

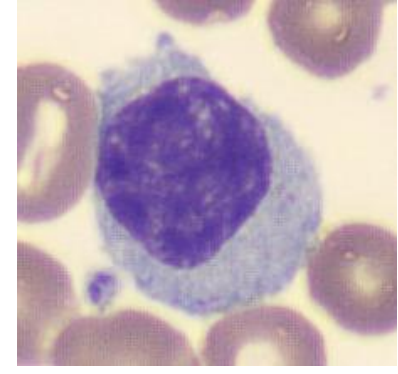
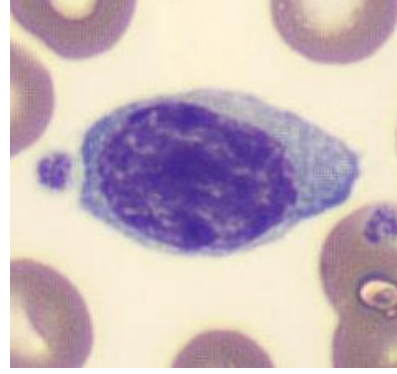
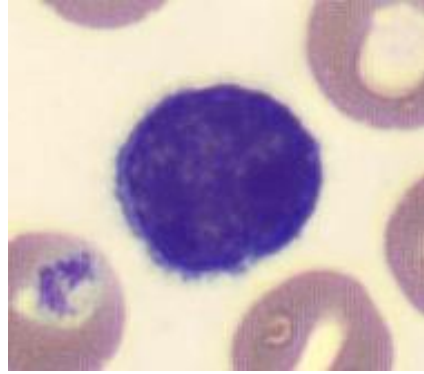
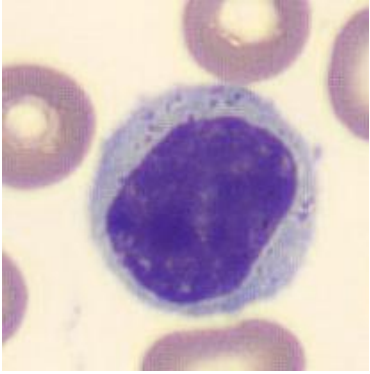


*Promjene brojnosti i morfologije krvnih stanica u
kroničnoj limfocitnoj leukemiji*

Ivana Lapić

Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb

Morfološki izgled zrelih limfocita



- Promjer 8 – 10 μm
- Velika jezgra koja zauzima veći dio stanice (visok omjer jezgra/citoplazma)
- Bez segmentacije jezgre
- Uski obodni sloj citoplazme
- Bez specifičnih granulacija

Referentni intervali za limfocite se razlikuju po dobi

DJECA

	Rezultat	Jedinica	Referentni interval
⌘(K) Leukociti	6.8	$\times 10^9/L$	6.0 - 16.0
⌘(K) Eozinofilni granulociti	2.8	%	0 - 6
⌘(K) Bazofilni granulociti	1.8	%	0 - 2
⌘(K) Segmentirani granulociti	11.9	%	23 - 66
⌘(K) Limfociti	79.8	%	18 - 60
⌘(K) Monociti	3.7	%	5 - 13
⌘(K) Eozinofilni granulociti	0.19	$\times 10^9/L$	0.00 - 0.70
⌘(K) Bazofilni granulociti	0.12	$\times 10^9/L$	0.00 - 0.20
⌘(K) Segmentirani granulociti	0.81	$\times 10^9/L$	2.50 - 7.20
⌘(K) Limfociti	5.43	$\times 10^9/L$	2.00 - 6.60
⌘(K) Monociti	0.25	$\times 10^9/L$	0.22 - 1.51

Limfociti >
Neutrofili

ODRASLI

	Rezultat	Jedinica	Referentni interval
⌘(K) Leukociti	6.3	$\times 10^9/L$	3.4 - 9.7
⌘(K) Eozinofilni granulociti	3.5	%	0 - 7
⌘(K) Bazofilni granulociti	1.3	%	0 - 1
⌘(K) Neutrofilni granulociti	53.0	%	44 - 72
⌘(K) Limfociti	32.1	%	20 - 46
⌘(K) Monociti	10.1	%	2 - 12
⌘(K) Eozinofilni granulociti	0.22	$\times 10^9/L$	0.00 - 0.43
⌘(K) Bazofilni granulociti	0.08	$\times 10^9/L$	0.00 - 0.06
⌘(K) Neutrofilni granulociti (ANC)	3.34	$\times 10^9/L$	2.06 - 6.49
⌘(K) Limfociti	2.02	$\times 10^9/L$	1.19 - 3.35
⌘(K) Monociti	0.64	$\times 10^9/L$	0.12 - 0.84

Limfociti <
Neutrofili

...a time i kriteriji za limfocitozu

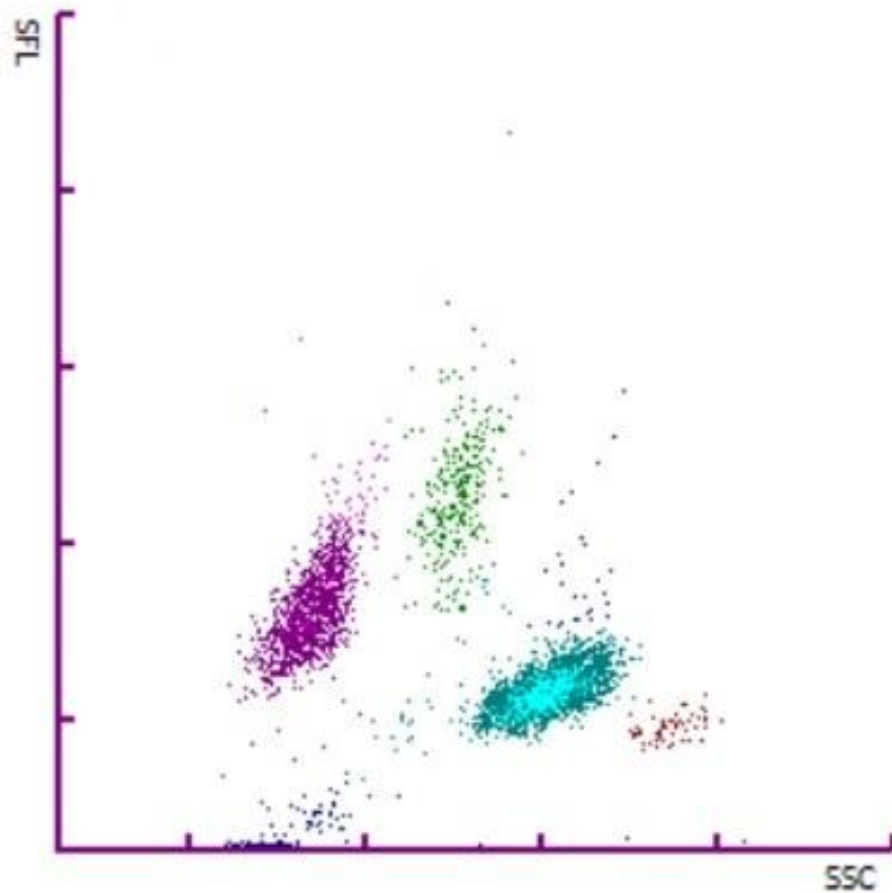
- Djeca > $11 \times 10^9/L$
- Odrasli > $4 \times 10^9/L$

Laboratorijska obilježja KLL-a

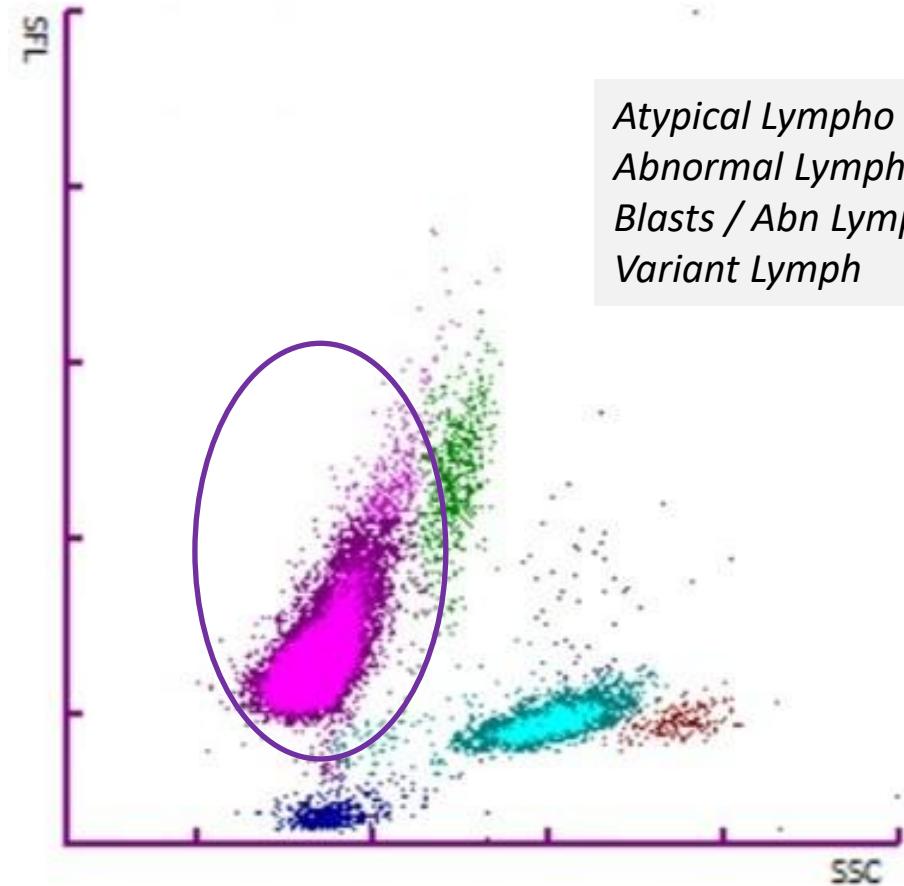
	Rezultat	Jedinica	Referentni interval
⌘(K) Eritrociti	4.89	x10 ¹² /L	3.86 - 5.08
⌘(K) Hemoglobin	130	g/L	119 - 157
⌘(K) Hematokrit	0.422	L/L	0.356 - 0.470
⌘(K) MCV	86.3	fL	83.0 - 97.2
⌘(K) MCH	26.6	pg	27.4 - 33.9
⌘(K) MCHC	308	g/L	320 - 345
⌘(K) RDW	16.4	%	9.0 - 15.0
⌘(K) Retikulociti	18.1	/1000 Erc	5.0 - 21.6
⌘(K) Retikulociti	89	x10 ⁹ /L	22 - 97
⌘(K) Indeks zrelosti retikulocita	0.22		0.02 - 0.18
⌘(K) Eritroblasti	0.0	x10 ⁹ /L	0.0
⌘(K) Eritroblasti	< 0.4	/100 Lkc	0
	Rezultat	Jedinica	Referentni interval
⌘(K) Leukociti	198.1 !	x10 ⁹ /L	3.4 - 9.7
⌘(K) Eozinofilni granulociti	0.5	%	0 - 7
⌘(K) Bazofilni granulociti	0.0	%	0 - 1
⌘(K) Segmentirani granulociti	10.0	%	44 - 72
⌘(K) Limfociti	88.5	%	20 - 46
⌘(K) Monociti	1.0	%	2 - 12
⌘(K) Eozinofilni granulociti	0.99	x10 ⁹ /L	0.00 - 0.43
⌘(K) Bazofilni granulociti	0.00	x10 ⁹ /L	0.00 - 0.06
⌘(K) Segmentirani granulociti	19.81	x10 ⁹ /L	2.06 - 6.49
⌘(K) Limfociti	175.32	x10 ⁹ /L	1.19 - 3.35
⌘(K) Monociti	1.98	x10 ⁹ /L	0.12 - 0.84
	Rezultat	Jedinica	Referentni interval
⌘(K) Trombociti	189	x10 ⁹ /L	158 - 424
⌘(K) MPV	9.5	fL	9.0 - 13.0
Napomena	Stanice u raspadu		

- **Leukocitoza** (> 10 x 10⁹/L, često > 100 x 10⁹/L)
- **Limfocitoza** (> 5 x 10⁹/L, često > 90 %)
- Relativni udio neutrofilnih granulocita snižen
- Apsolutni broj neutrofilnih granulocita ovisi o broju leukocita (↓, N ili ↑)
- U početnoj fazi bolesti nema anemije i trombocitopenije, razvijaju se kasnije

Promijenjen citogram



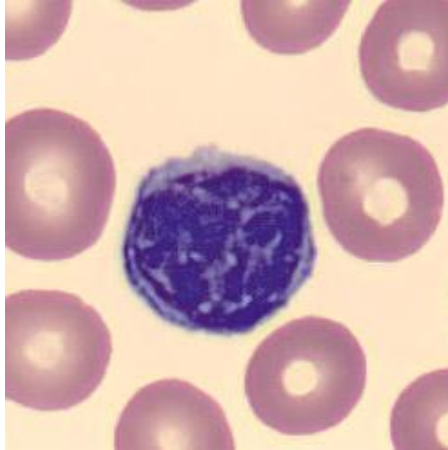
Uredan citogram



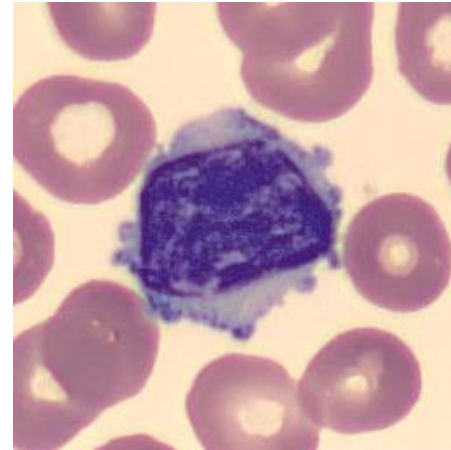
Citogram kod KLL-a

Morfologija limfocita kod KLL-a

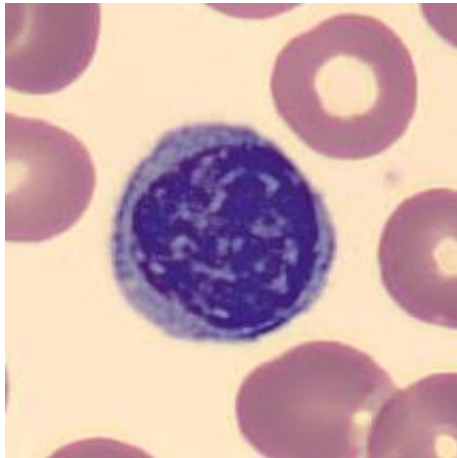
*mali, naizgled zreli
limfociti pravilnog
okruglog oblika*



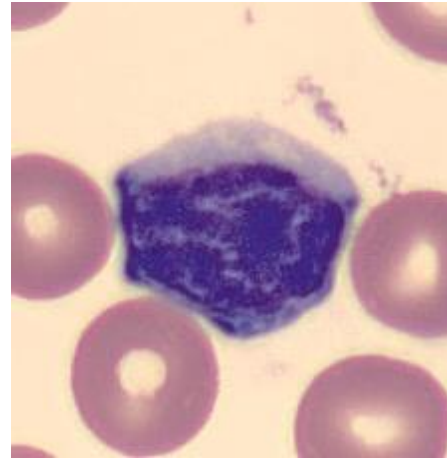
*oskudna, blago bazofilna
citoplazma vidljiva
uglavnom samo s jedne
strane stanice;
vrlo visok omjer jezgre i
citoplazme*



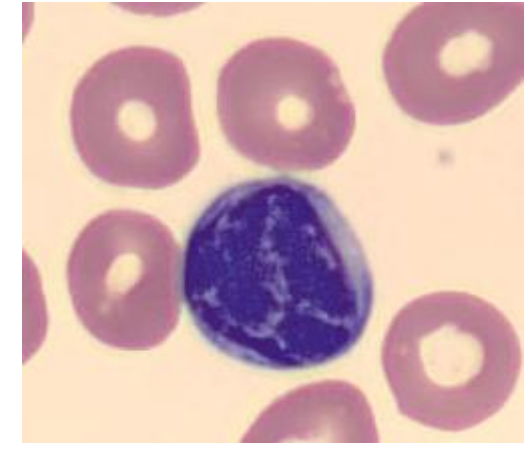
*unutar jezgre je vidljiv gust,
kondenziran kromatin
organiziran u guste, tamno
obojene grudice razdvojene
svjetlijim pukotinama, a
jezgrice su neprimjetne*



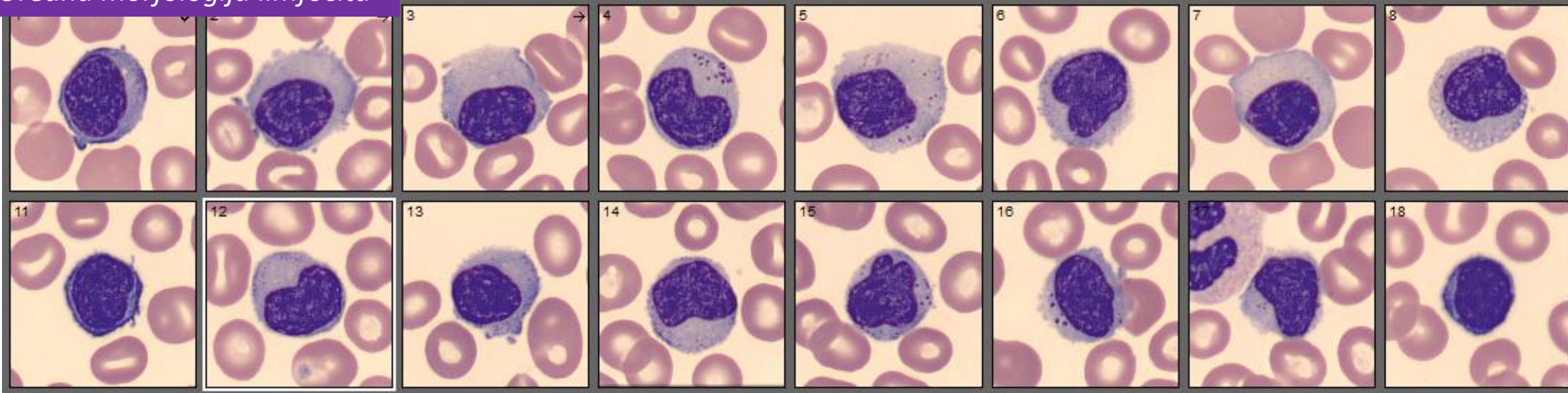
*jezgra je najčešće
okrugla,
ekscentrično
položena i tamno
plava*



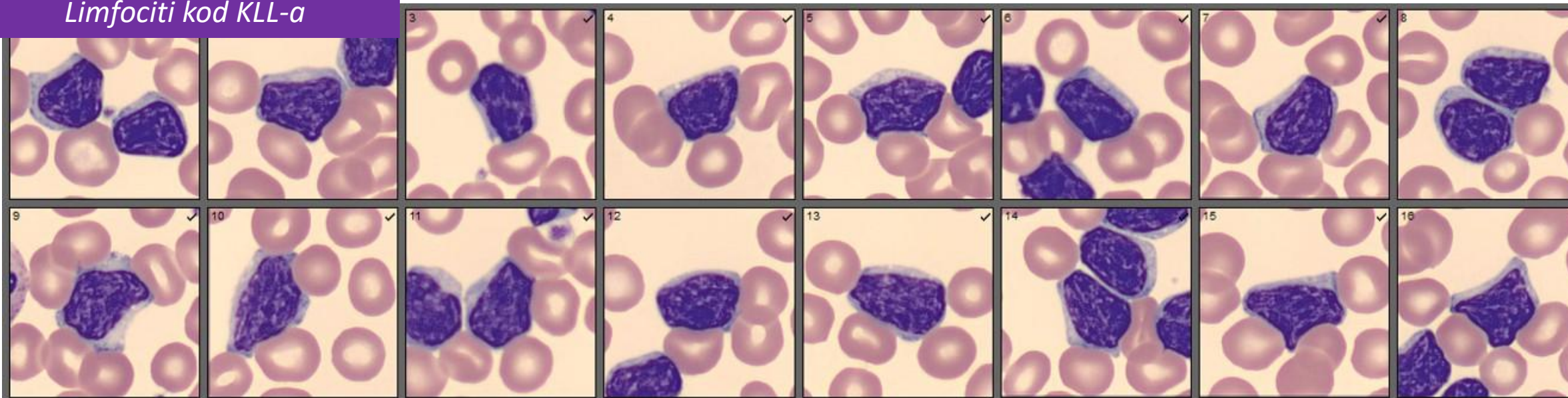
*citoplazma može
imati manje
nepravilne,
vezikularne izdanke*



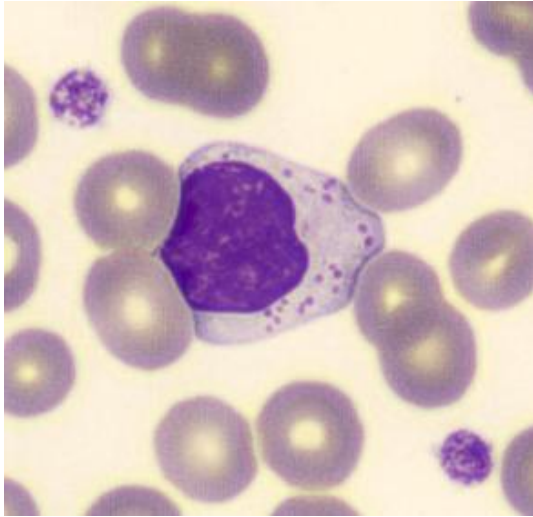
Uredna morfologija limfocita



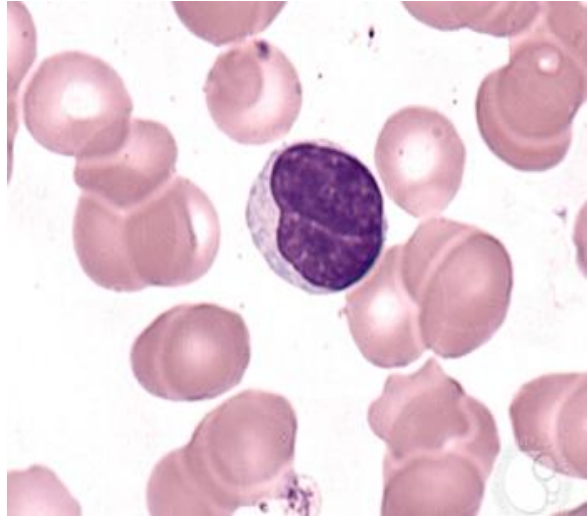
Limfociti kod KLL-a



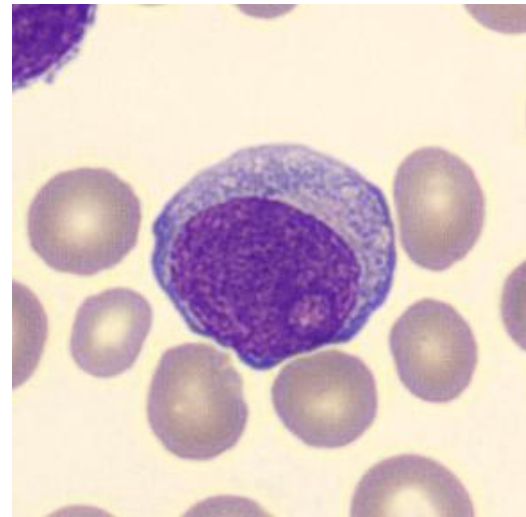
Do 10 % varijantnih limfocita



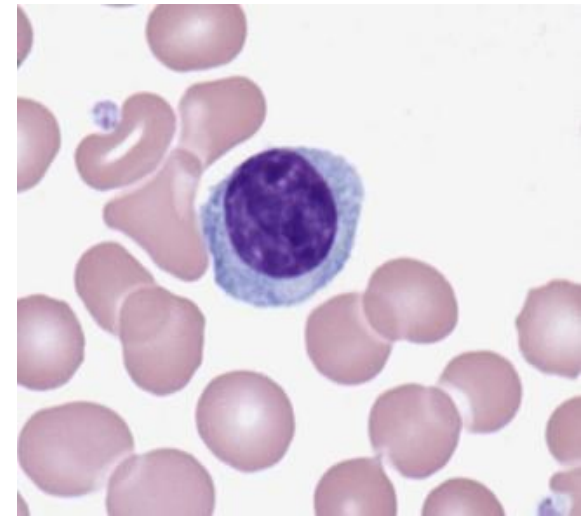
Veliki granulirani limfociti
(engl. *large granulated lymphocytes, LGL*)



Mali zarezani limfociti

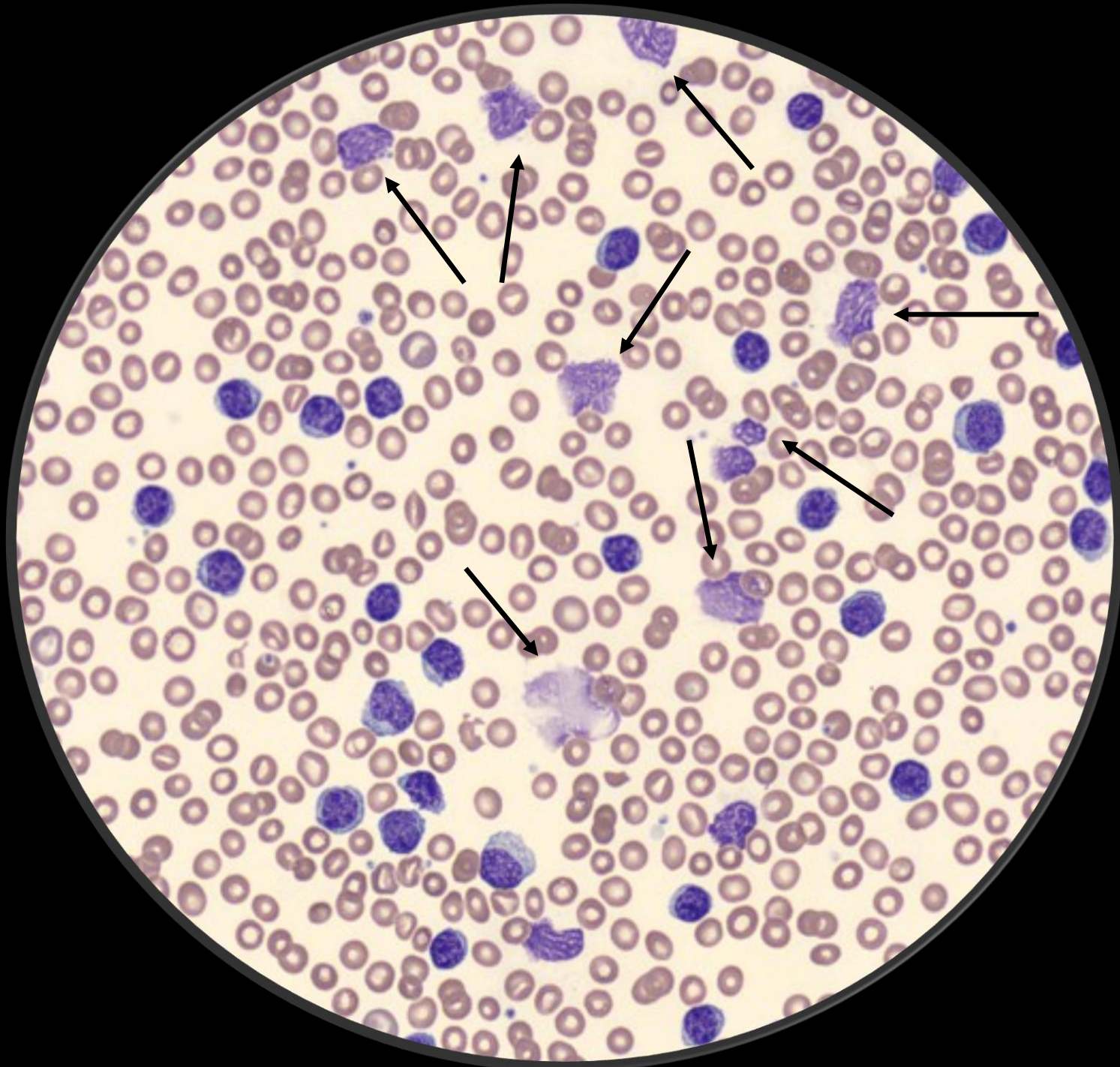


Prolimfociti



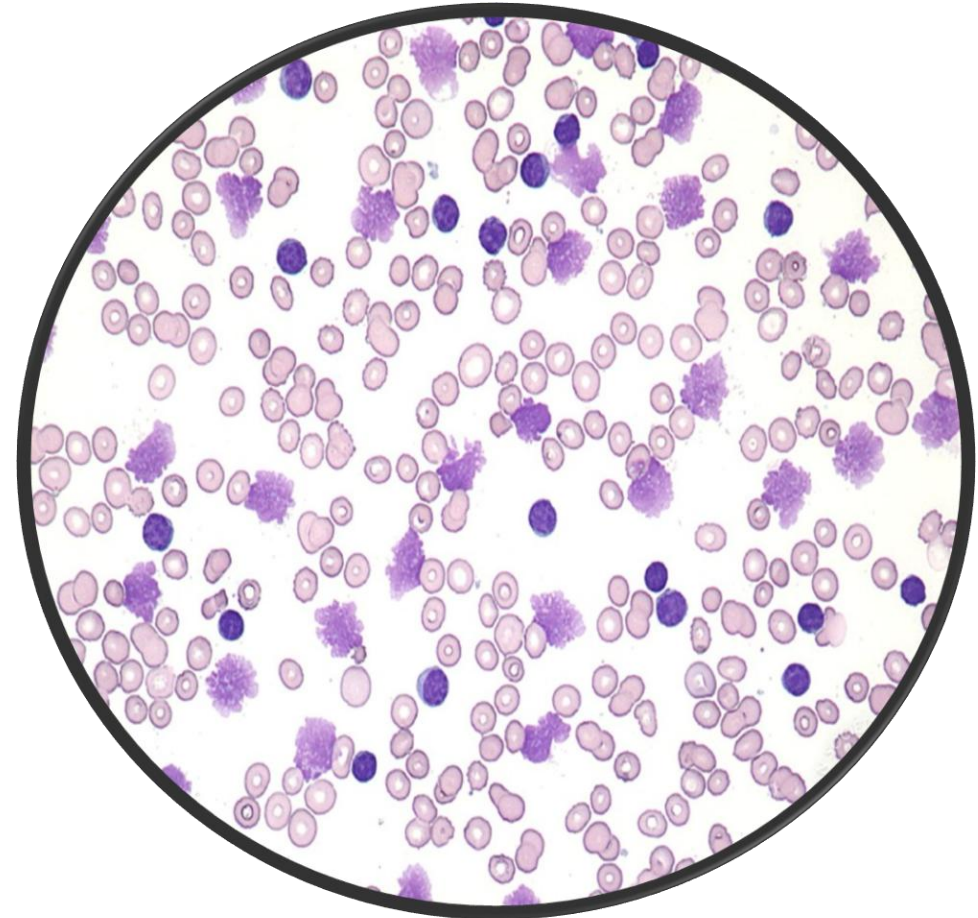
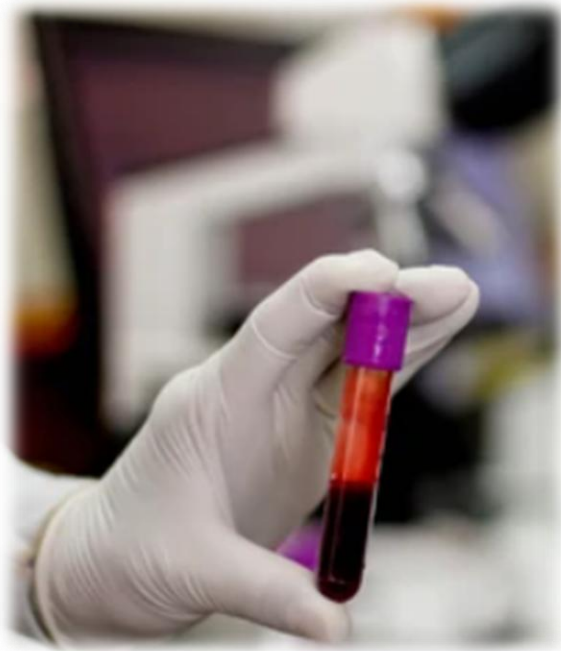
Limfoplazmocitoidne
stanice

Blasti, ako su prisutni, ne prelaze udio od 5 %

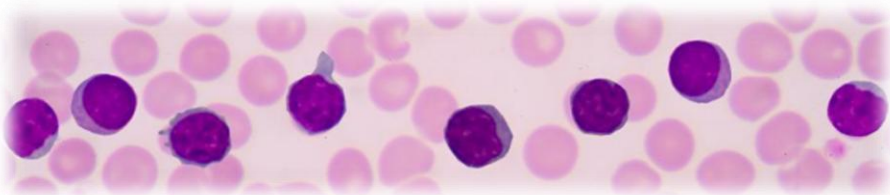


Gumprechtove sjene

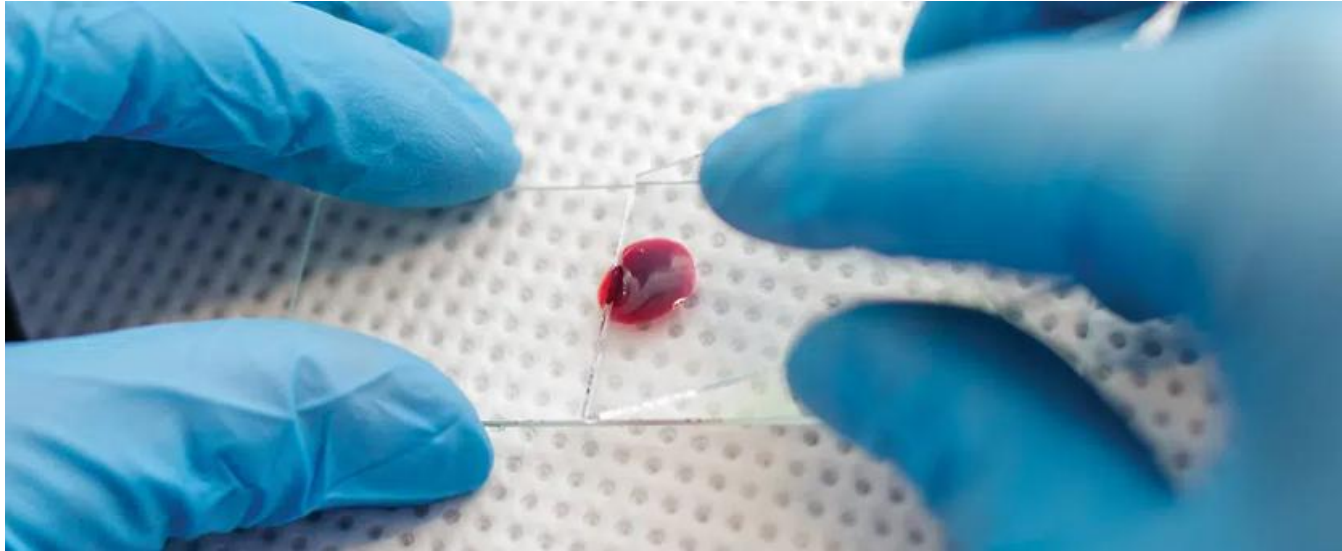
U uzorku pune krvi su prisutni
intaktni limfociti...



...dok su **Gumprechtove sjene** prisutne isključivo
u razmazu periferne krvi.



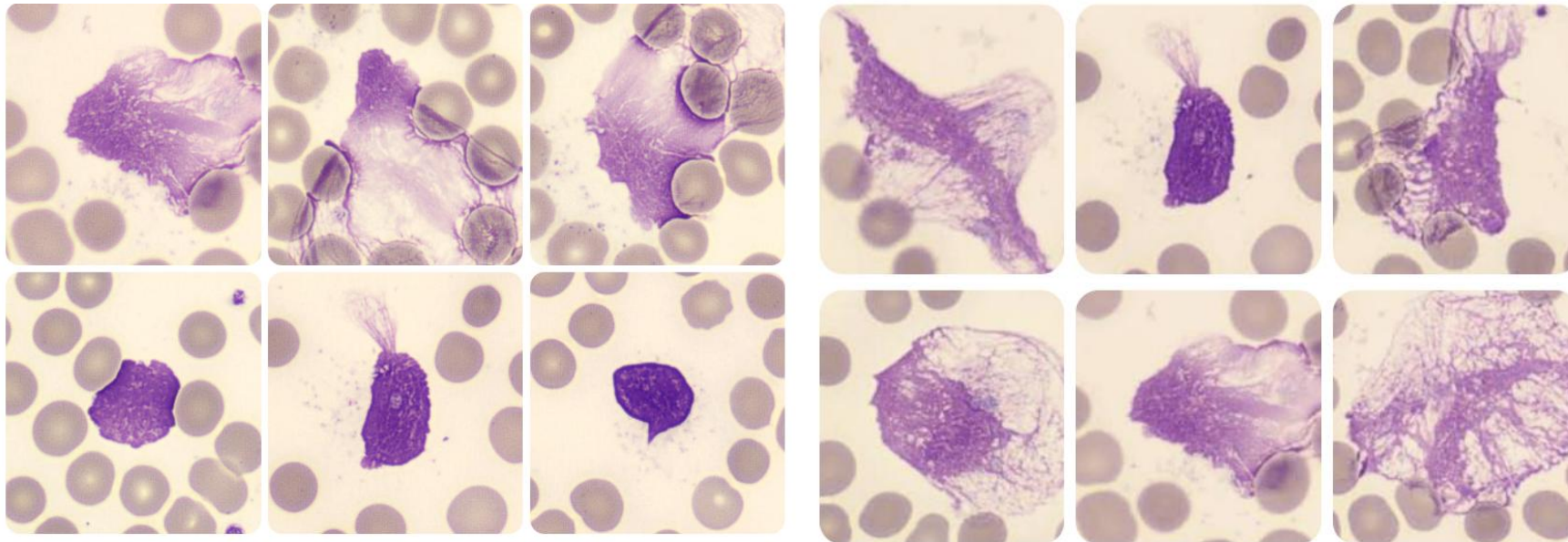
Gumprechtove sjene



Povećana osjetljivost membrane na mehaničke sile *in vitro*



Oštećene, raspuknute ili fragmentirane tvorbe



ne smatraju se patognomoničnim obilježjem niti su dio dijagnostičkih kriterija KLL-a

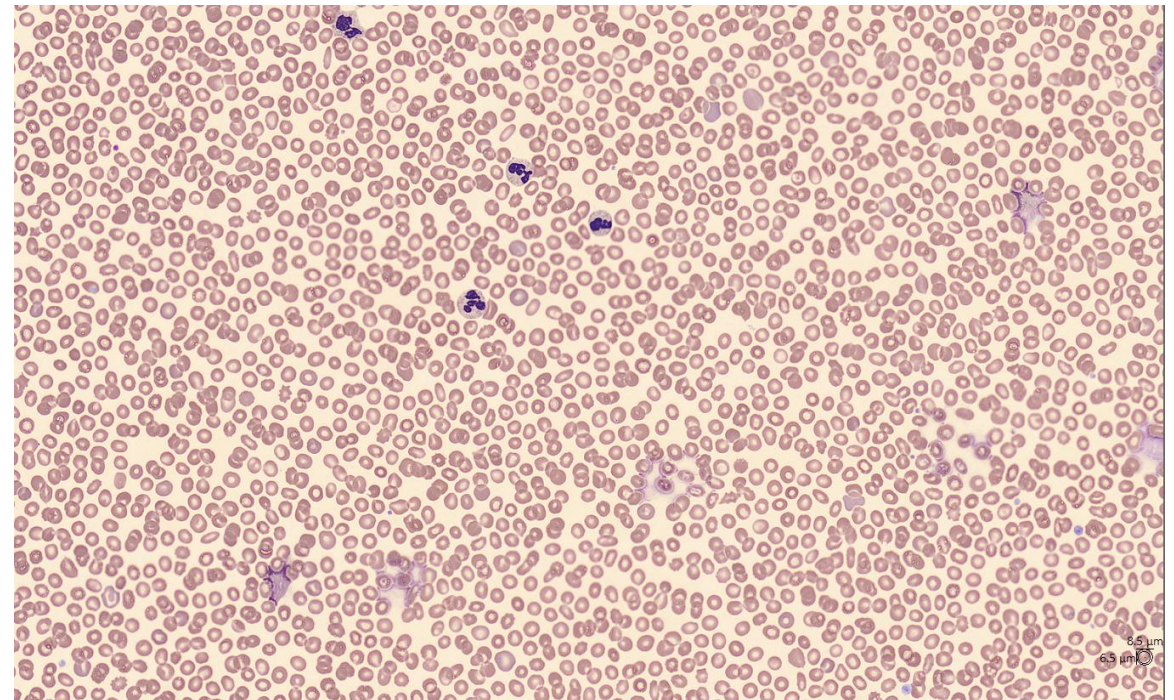
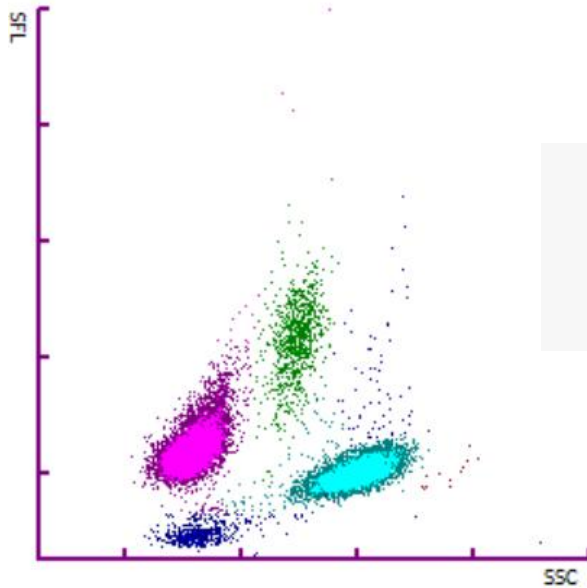
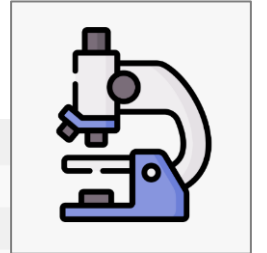
Gumprechtove sjene



NEUT%	34.1	%
LYMPH%	61.8	%
MONO%	3.9	%
EO%	0.0	%
BASO%	0.2	%



WBC	Count	%
• Band neutrophil	1	0.5
• Segmented neutrophil	161	80.1
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	28	13.9
• Monocyte	11	5.5



*utjecaj na nalaz mikroskopske diferencijalne krvne slike (DKS),
ali ne i DKS određenu automatiziranom metodom na hematološkom uređaju*

Preporuke za izvještavanje

Kada je očito o kojim se stanicama radi, raspadnute stanice se **uvrštavaju u stanice iz kojih su nastale**. Preporučuje se **izdavanje DKS s uređaja, uz komentar** o prisutnosti raspadnutih stanica.

Palmer L, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. Int J Lab Hematol. 2015;37:287-303.

Ako **nije moguće identificirati** iz kojih su stanica nastale, raspadnute stanice se **ubrajaju u zasebnu kategoriju** i tako izvještavaju, **uz komentar** na nalazu.

CLSI H20-A2, 2007

Izdaje se **DKS s uređaja, uz komentar** o prisutnosti raspadnutih stanica.

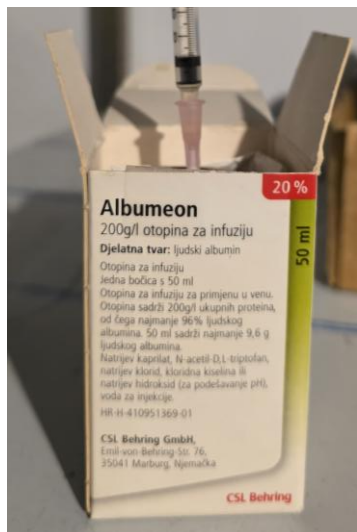
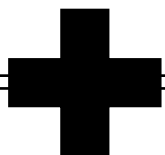
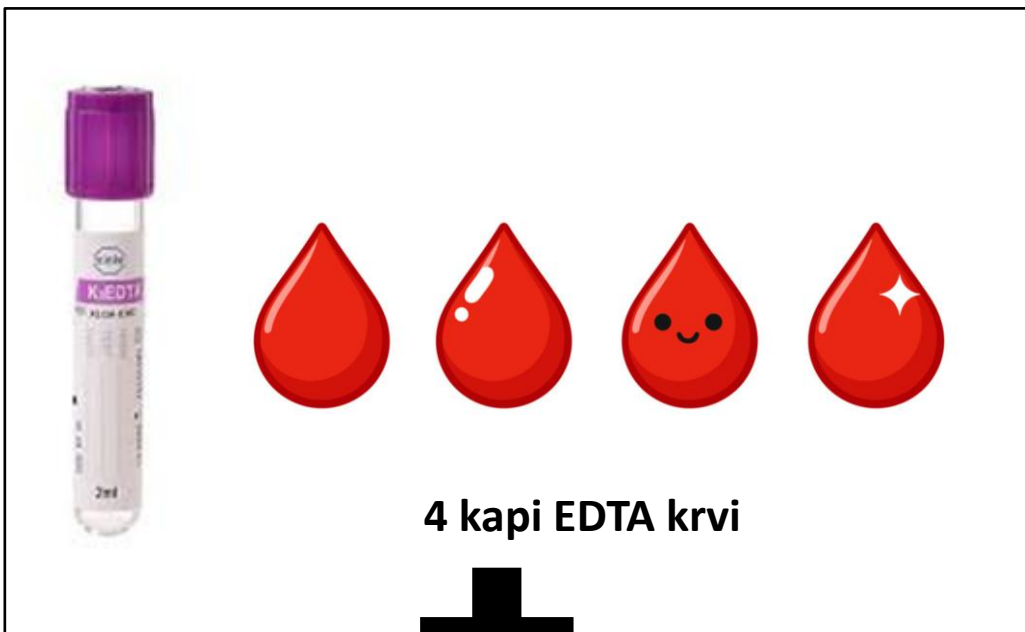
Ako DKS s uređaja nije dostupan, raspadnute stanice se **ubrajaju u limfocite** i izdaju **uz komentar, ili kao zasebna subpopulacija**.

Hematology Committee, Quality Management Program—Laboratory Services, Ontario. Broadsheet: Good Practice Guidelines on the Reporting of Smudge Cells. Toronto, Ontario: QMP-LS; June 26, 2001. Hematology-Morphology Binder: vol 3;sect 1.2:1.

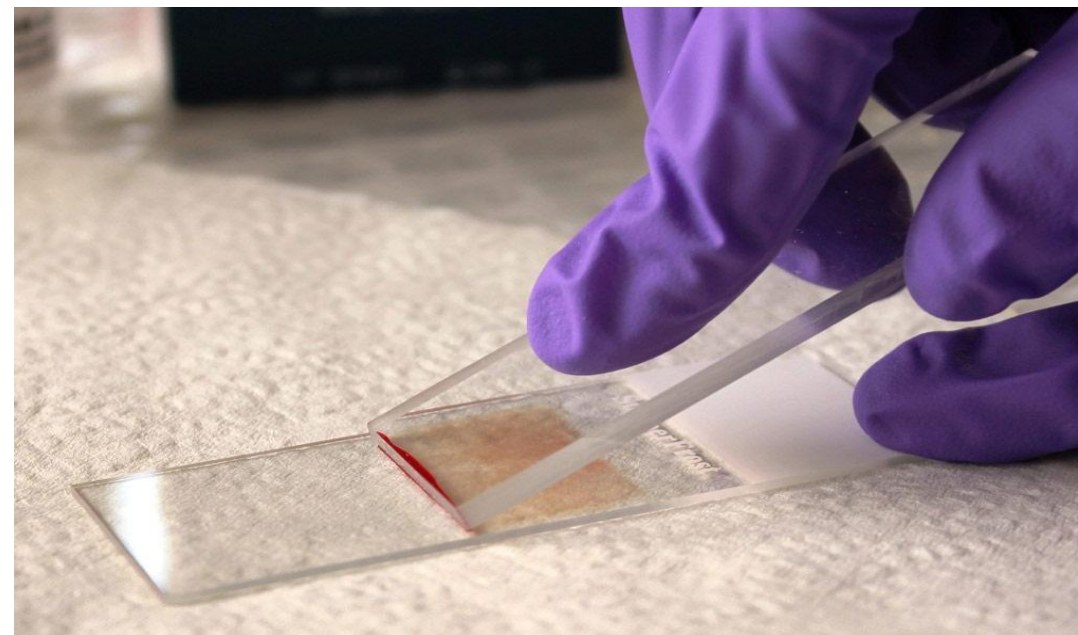


**NE izdavati samo mikroskopski DKS
ako su prisutne raspadnute stanice**

Preodbrada uzorka s albuminom



1 kap 20%-tne
otopine albumina



- ✓ kod bolesnika s KLL-om ako postoje kvalitativne poruke upozorenja s hematološkog uređaja
- ✓ ako su uz limfocite prisutni blasti ili više od 10 % ostalih limfoidnih stanica

Diferencijalna dijagnoza

REAKTIVNE LIMFOCITOZE

- virusne infekcije (EBV, CMV)
- primjena određenih lijekova
- traume
- akutni stresni događaji

NON-HODGKINOV LIMFOM

OSTALI LIMFOPROLIFERATIVNI POREMEĆAJI

- Prolimfocitna leukemija
- Leukemija vlasastih stanica
- Leukemija velikih granuliranih limfocita
- Sézaryjev sindrom
- Splenični B-stanični limfom

AKUTNA LIMFOBLASTIČNA LEUKEMIJA

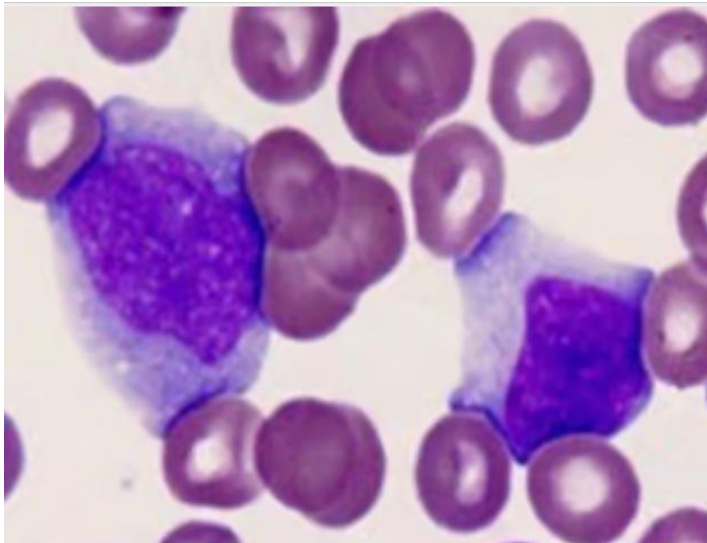
Infektivna mononukleoza

KARAKTERISTIČNI NALAZI

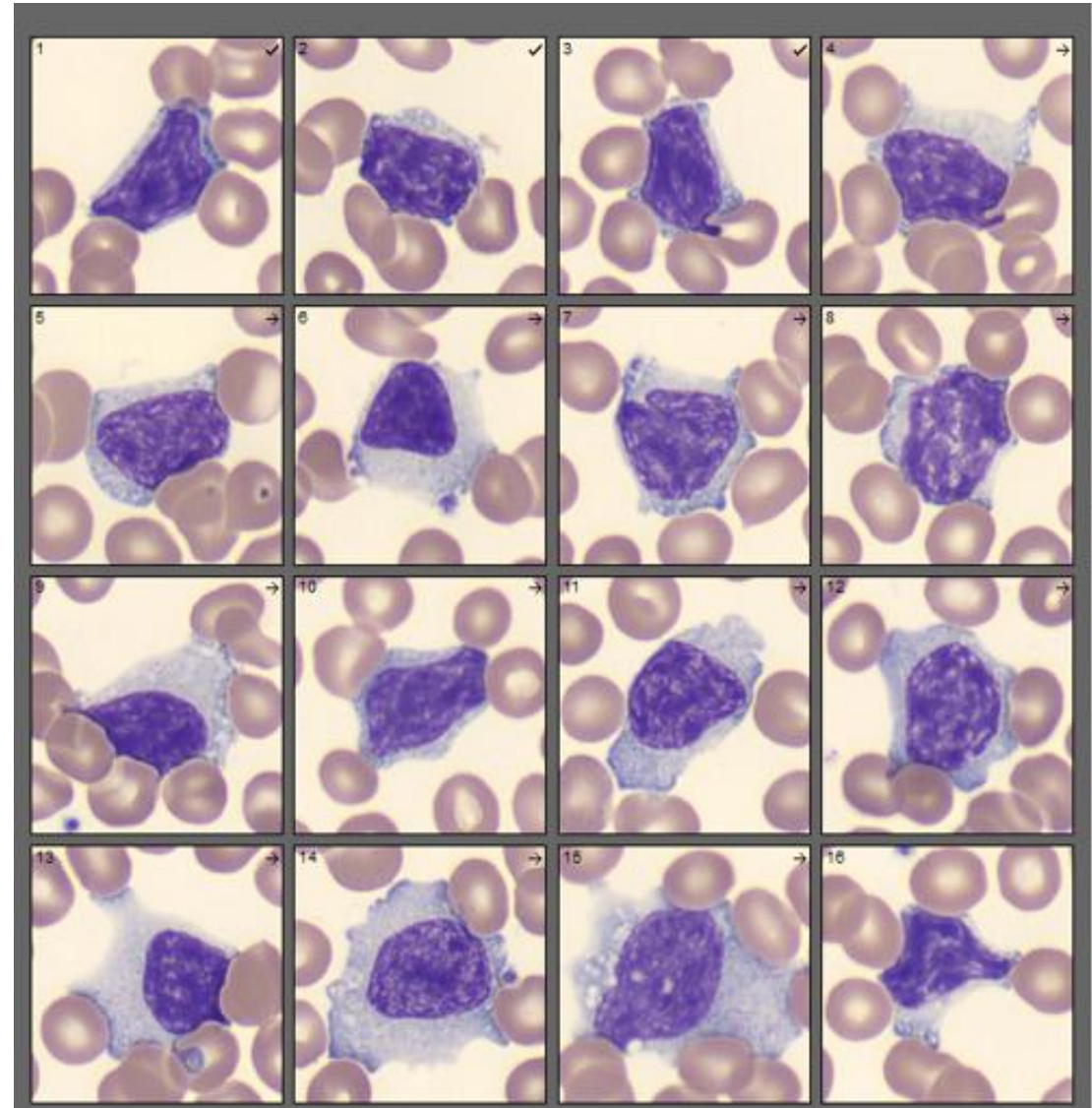
Leukocitoza ($12-25 \times 10^9/L$)

Pleomorfna limfocitoza (>20% reaktivnih ly)

Jetreni enzimi (LDH, AST, ALT) ↑



- 2-3x veći volumen od zdravih limfocita (promjera > 30 μm)
- zrela jezgra
- obilna, bazofilna citoplazma s izraženijim bazofilnim rubom



Learning from Error

Mark L. Graber*, Dan Berg, Welcome Jerde, Phillip Kibort, Andrew P.J. Olson and Vinita Parkash

Learning from tragedy: the Julia Berg story

Table 1: Laboratory results – Julia Berg.

Normal range		5-Aug	6-Aug	7-Aug	8-Aug	9-Aug	10-Aug	
							Pre-op	Post-op
0.2–1 mg/dL	Bili	7.2	7	6.7	7.6		9.6	
0.0–0.3 mg/dL	Direct bili	5.2	5.3	5.2	5.7		7.3	
3.5–4.9 g/dL	Alb		2.5	2.1			2	
42–168 U/L	Alk Phos	393	431	451	561	604	659	
30–65 U/L	ALT	249	184	161	161	180	179	
23–60 U/L	AST	189	132	130	163	209	211	
20–210 U/L	Gamma GT		290	271	280			
114–286 U/L	Lipase		406	320	321	383	80	
20–110 U/L	Amylase	39	85	59	46	45	102	
12–16 g/dL	Hgb		11.9	12.2	12.7	12.3	11.4	5.8
150–450 K/ μ L	Plts		134	134	151	157	147	
4.5–11.3 K/ μ L	WBC		25	28 ^a	39.9 ^a	47 ^a	43.8	
25–45%	% Lymphs		86%	93%	94%	94%	92%	

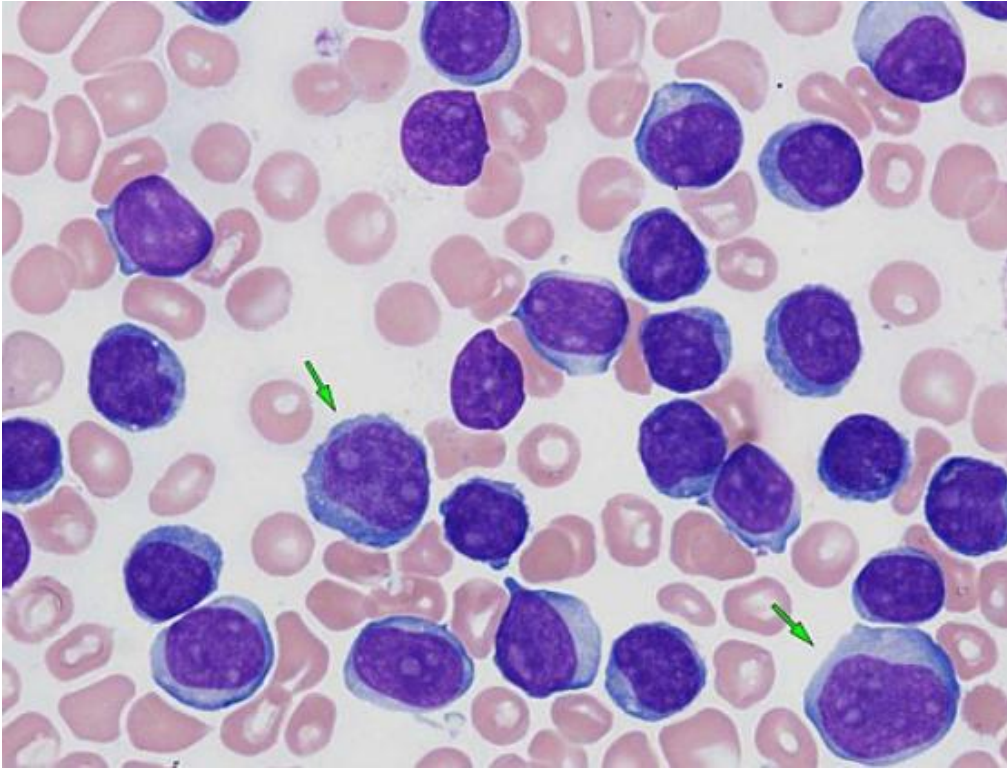
^aWBC values phoned to ward and read back by nurses Aug 7–9.

Reaktivne vs. maligne limfocitoze

Table 2 Most common features of reactive and malignant lymphocytosis

Feature	Reactive	Malignant
Patient's age	<30	>50
Lymphadenopathy	Typically absent	May be present
Absolute lymphocyte count	Usually increased	Increased, normal, or decreased
Percent lymphocytes	Usually increased	Increased, normal, or decreased
Platelets	Normal	Normal or decreased
Anemia	Absent or mild	Usually present
Cell heterogeneity	Present	Absent
Cell size	Variable but usually large	Small to medium to large
Nucleus	Irregular	Round, indented, folded, irregular
Nucleolus	Absent or inconspicuous	Absent, prominent
Cytoplasm	Abundant, radiating basophilia	Scant to moderate
Cytoplasmic projections	Absent	May be present *
Red blood cell molding	Present	Usually absent
Smudge cells	Absent	May be present **

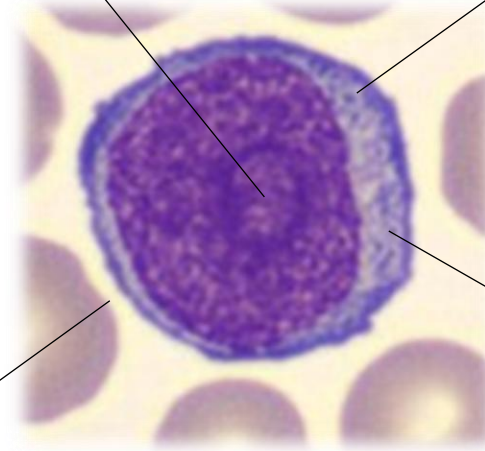
Prolimfocitna leukemija



- izrazita leukocitoza ($> 300 \times 10^9/L$), anemija i trombocitopenija
- prolimfociti $> 55\%$ stanica limfocitnog reda

Kondenziran i heterogen kromatin s jednom jezgri u središnjem dijelu jezgre

Okrugla jezgra pravilnog obruba

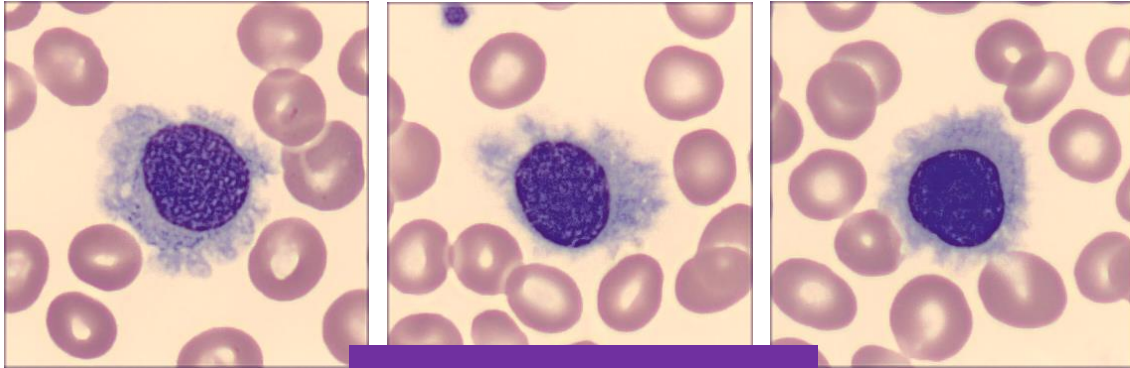


Veći volumen stanice od limfocita u KLL-u, manji omjer jezgre i citoplazme

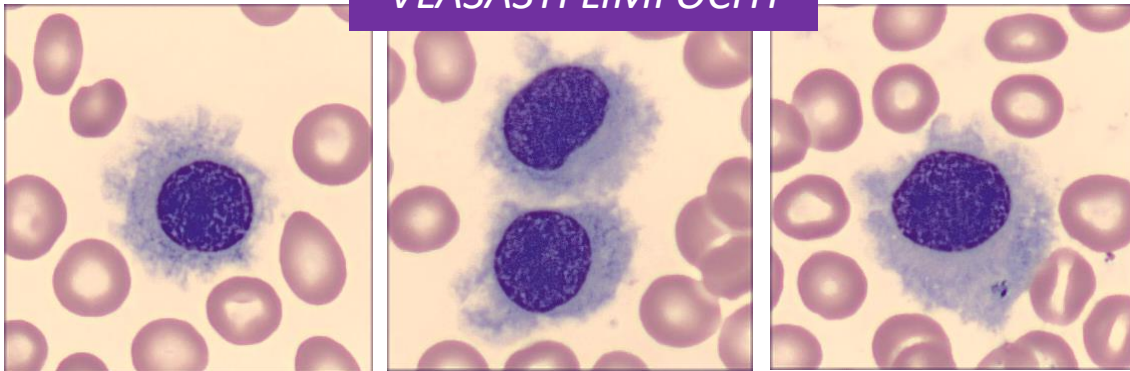
Citoplazma bazofilna i bez granula, obilnija nego u zrelih limfocita

Vlasasti vs. vilozni limfociti

LEUKEMIJA VLASASTIH STANICA

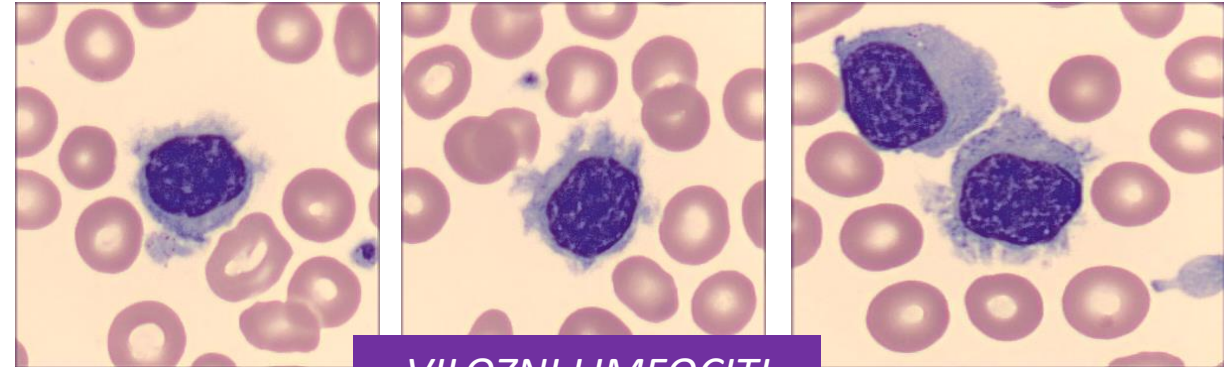


VLASASTI LIMFOCITI

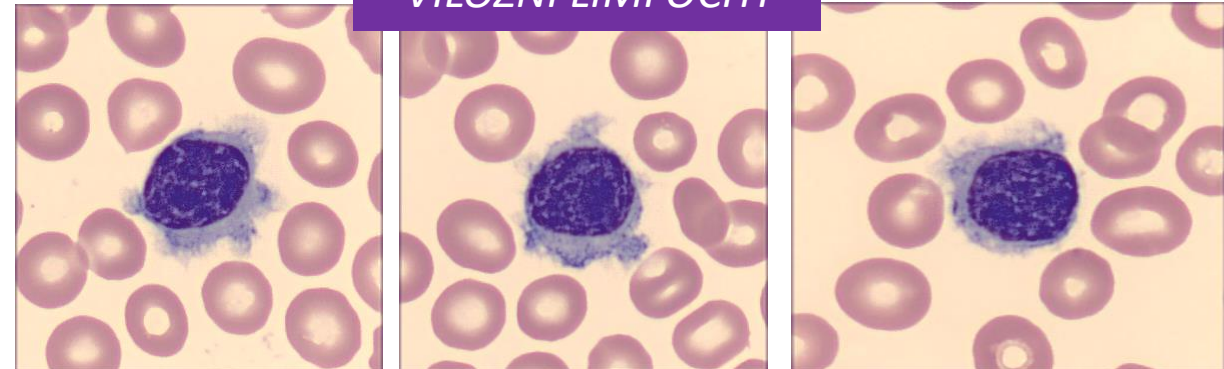


- obilna, blijedo-plava citoplazma iz koje se zrakasto pružaju vlasasti izdanci
- jezgra je okrugla ili bubrežasta, s nježnim kromatinom
- najčešće pancitopenija

SPLENIČNI B-STANIČNI LIMFOM MARGINALNE ZONE



VILOZNI LIMFOCITI

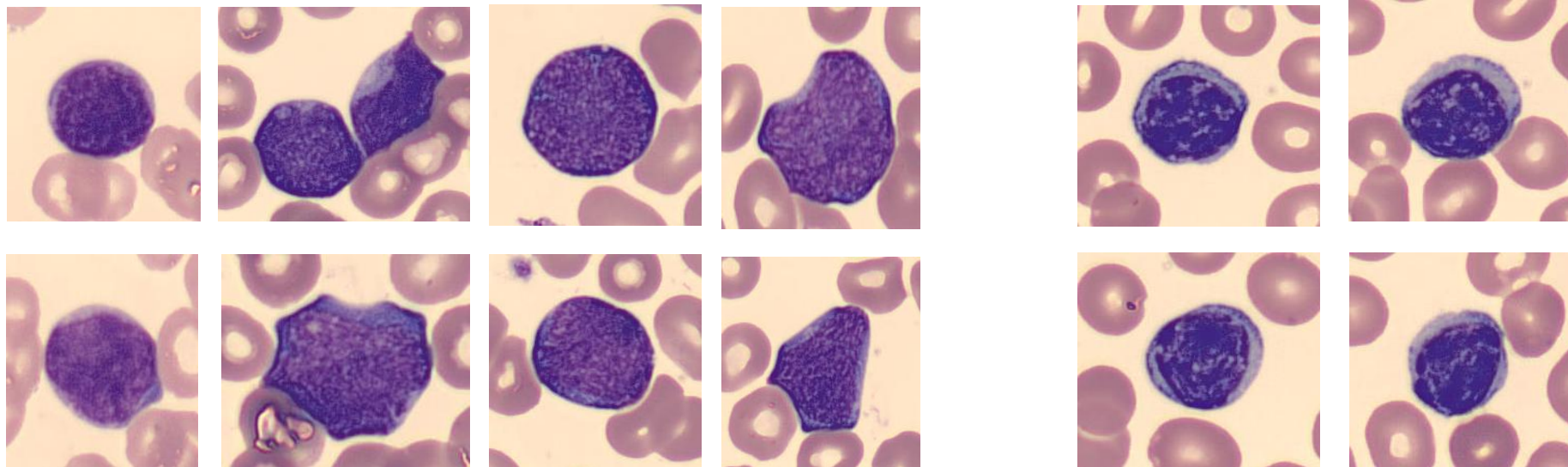


- limfociti su ovalnog oblika, s okruglom ili ovalnom jezgrom centralno položenom
- zgusnuti kromatin organiziranim u područja veće gustoće i vidljivom malom jezgrićom
- citoplazma je srednje obilna i umjereno bazofilna, s izdancima koji su prisutni ili izraženiji samo s jedne strane stanice

Akutna limfoblastična leukemija

	Akutna limfoblastična leukemija	Kronična limfocitna leukemija
Predominantne stanice	(limfo)blasti	Zreli, neoplastični limfociti
Progresija	Rapidno, agresivna bolest	Sporo
Dob	Djeca i mlađi odrasli	> 50 godina

- LEUKOCITI: N, ↑ ili ↓
- ANEMIJA
- TROMBOCITOPENIJA



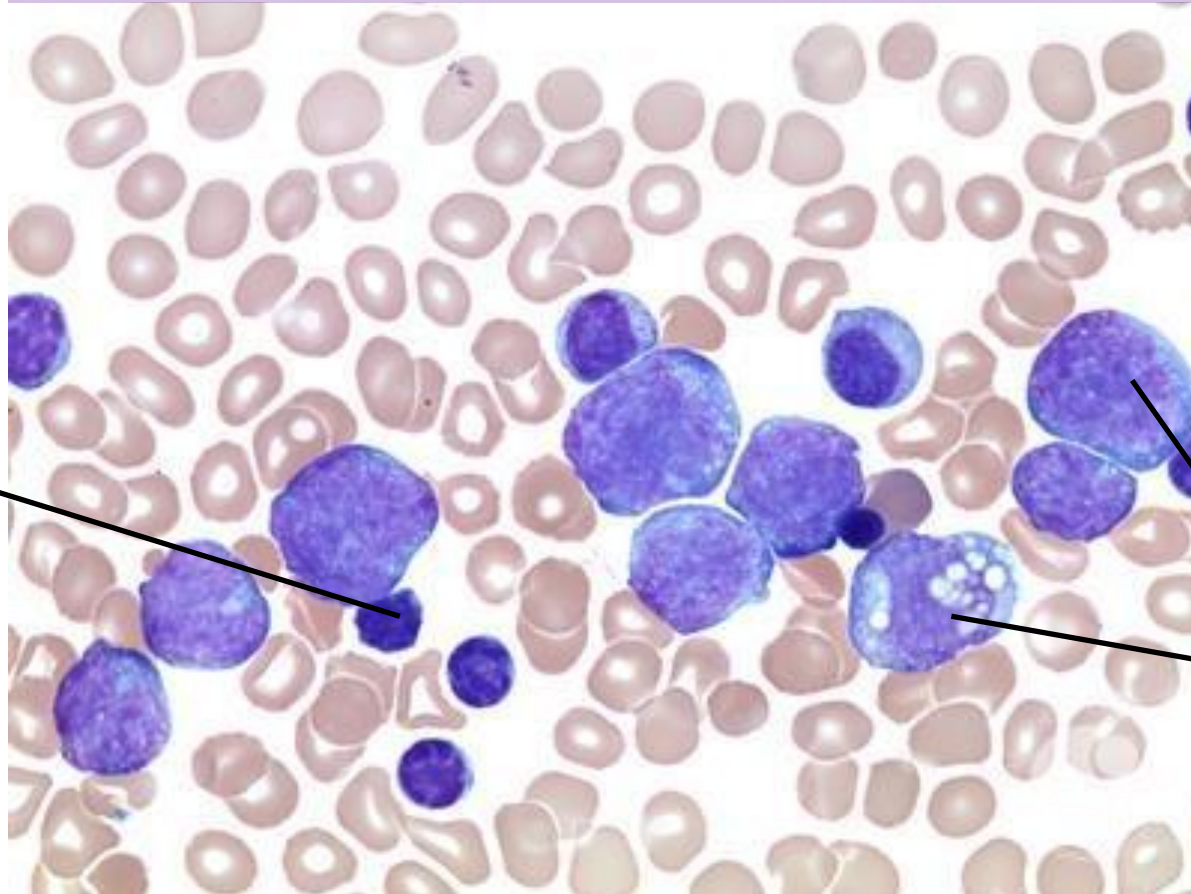
- visok omjer jezgre i citoplazme
- jezgra je okrugla i položena u središnjem dijelu stanice
- kromatin je nježan i raspršen
- jezgrice nisu vidljive

Transformacija KLL-a

- 2 – 15 % slučajeva
- Hodgkinov limfom - rijetko
- Difuzni B-limfom velikih stanica (engl. *diffuse large B-cell lymphoma*, DLBCL) – **Richterova transformacija**

HETEROGENA POPULACIJA STANICA LIMFOIDNOG REDA

mali limfociti s grudastim kromatinom i oskudnom citoplazmom karakteristični za KLL



velike pleomorfne stanice s obilnom, vakuoliziranom citoplazmom i vidljivim jezgricama unutar jezgre

Umjesto zaključka

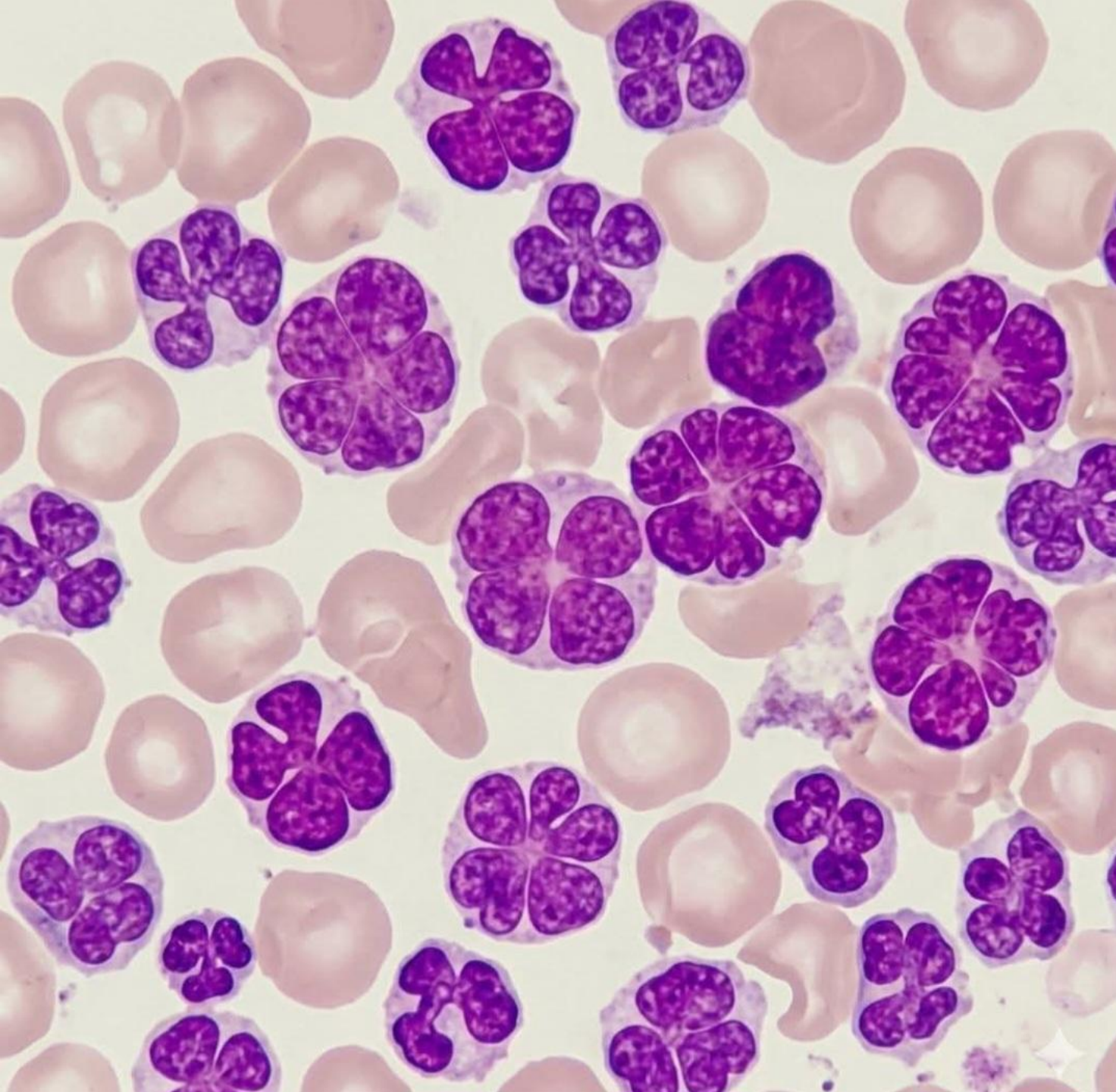
„**Peripheral blood lymphocyte atypia is hard to define** due to its variability and significant overlap between reactive and neoplastic conditions.

Atypical lymphocytosis is a nonspecific descriptor with poor concordance among laboratory professionals categorizing lymphocytes.

Our approach as hematopathologists to a peripheral blood smear review for atypical lymphocytes includes **microscopic evaluation** along with review of CBC and other available laboratory data in the clinical context.

Morphologic features of lymphocyte populations can help favor a reactive versus a malignant process.

Peripheral smear review by an experienced pathologist can **help expedite further diagnostic workup** or avoid unnecessary ancillary testing.”



***Hvala na
pažnji!***