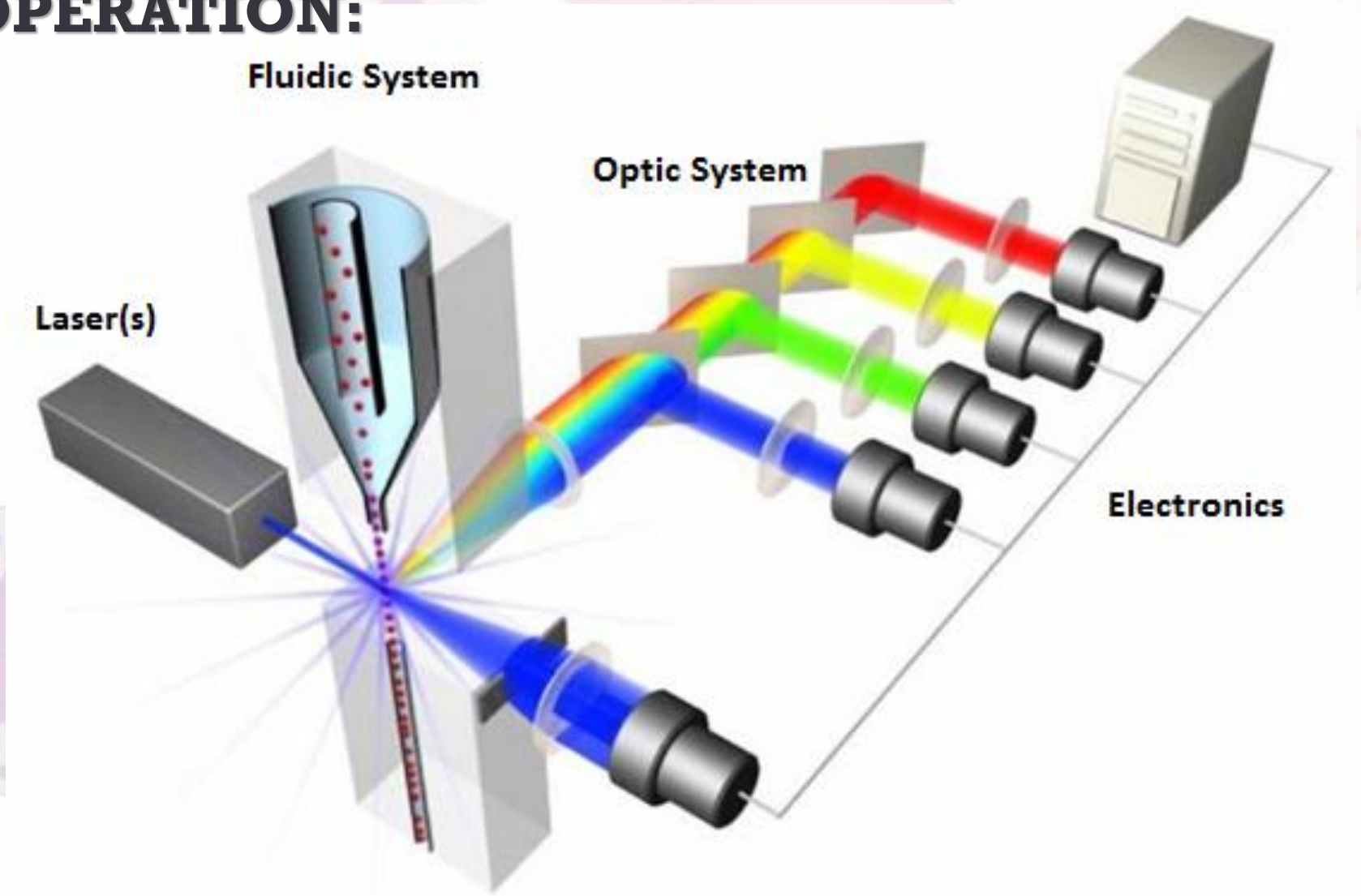






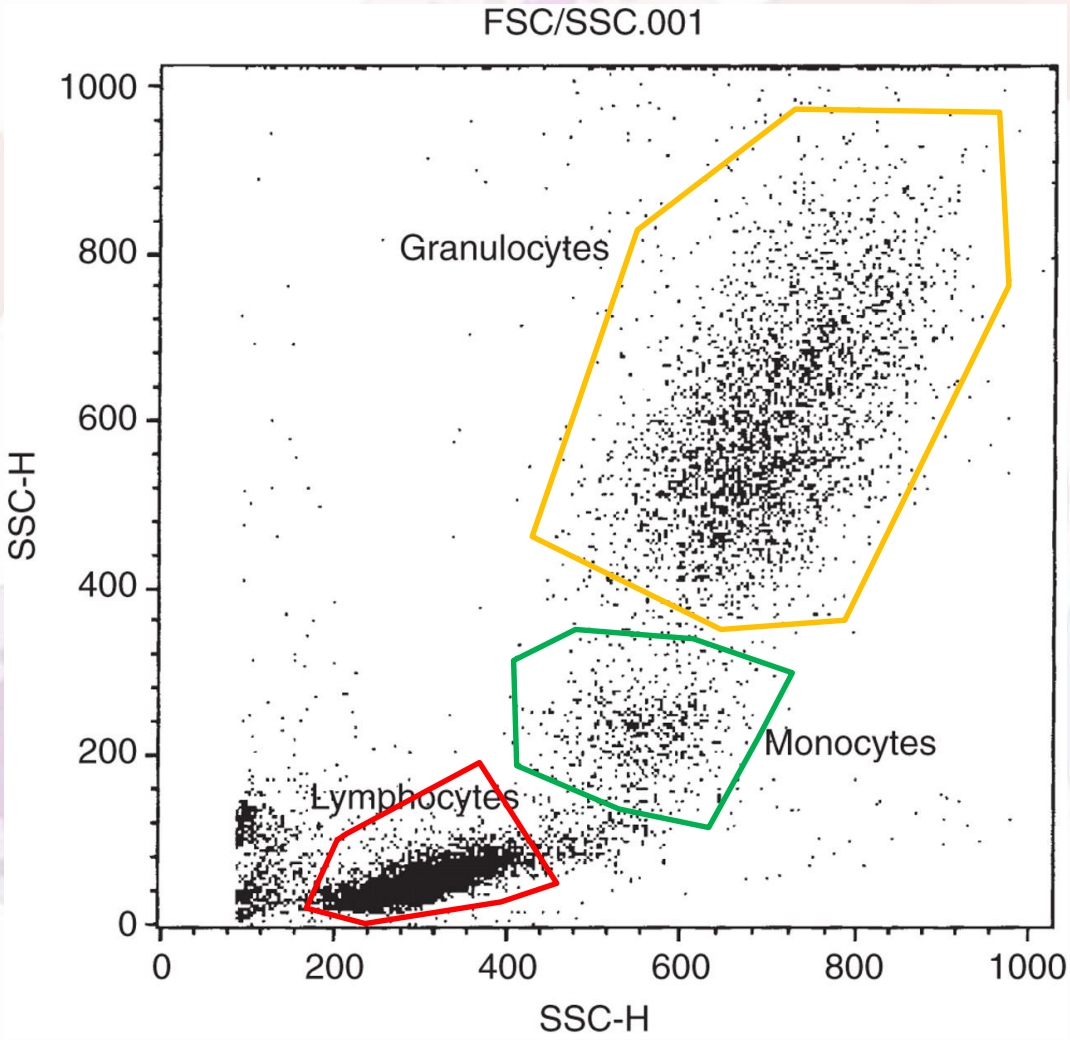
Flow cytometry 2.

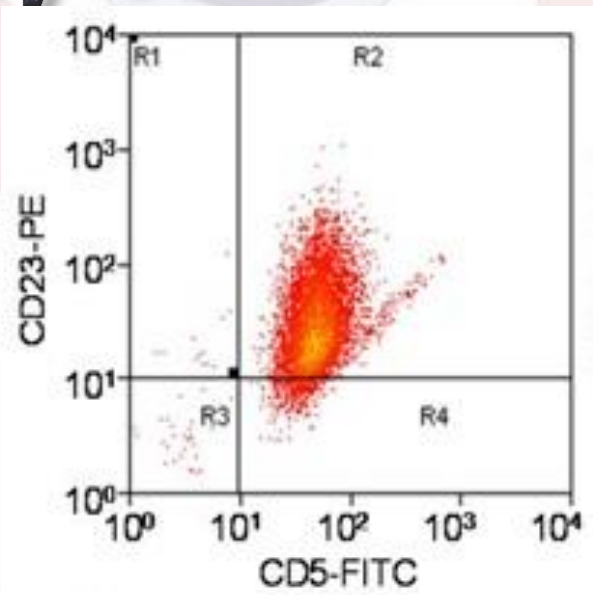
OPERATION:

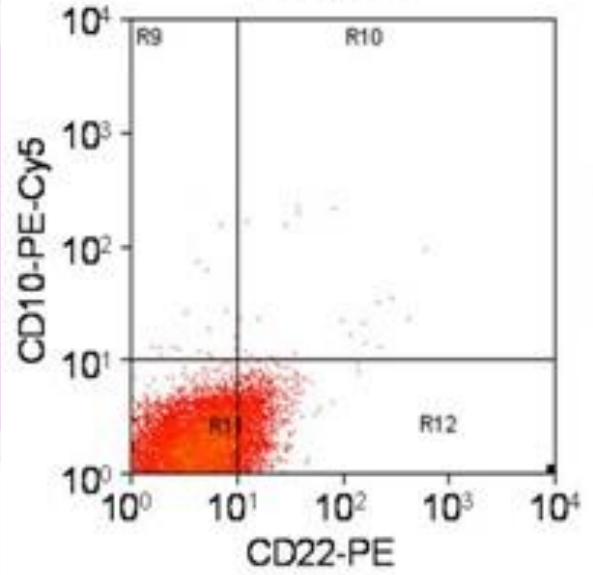


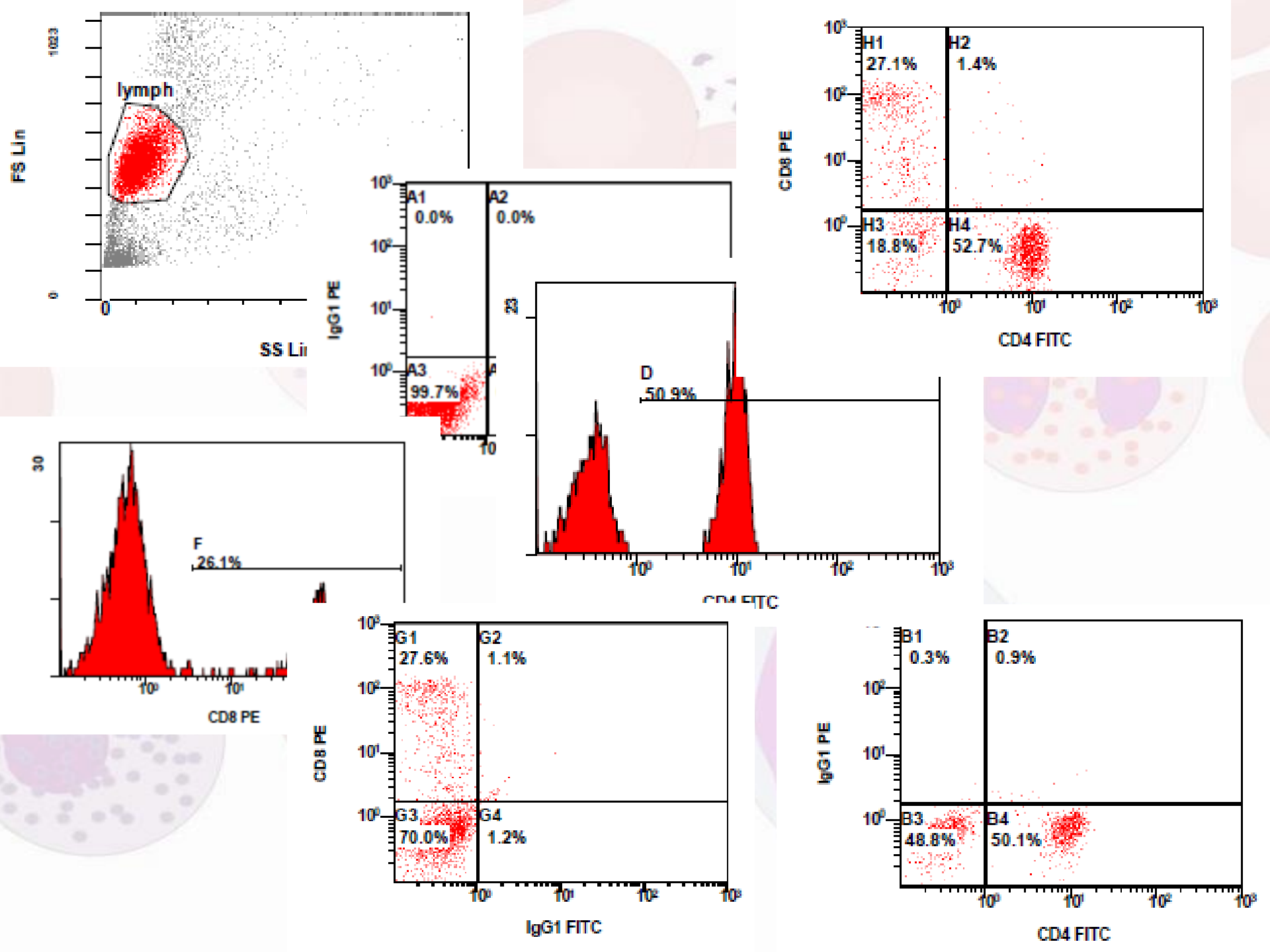
Flow cytometry 3.

EVALUATION:








lymph
FS Lin
SS Lin
IgG1 PE
A1
0.0%
A2
0.0%
A3
99.7%
CD8 PE
H1
27.1%
H2
1.4%
H3
18.8%
H4
52.7%
CD4 FITC
D
50.9%
F
26.1%
CD8 PE
G1
27.6%
G2
1.1%
G3
70.0%
G4
1.2%
IgG1 FITC
B1
0.3%
B2
0.9%
B3
48.8%
B4
50.1%
IgG1 PE
CD4 FITC

CASE STUDY

- 5 y.o. boy
- somnolent, exhaustible for a week, subfebrility
- started to vomit and had fever from a few days (38,2 °C)
- from that he cannot be fed, vomited again
- stool and urine was ok
- practitioner: hepatosplenomegaly
- requesting blood test

Születési dátum...: 2007.10.20 Esetszám: 6143436
Anyja neve.....: Kiss Helga Telj. AZ: 5104335
Lakcím.....: 7030 Paks, Vak Bottyán u. 23. TAJ.....: 126-643-392
Vizsgálatkérő.int.: GYF2 024211207 11207 Gyerm.Kl. Onkológia
Vizsgálatkérő.orv.:
69637 Györei Eszter Dr.
Iránydiagnózis....: U9990
Minta visszaigazolás: 2012.02.28 18:38

Megnevezés	Érték	Abn	Egység	Referencia tart.
Vérkép automatával:				
Fehérvérsejt	1420,000	H	Giga/l	5,000-14,500
WOC=1192 G/l				
Vörösvértest	2,81	L	T/l	3,60-5,80
Hemoglobin	113		g/l	108-156
Hematokrit	23,5	L	%	31,0-45,0
MCV	83,5		fl	77,0-89,0
MCH	40,2	H	pg	25,0-31,0
MCHC	481	H	g/l	320-360
A magas fehérvérsejtszám miatt nem értékelhető				
RDW	18,8	H	%CV	11,6-14,4
Trombocita	38,0	L	Giga/l	286,0-509,0
MPV	9,30	L	fl	9,40-12,40
Kenet #	:	X		

NEU: 4% LY: 20% MO: 0% EO: 0% BASO: 0% EGYÉB: 2% Promyelocyta: 74% Blast karaktrű sejt, Necrocyta 25-30/100 fvs.
A kenet leolvasást nehezíti a rendkívül magas fvs. szám, a megadott %-os fvs. számok megközelítőek.

Ellenőrizte: X
Vassné Lakatos Ágnes 2012.02.29 10:05

Vizsgált szerv:
perifériás vér

Minta típusa:
Natív Anyag, Kenetek

Klinikai diagnózisok:
ALL in obs

Klinikai adatok:
Háziorvosánál 02.27-én jelentkezett subfebrilitás és fáradékonyság miatt, hepatosplenomegáliát véleményezett és laboratóriumi vizsgálatot kért, mely eredményének tükrében (fvs: 1,6 millió, HgB: 66, Htk: 19, tct: 36000) kéri osztályos felvételét.

Panaszai 1 hete szerdán kezdődtek (2012.02.21-én). Fáradékonyságot, alvászékonyságot észleltek nála. Hőemelkedése jelentkezett. Pénteki napon hányt először. Hétvégén panaszai fokozódtak, vasárnap belázasodott (38,2°C). Hányás ismét jelentkezett, mai napon nem volt per os táplálható , az elfogyasztott fél liter folyadékot is kihányta. Székletet szombaton ürített utoljára, kis mennyiségűt, normál állagút. Mai napon 1 alkalommal ürített kevés vizeletet, mely nem véres, nem barna, nem csíp.

Makroszkópos leírás
A: 3ml EDTAs vér, B: 5db perif kenet (saját), C: 13db perif kenet (küldött)

Mikroszkópos leírás
02.29.
A kenetekben extrém leukocytosis figyelhető meg, a sejtek 98%-a lymphoblast, melyek többsége közepes méretű, a sejtek peroxidáz negatív cytoplasmával rendelkeznek.
A minta áramlási citometriai vizsgálata során 4% myeloid és 2% lymphoid sejt mellett 94% blast karakterű sejt mutatkozott. A blastok CD58, CD38, CD99, CD19 expressziót mutattak, 30%-ban CD34 jelölődés, 6%-ban minimális intenzitású cytoplasmicus IgM expresszió mellett. A sejtek CD20, CD10 és TdT negatívak, a sejtfelszínen könnyűláncok nem mutatkoztak, myeloid, illetve T-sejtes markerek nem voltak jelen.

Diagnózisok:
ACUT LYMPHOBLASTOS LEUKAEMIA
CD19+, CD38+, CD58+, CD99+, CD20-, TdT-, CD10-, CD34-/+
BNO: C9100 Heveny lymphoblastos leukaemia

THANK YOU FOR YOUR ATTENTION!