



GRAN SEMINARIO INTERACTIVO DE CITOPATOLOGÍA

Dra. Carmela Iglesias

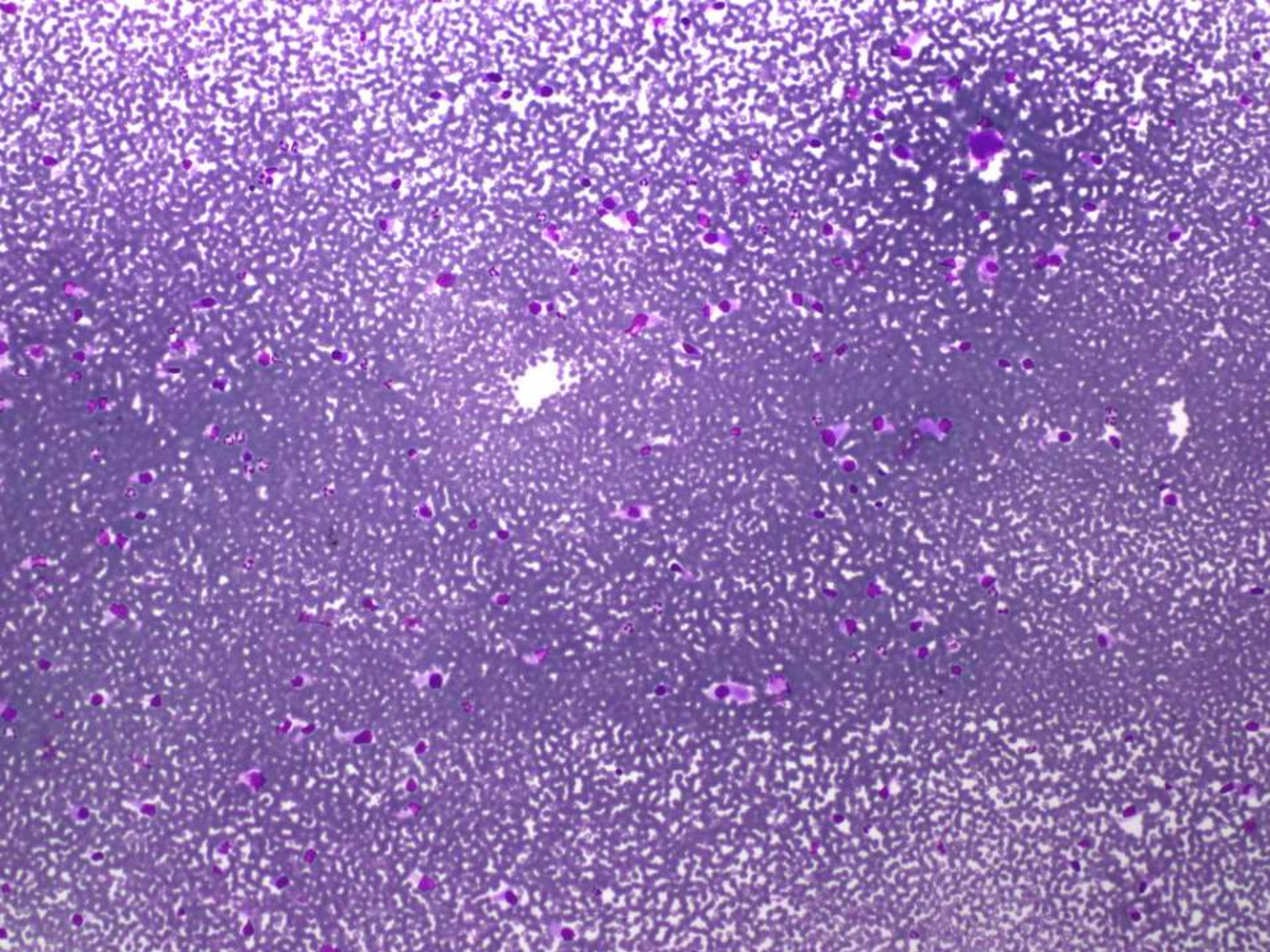
Hospitals Vall d'Hebron, Barcelona

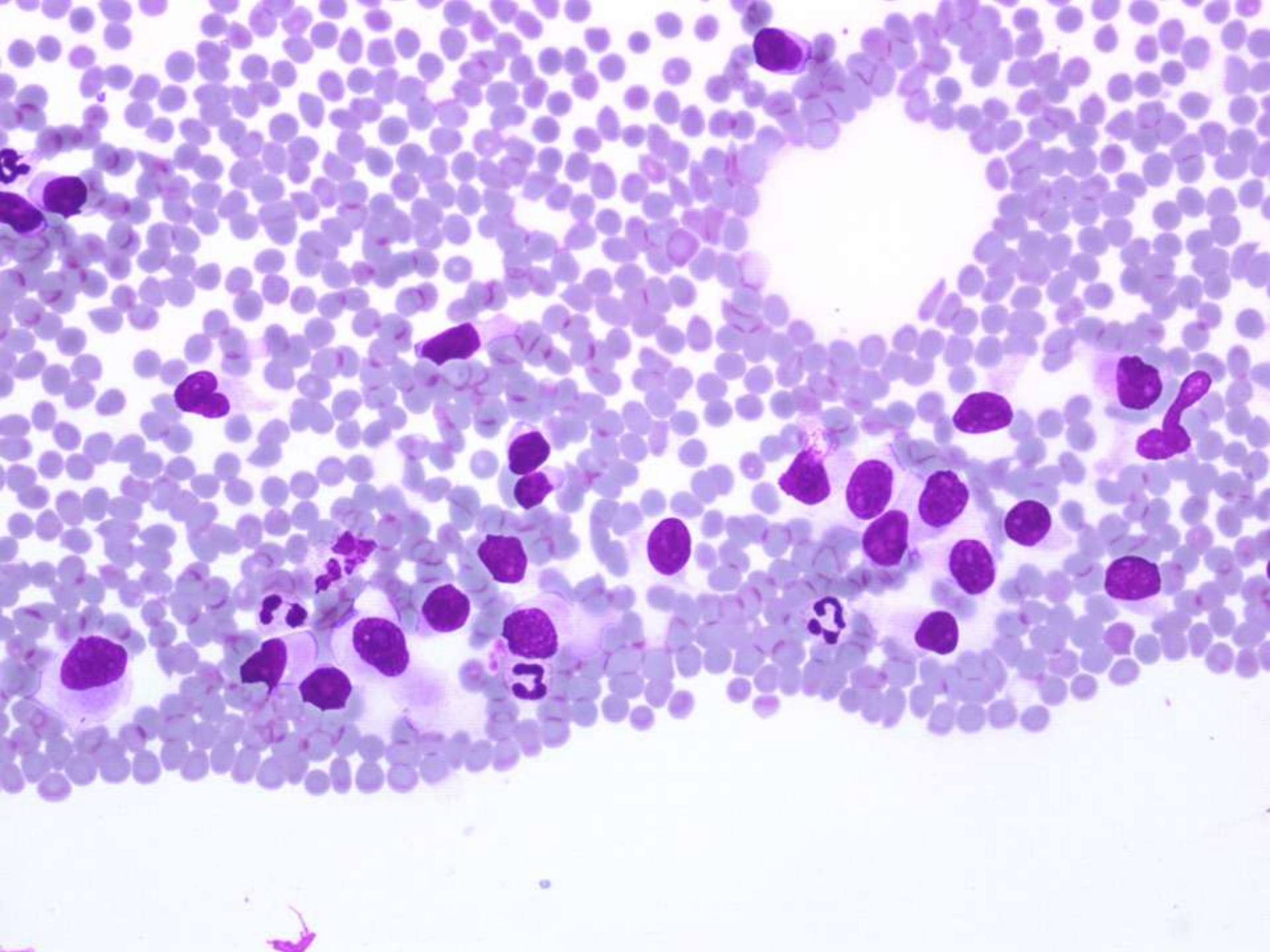


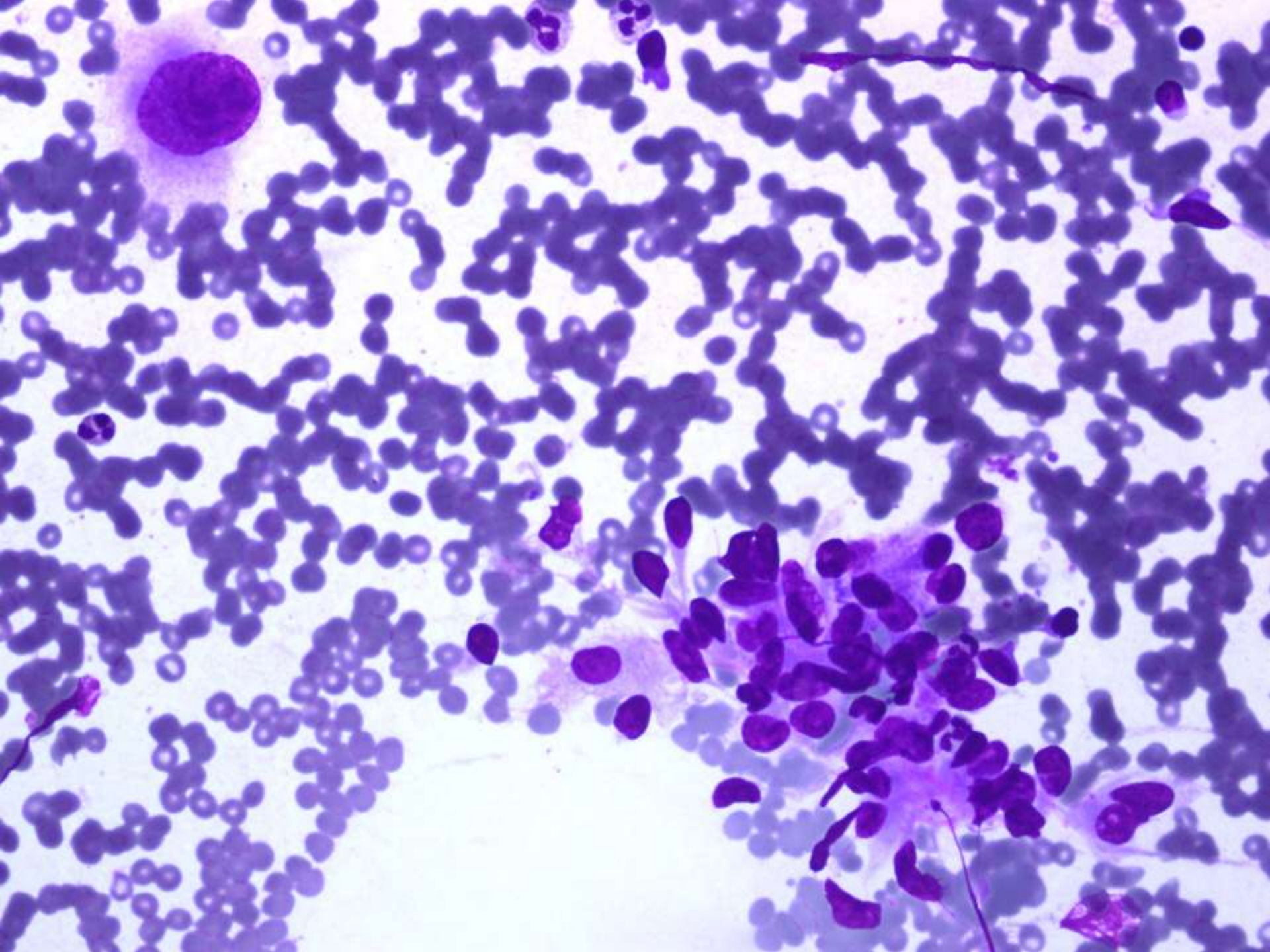
Universitat Autònoma de Barcelona

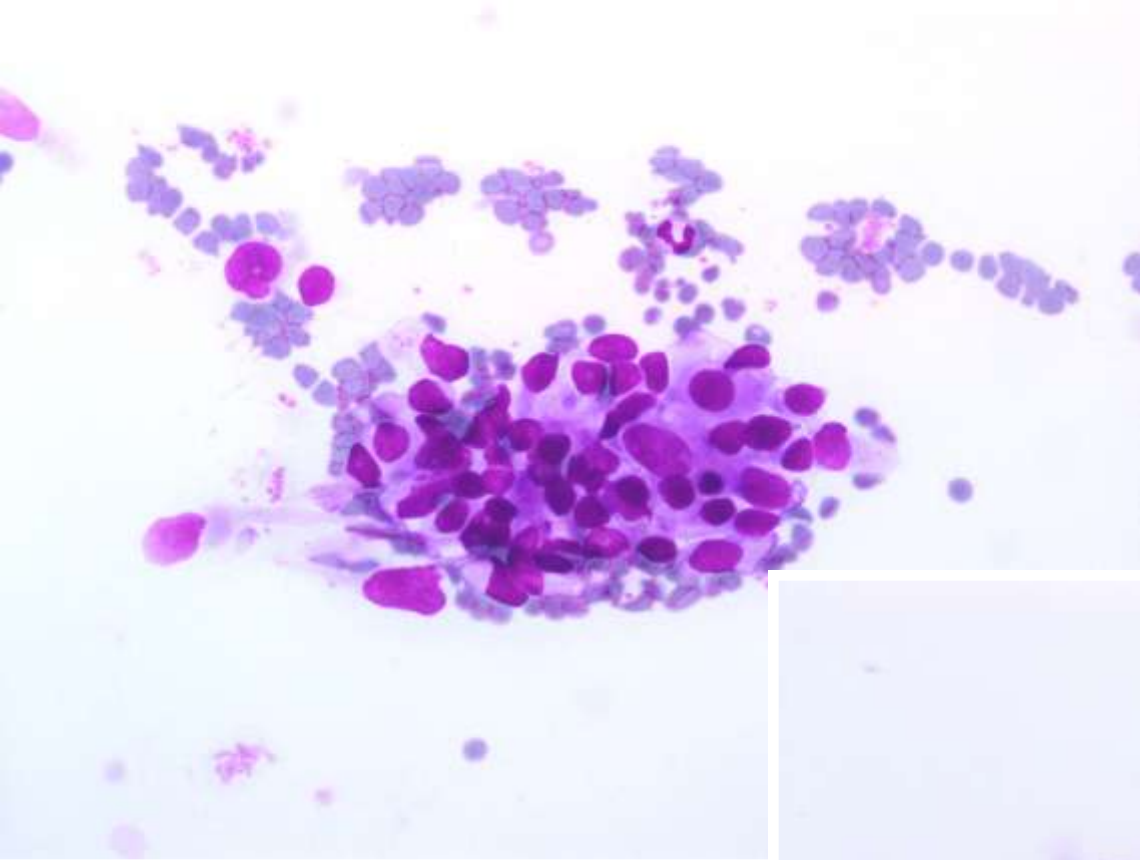
PRESENTACIÓN CLÍNICA

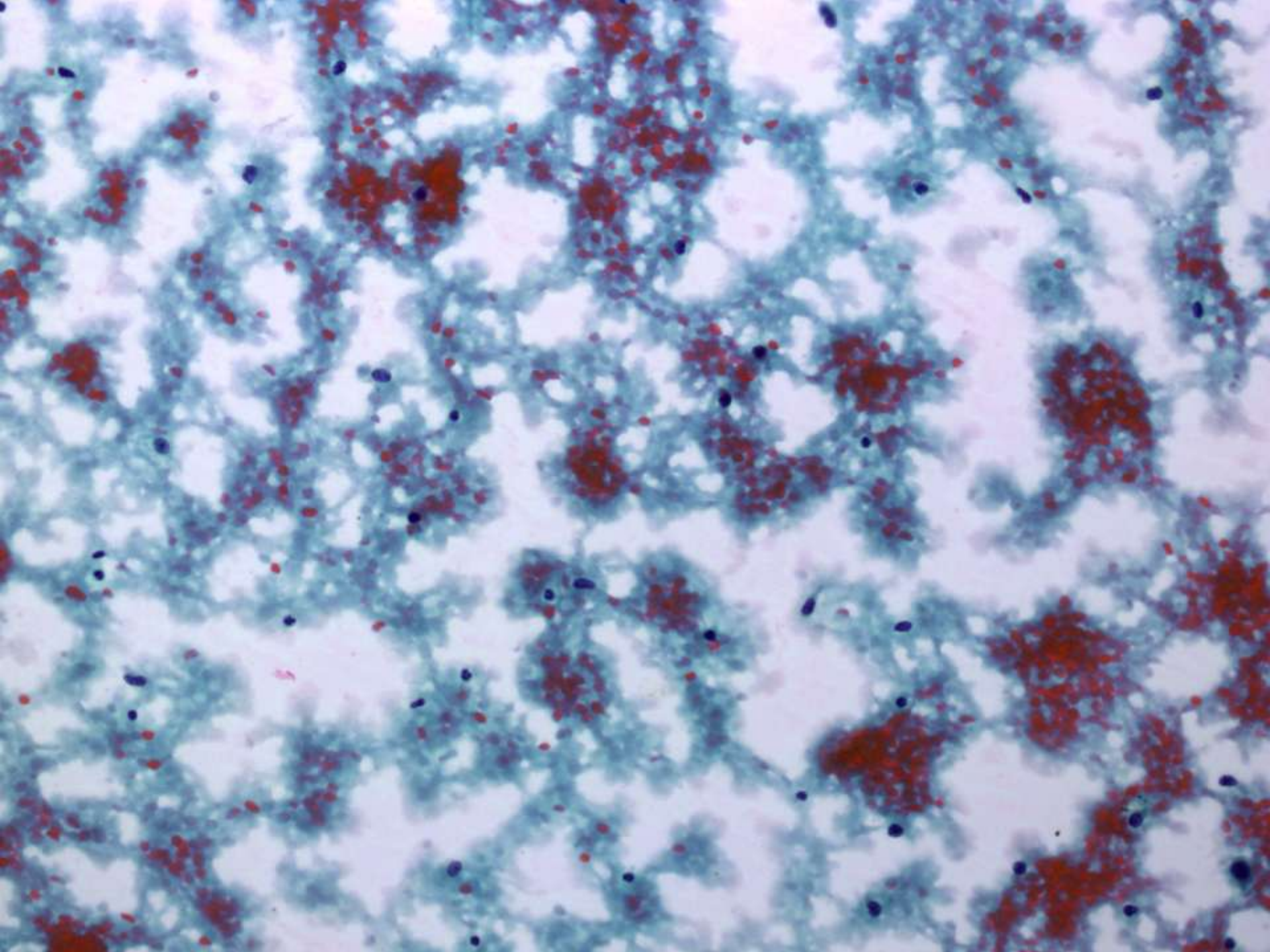
- Hombre de 69 años que es remitido a las CCEE de citología para realización de PAAF de adenopatía inguinal izquierda
- El examen físico pone de manifiesto la presencia de una lesión cutánea ulcerada en la planta del pie izquierdo, de la que no se hace referencia en la hoja de solicitud
- Sin otros antecedentes conocidos

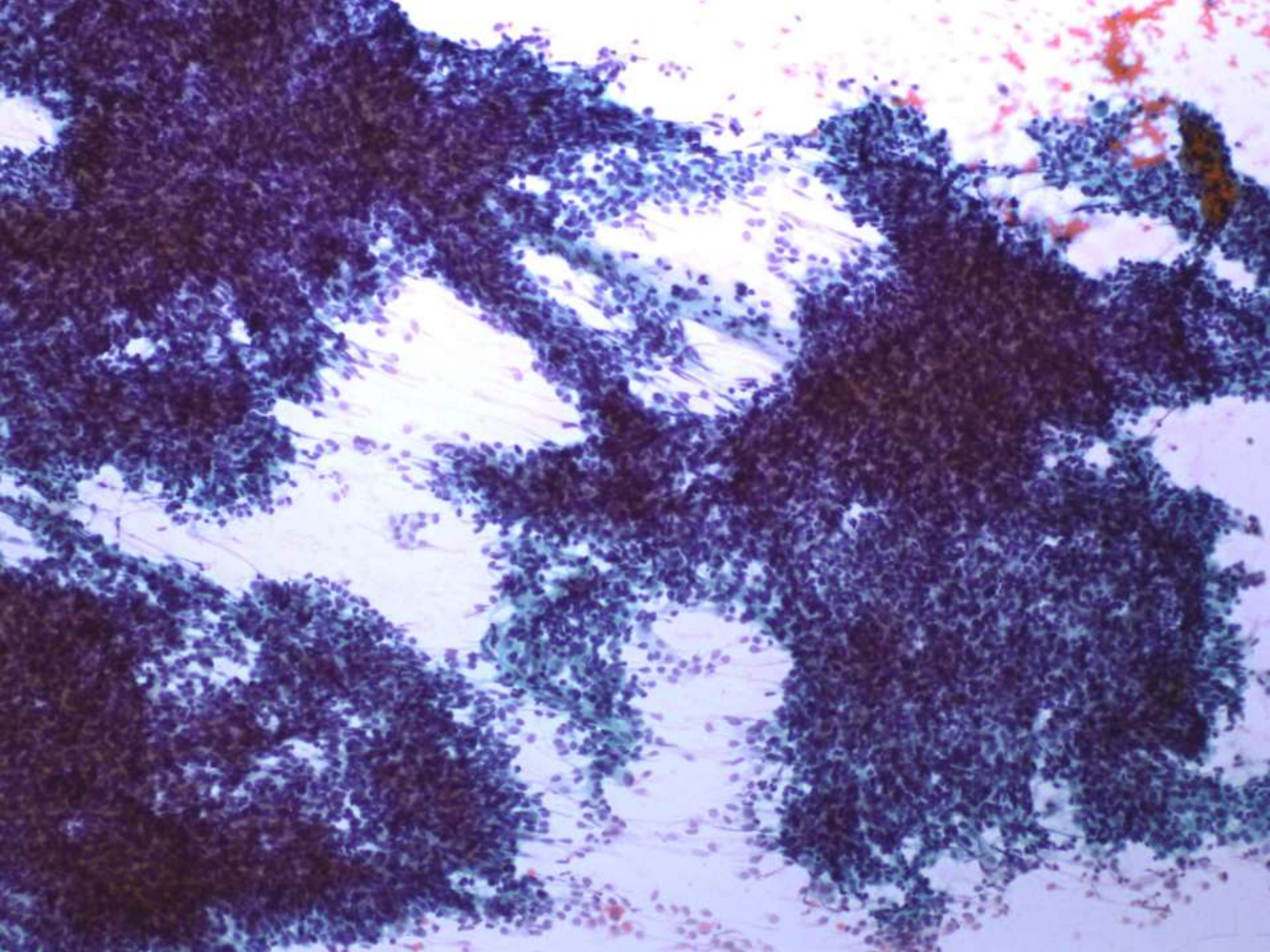


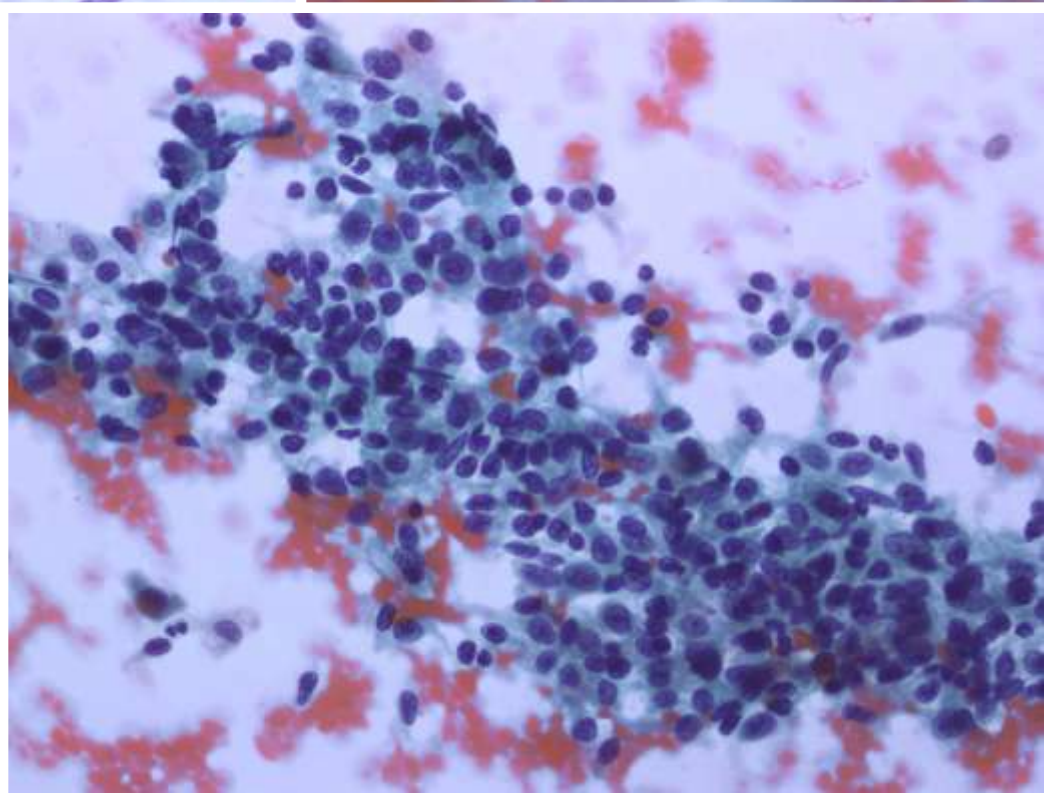
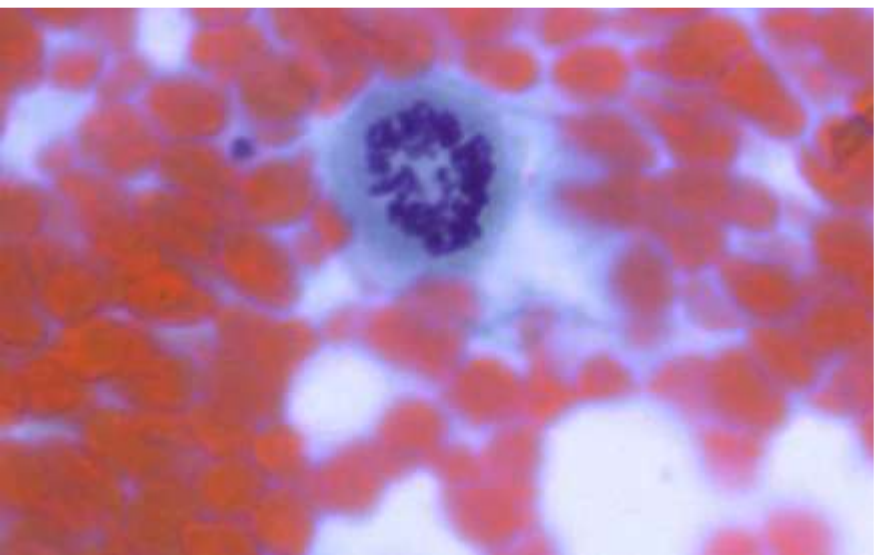
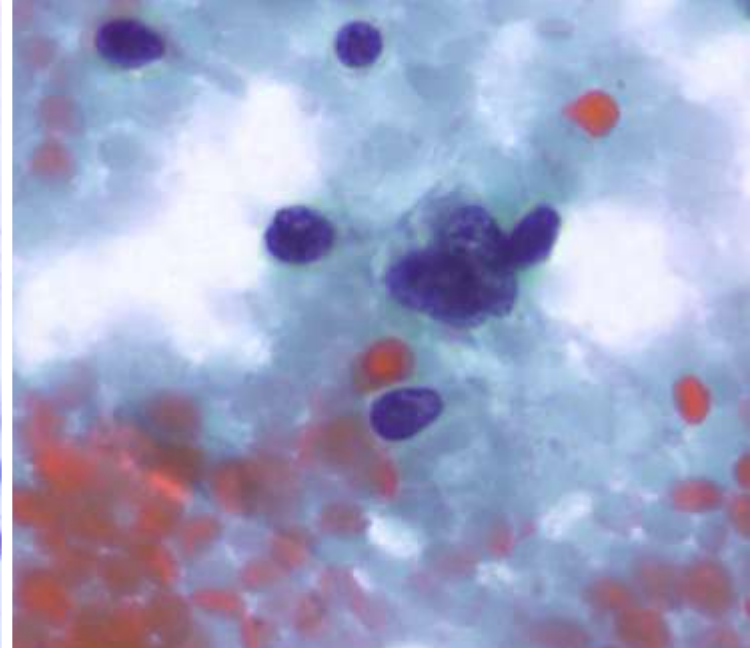
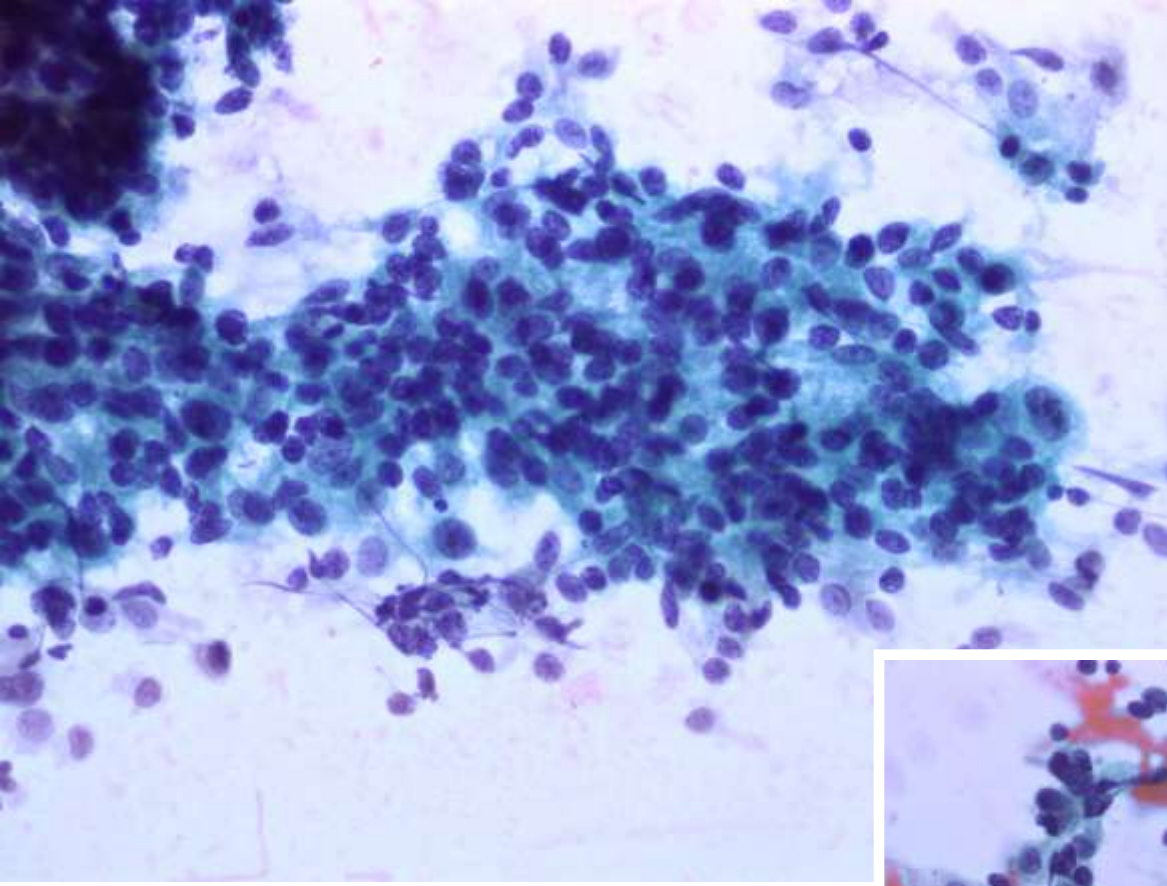


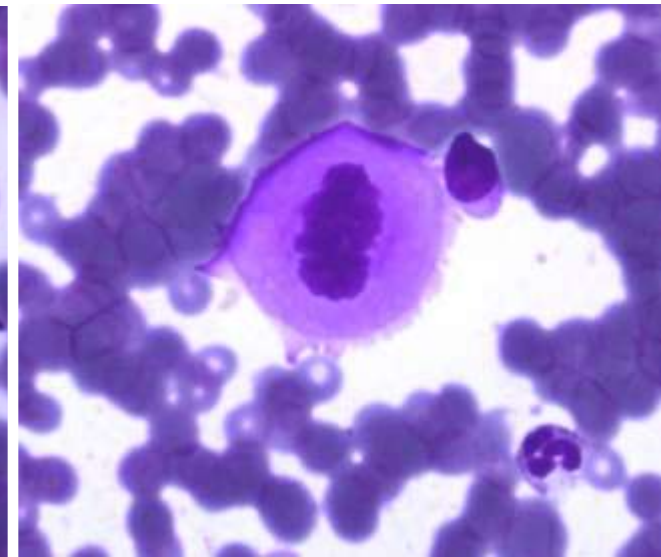
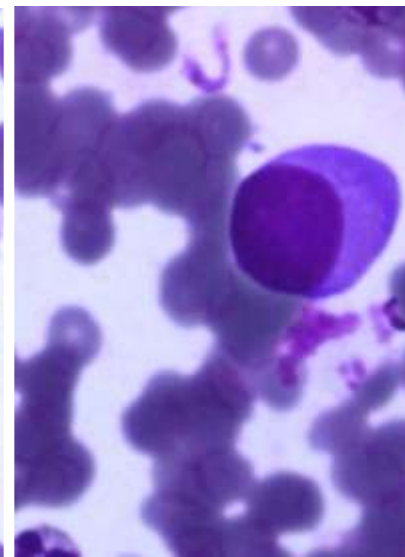
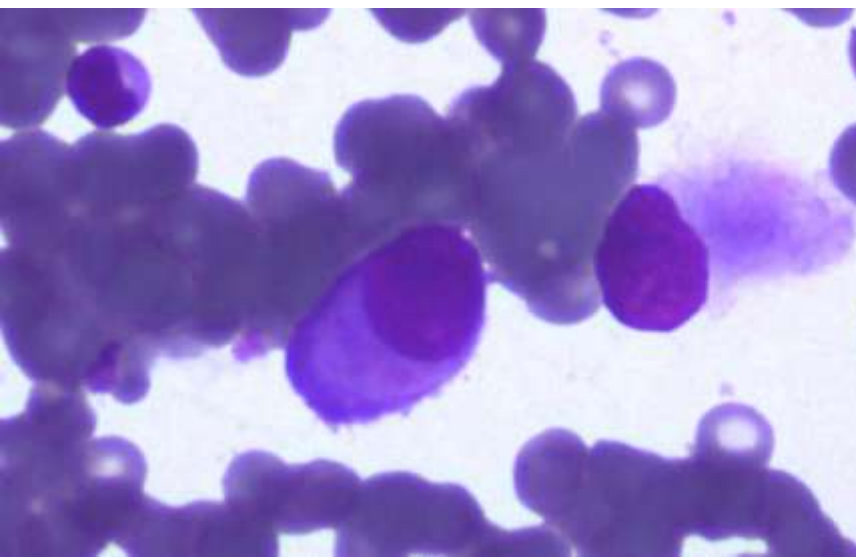
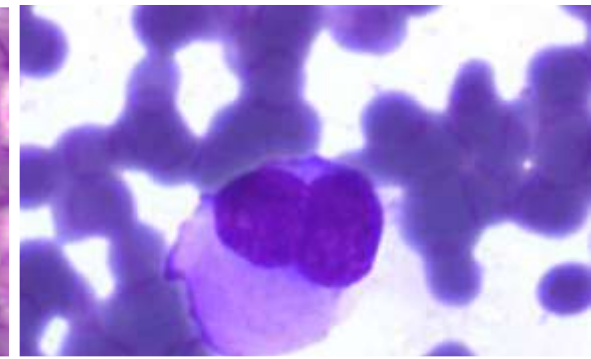
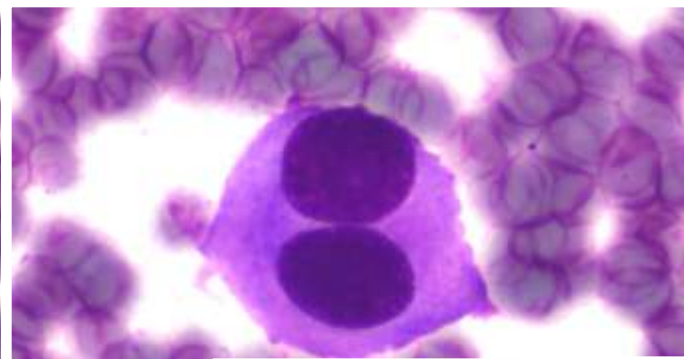
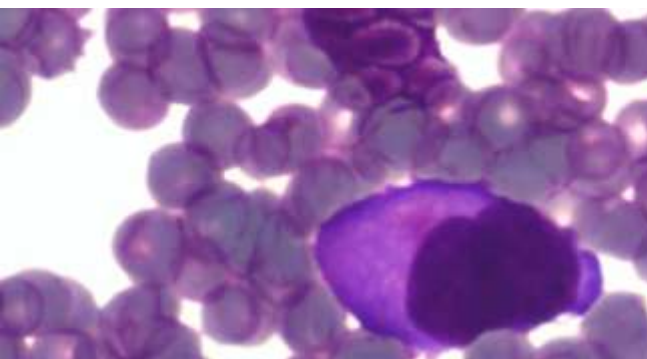
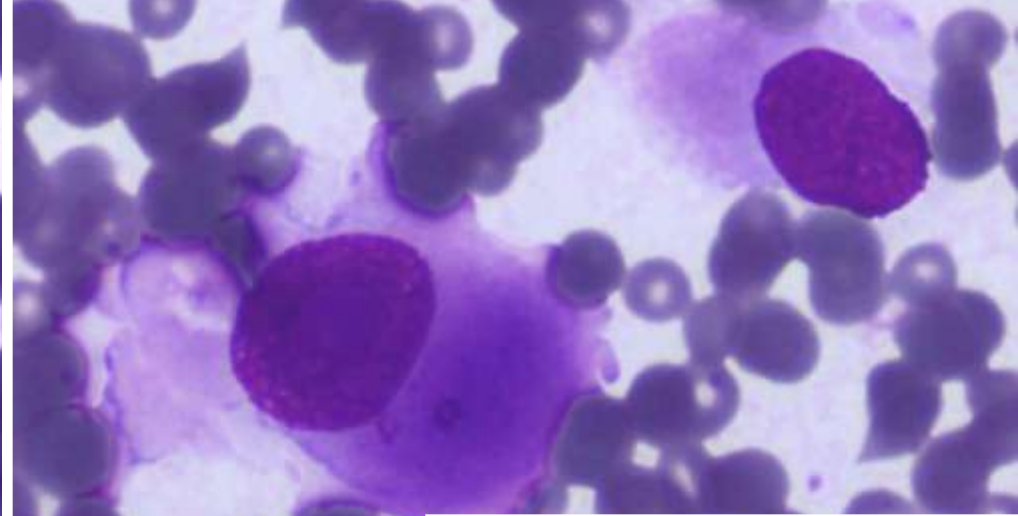
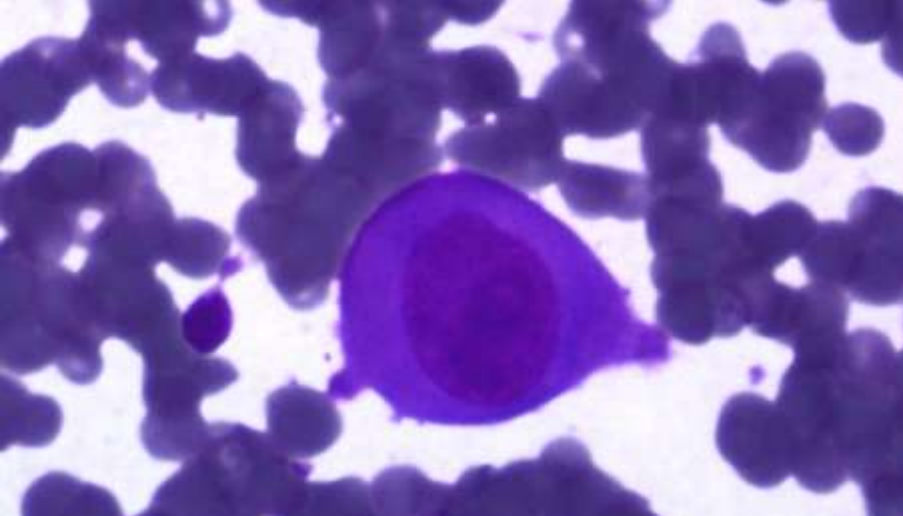


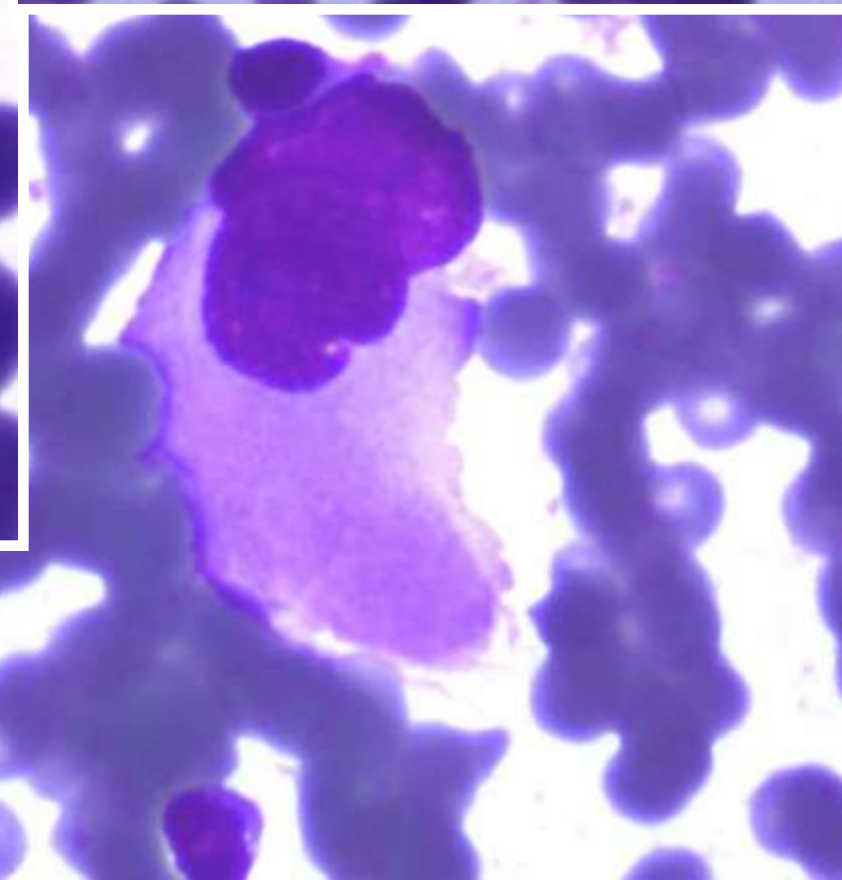
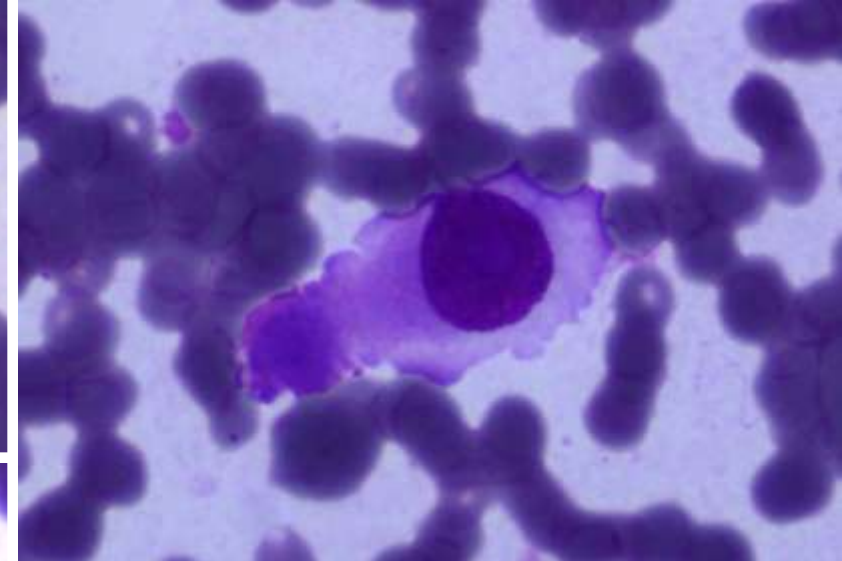


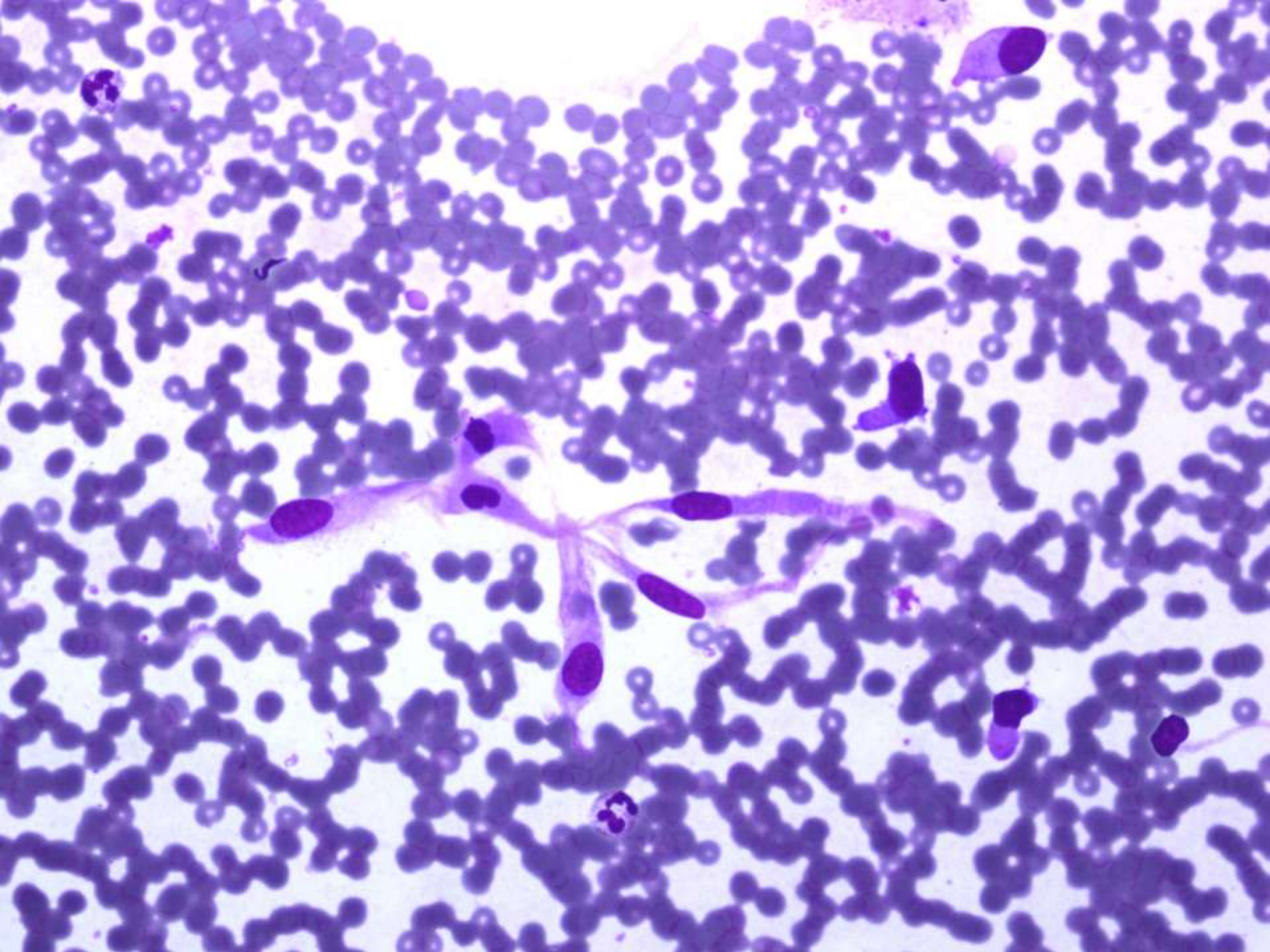


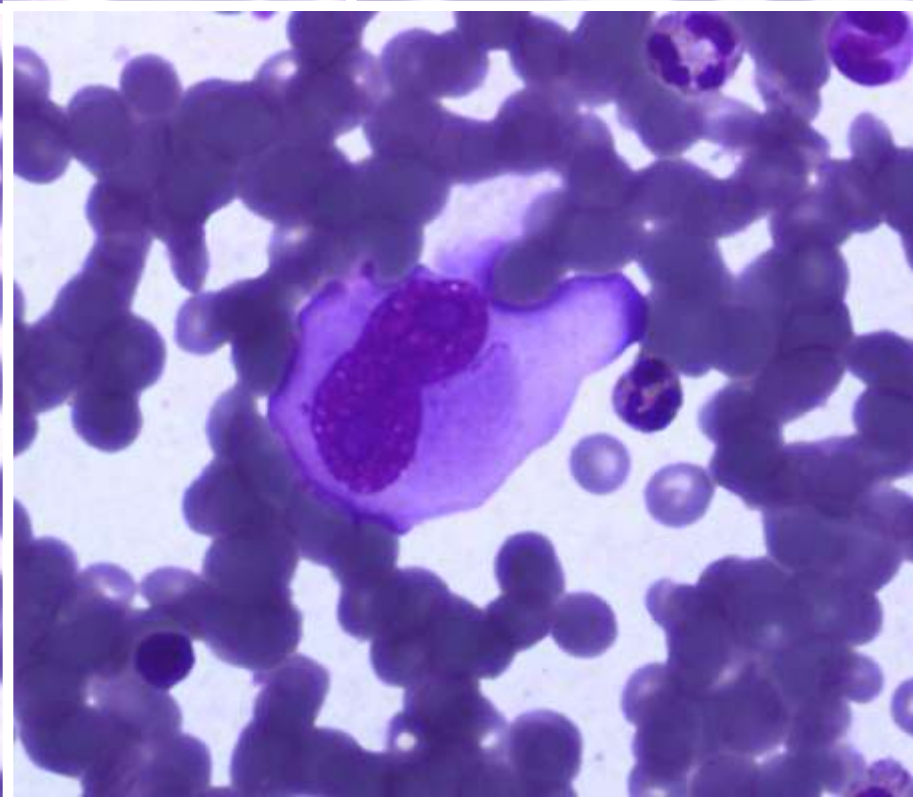
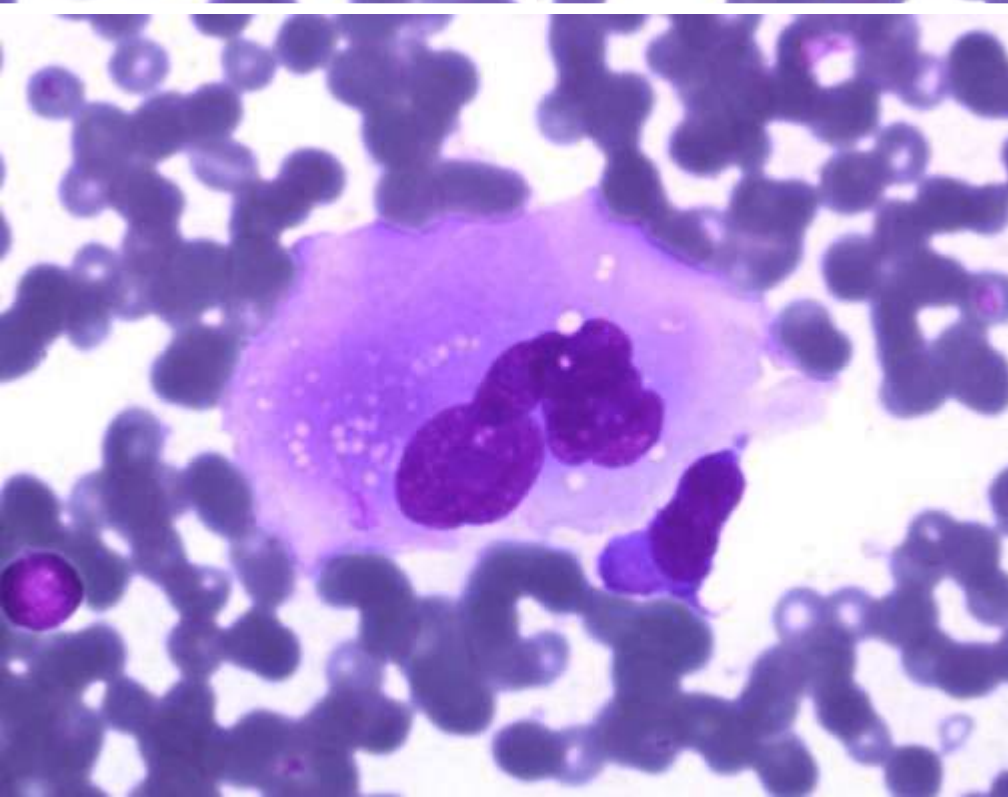
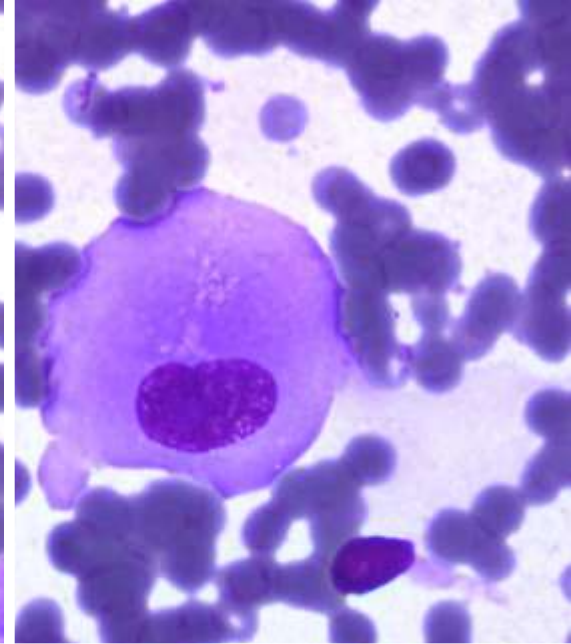
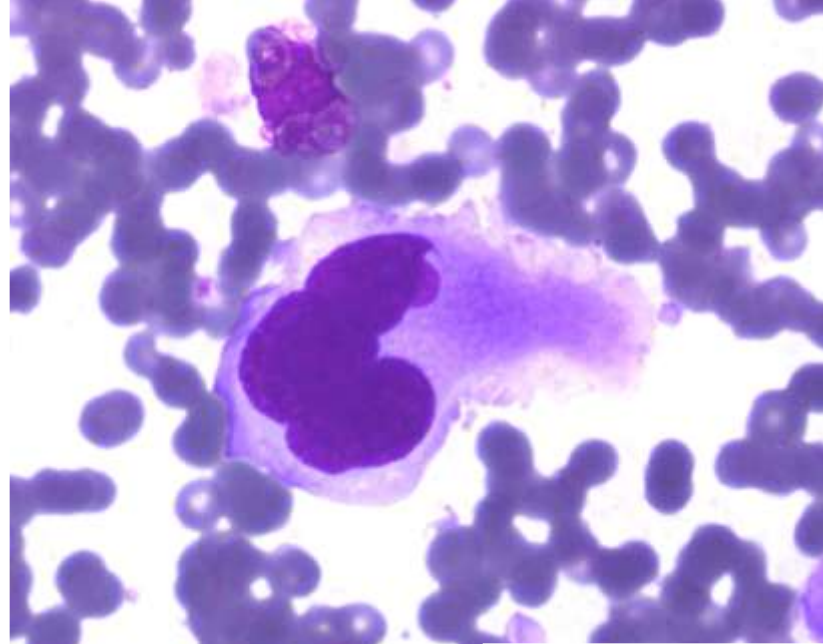
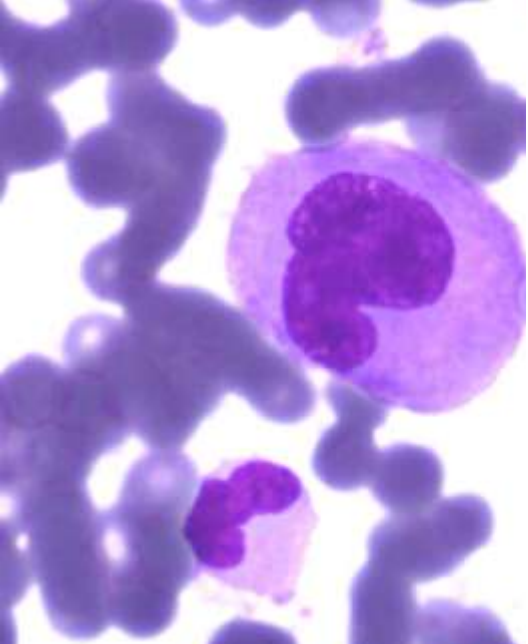


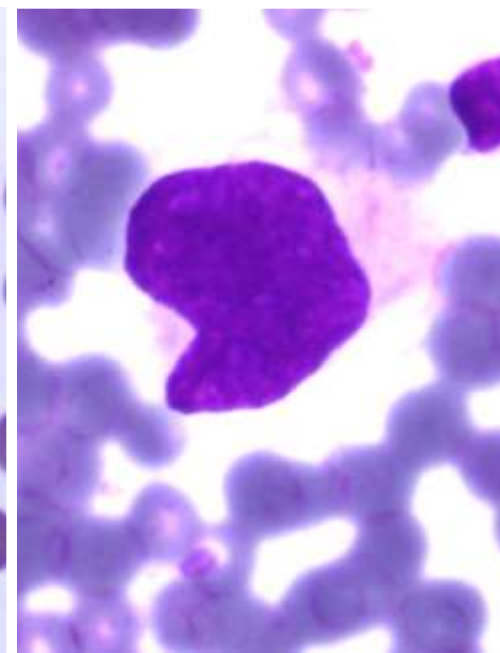
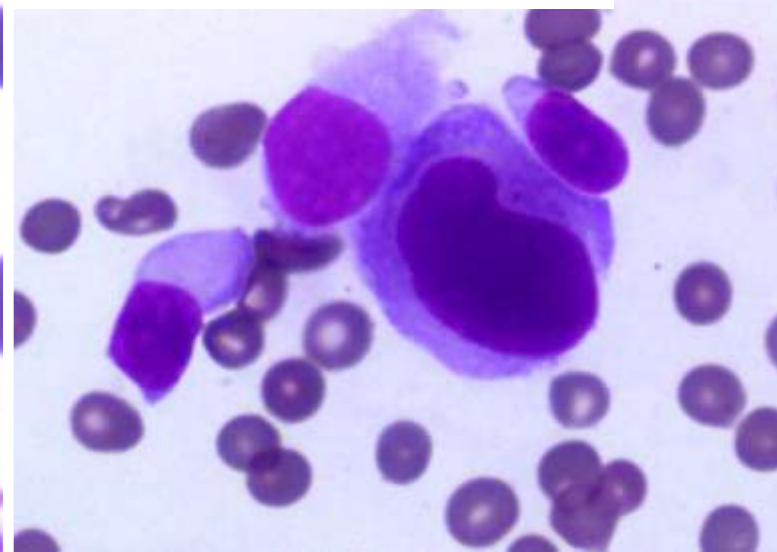
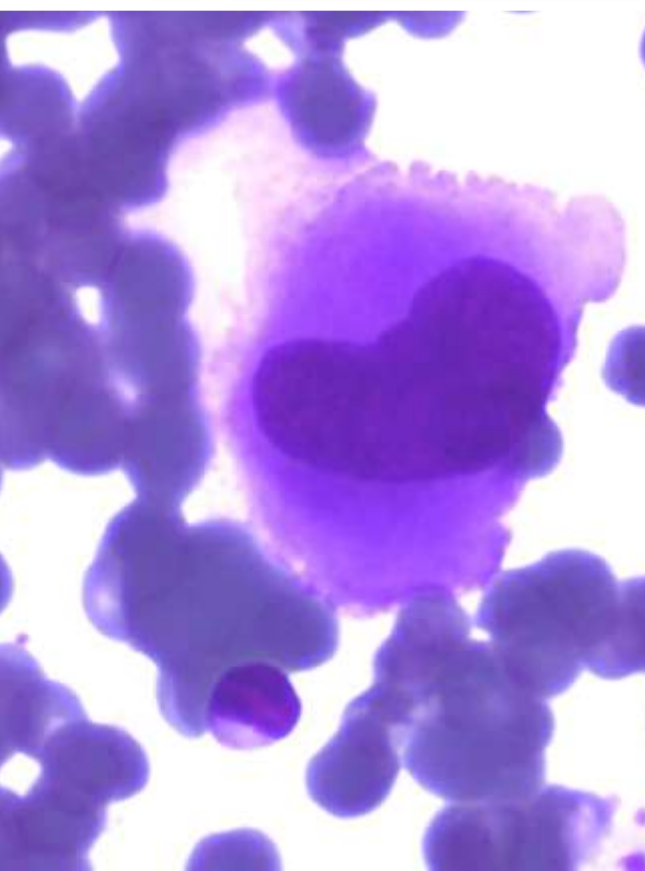
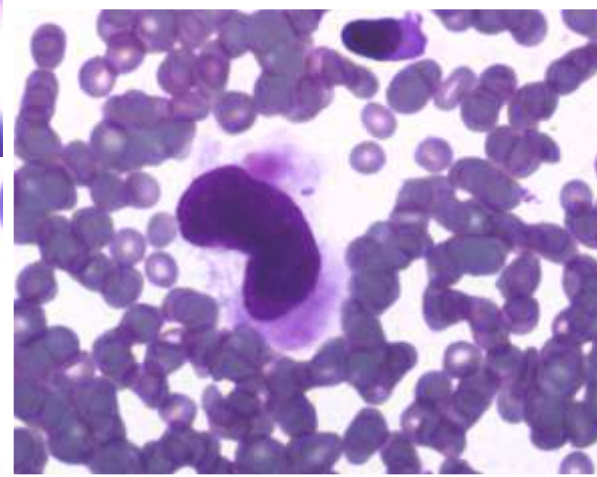
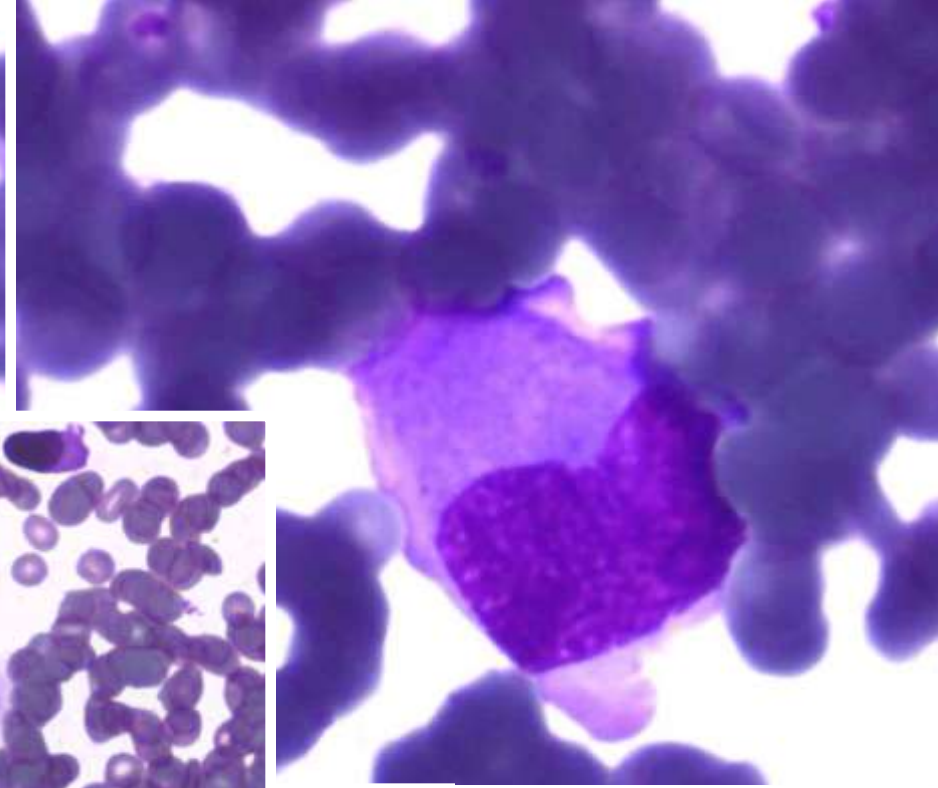
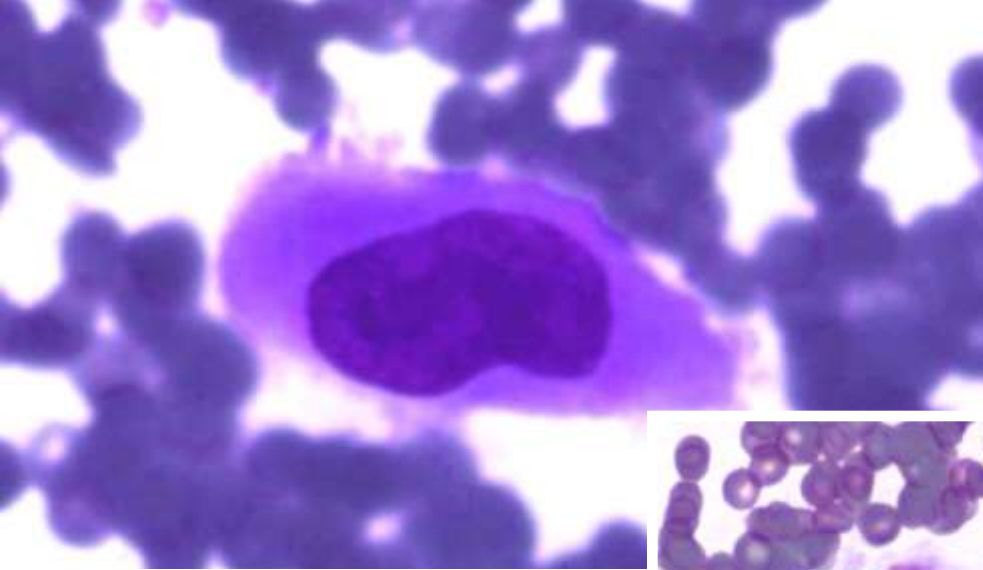


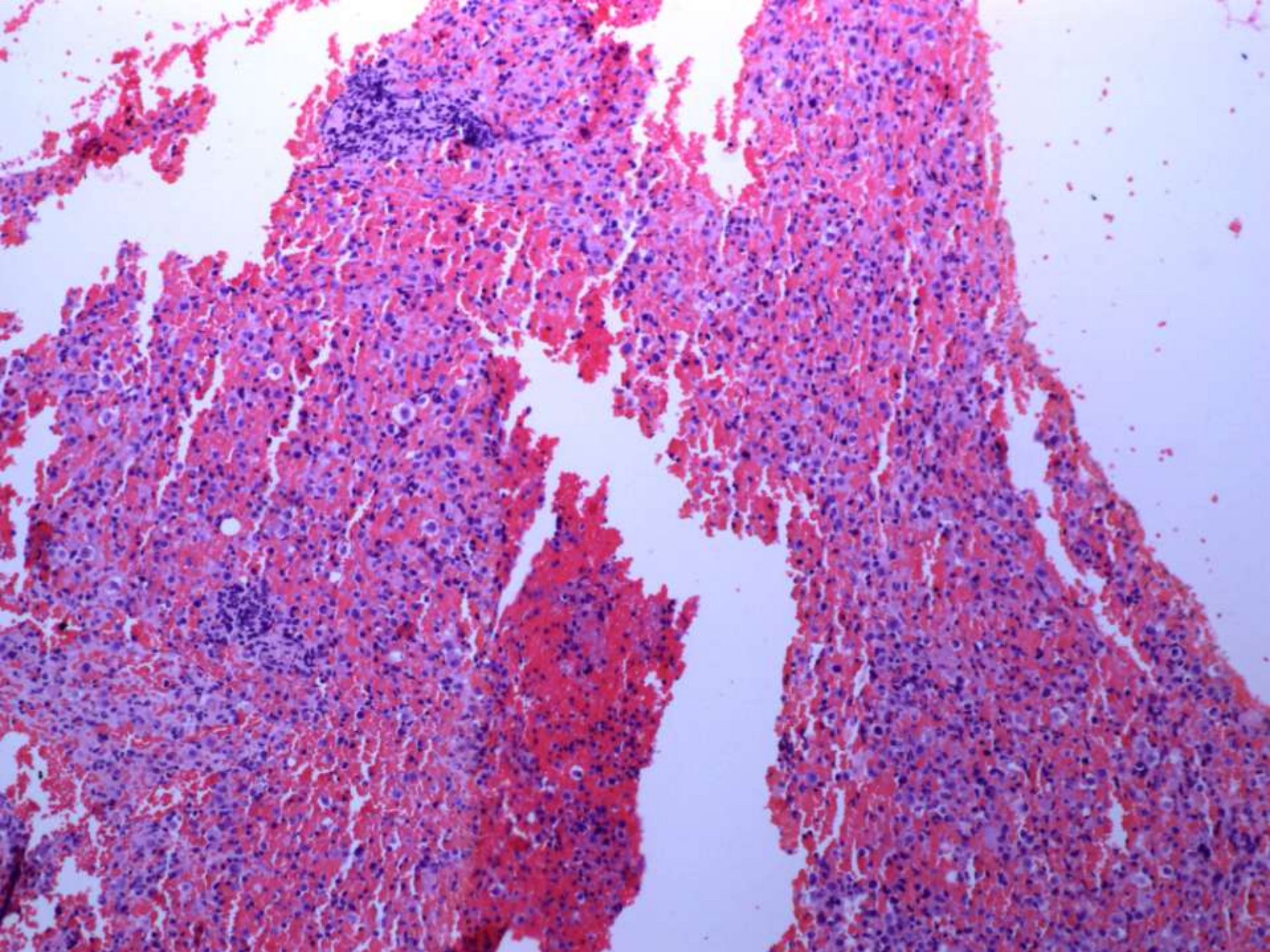


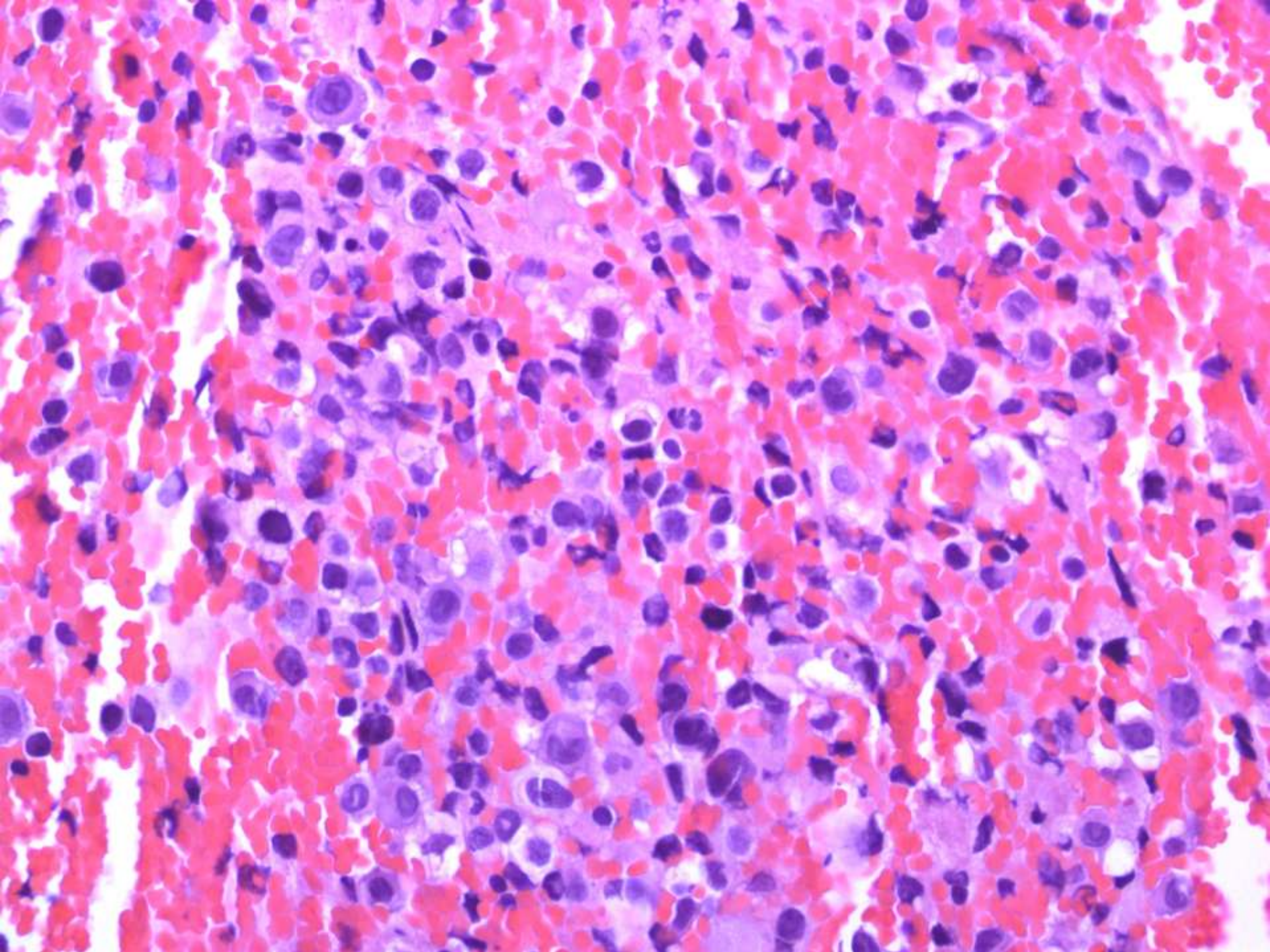












ORIENTACIÓN DIAGNÓSTICA MORFOLÓGICA

- ¿Melanoma maligno?
- ¿Carcinoma poco diferenciado?
- ¿Linfoma?
- ¿Sarcoma?

PANEL INMUNOHISTOQUÍMICO

- Marcadores negativos:

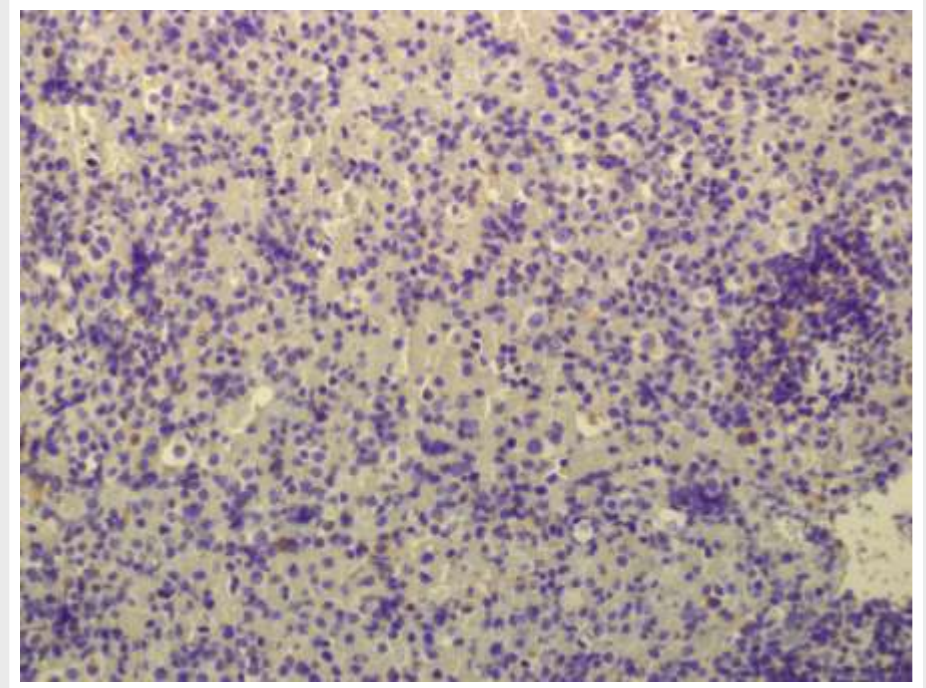
- Citoqueratinas
(5/6/8/18)

- EMA

- S100

- HMB45

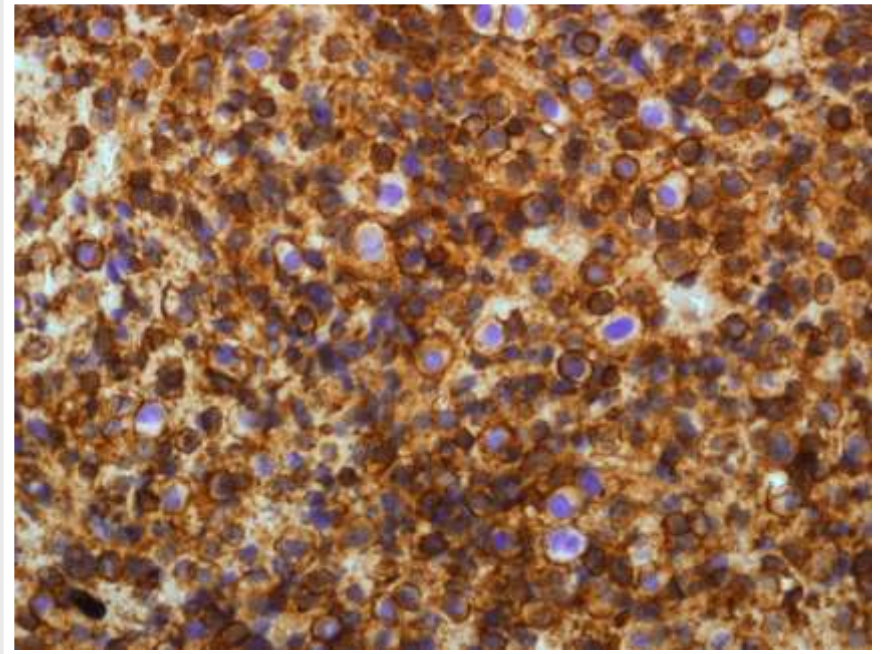
- Melan-A



PANEL INMUNOHISTOQUÍMICO

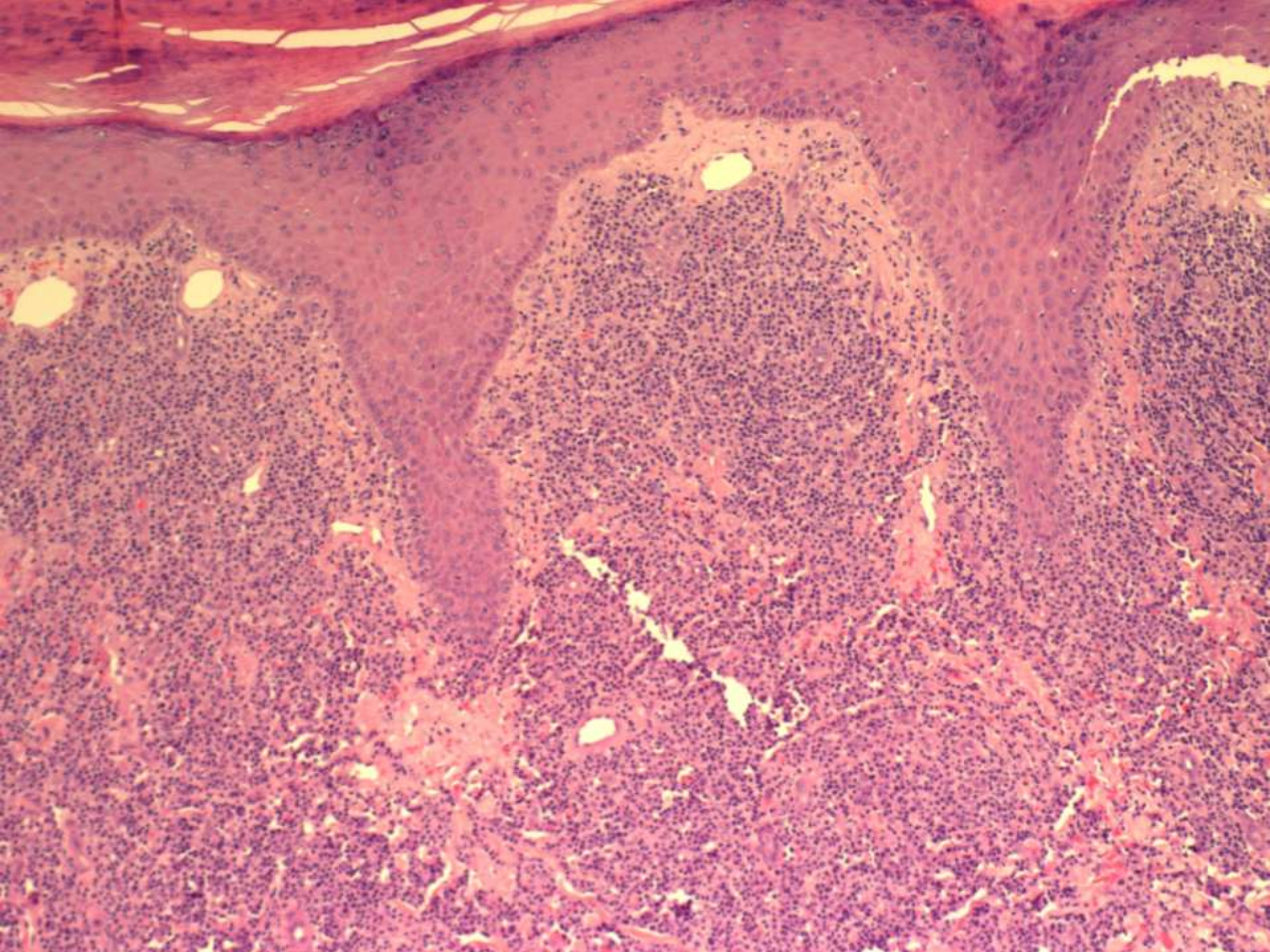
- Marcadores positivos:

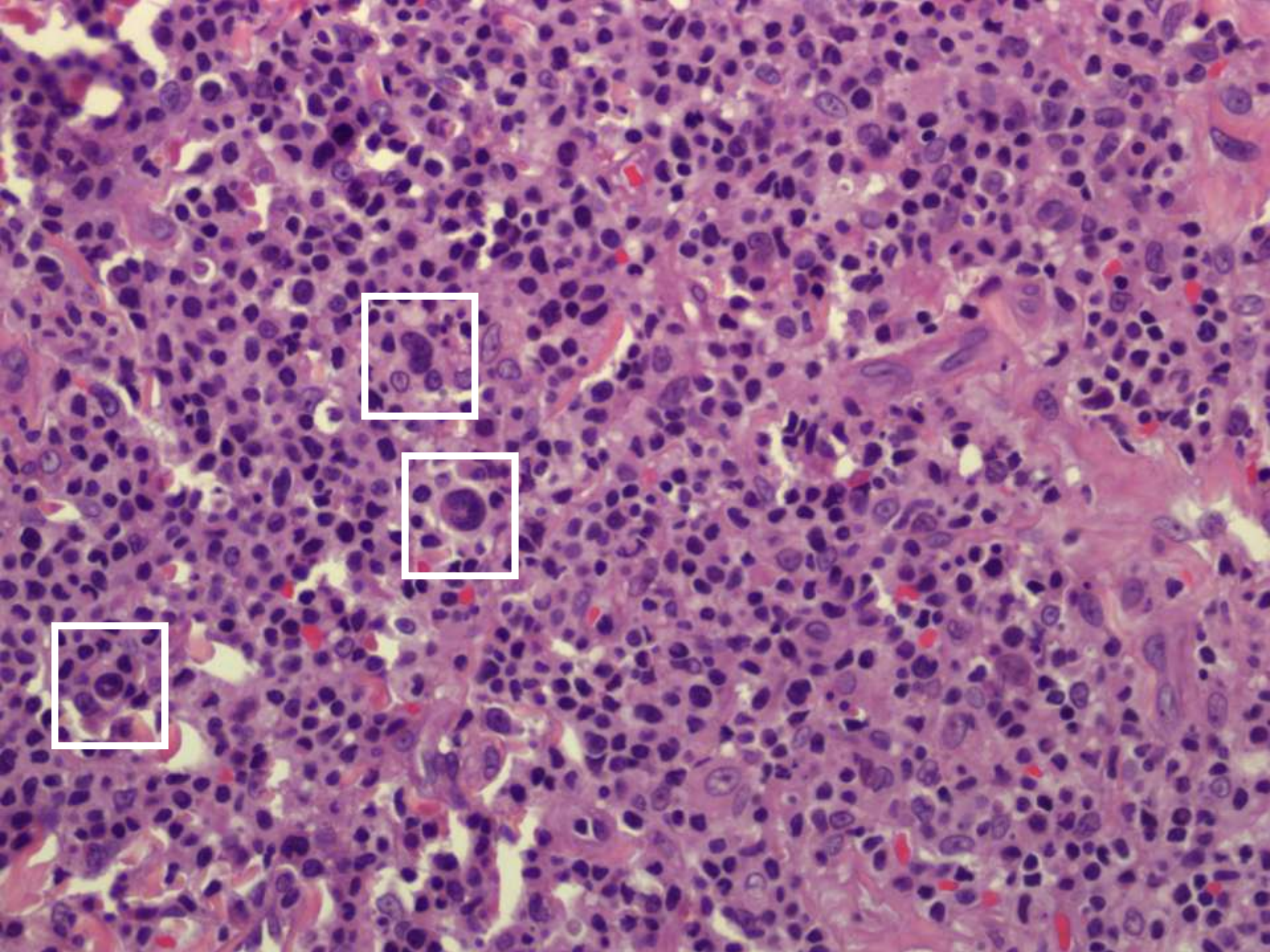
- Vimentina
- CD45 (ALC)

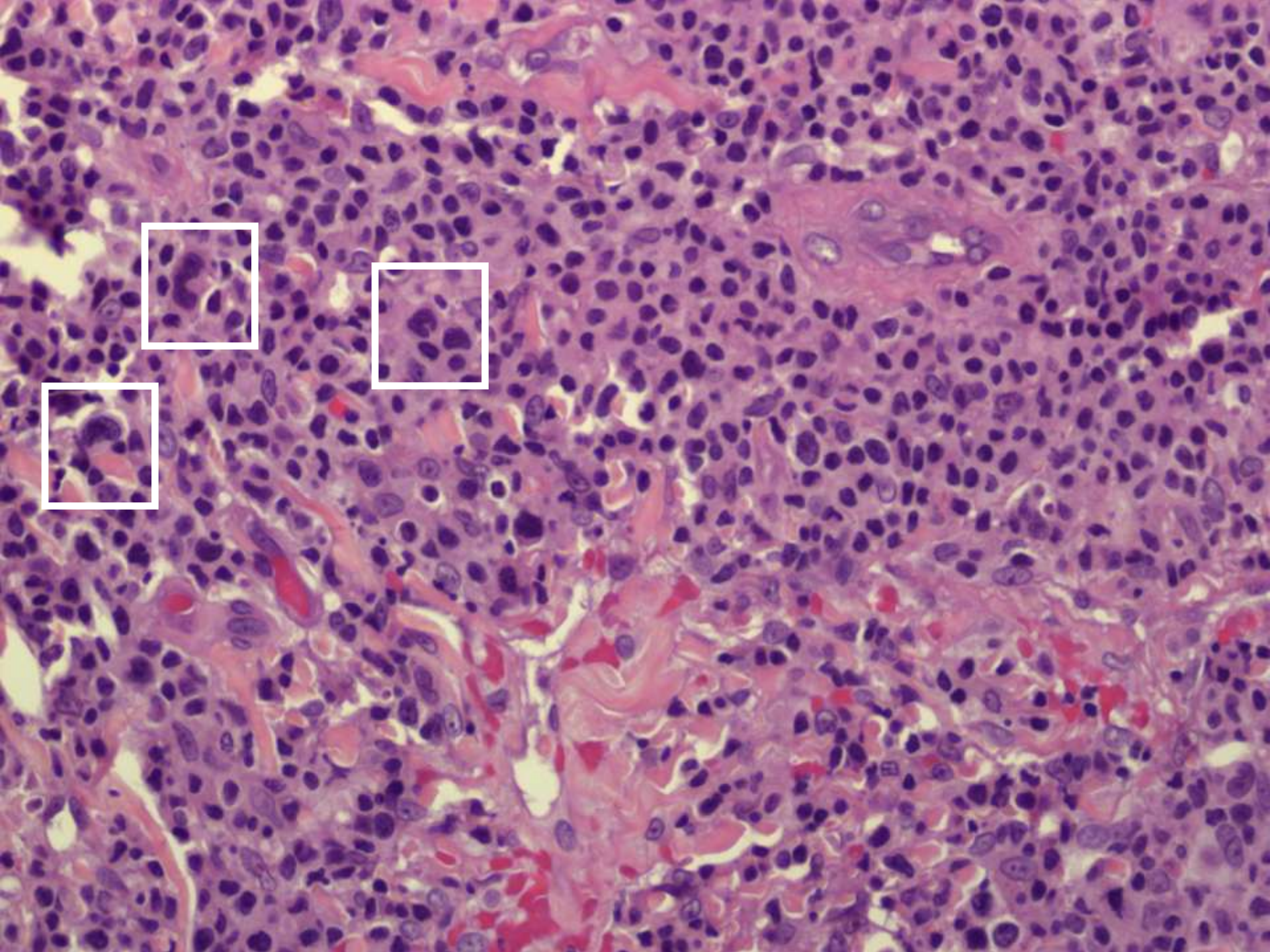


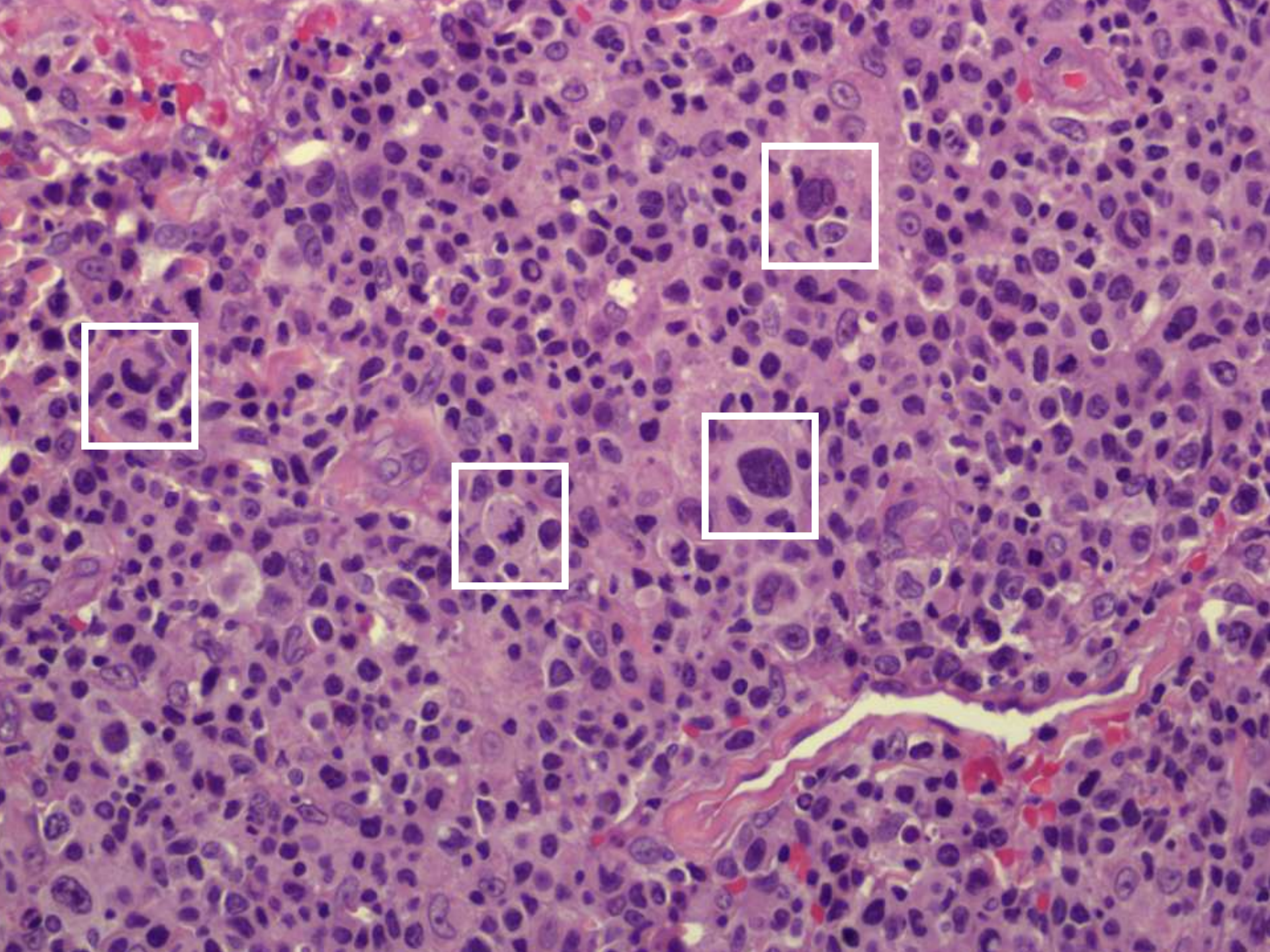
DIAGNÓSTICO CITOLÓGICO

- **POSITIVO PARA CÉLULAS MALIGNAS.
TUMOR MALIGNO PLEOMÓRFICO**
- NOTA ADICIONAL:
- La positividad para CD45 plantea la posibilidad de linfoma, aunque desde el punto de vista morfológico la celularidad no sugiere dicho diagnóstico
- Se aconseja biopsia de la lesión cutánea

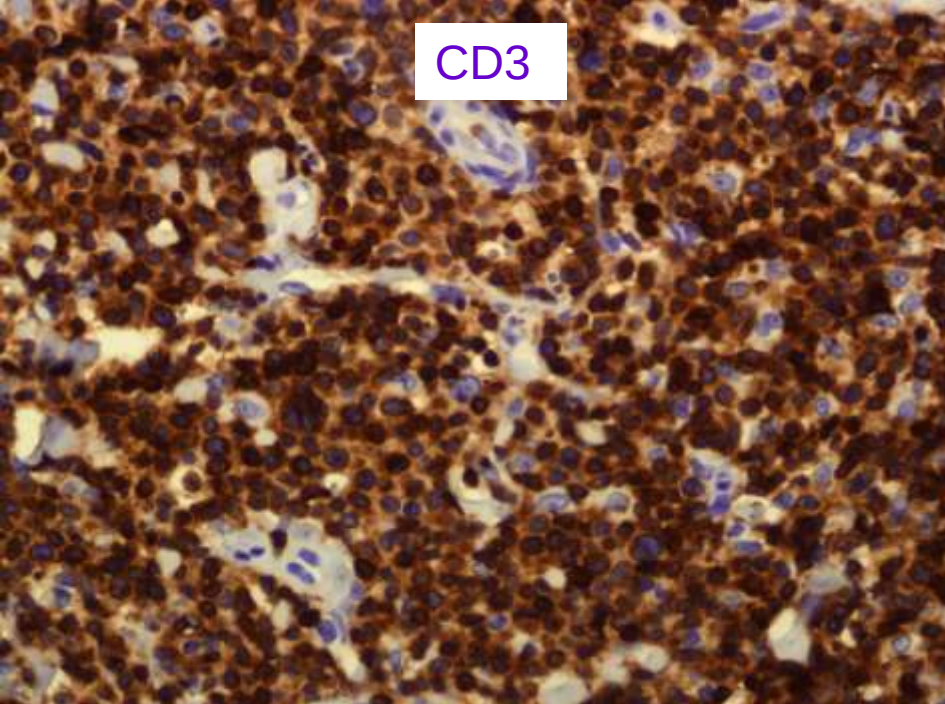




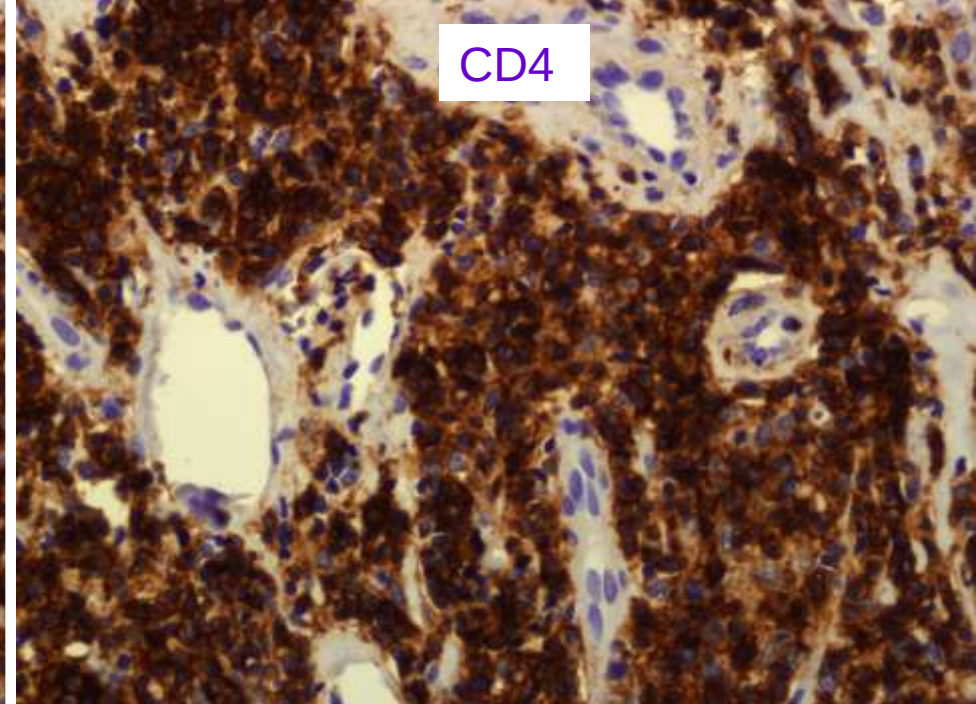




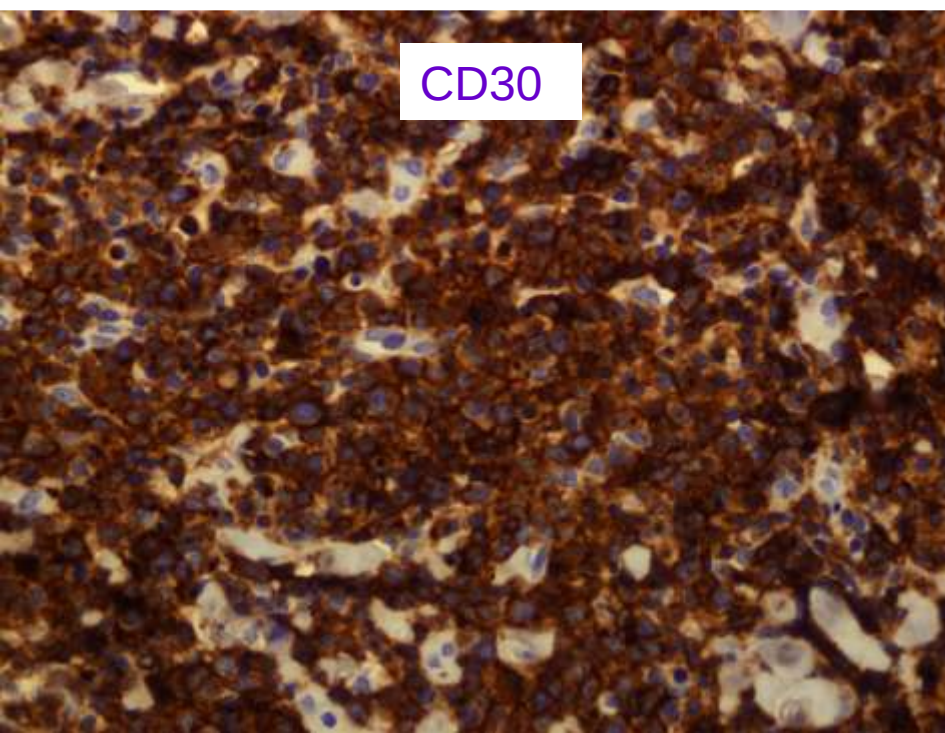
CD3



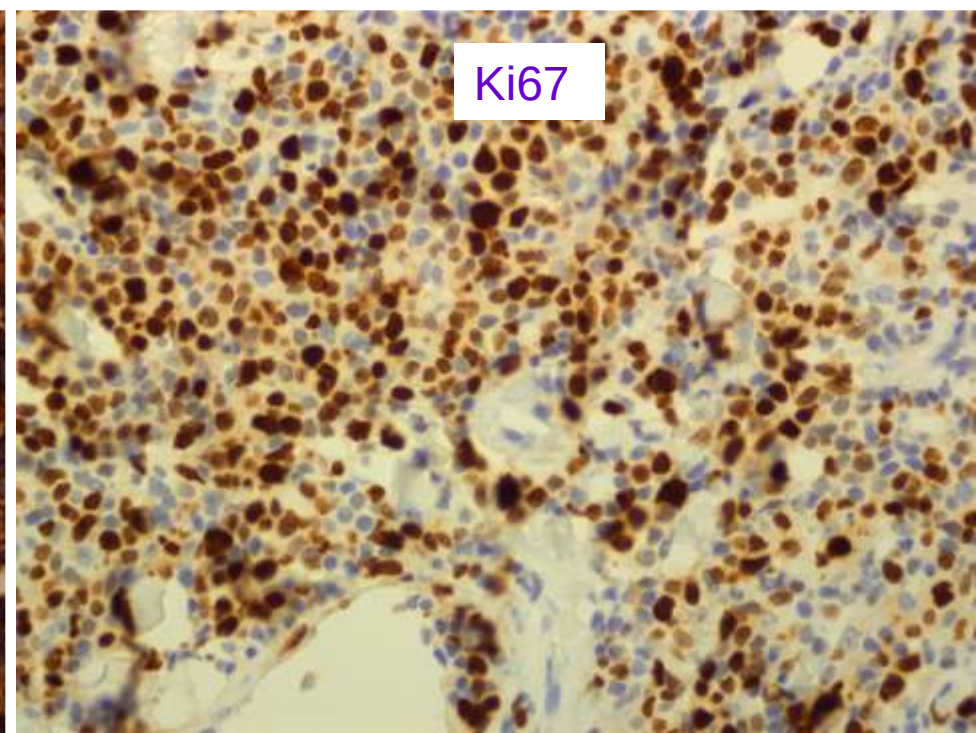
CD4



CD30



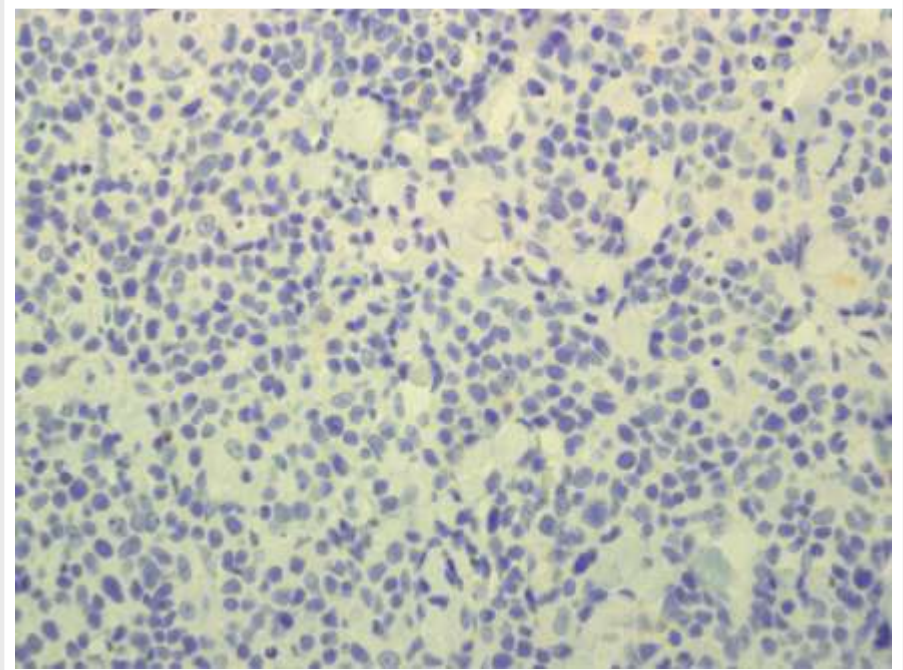
Ki67



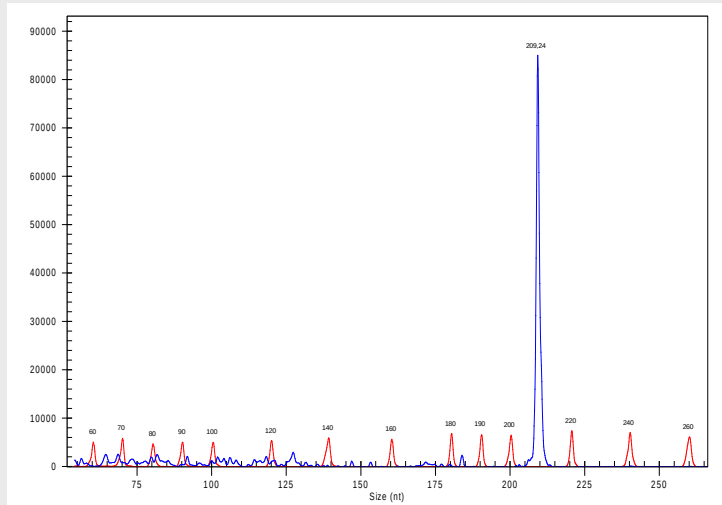
PANEL INMUNOHISTOQUÍMICO

- Marcadores negativos:

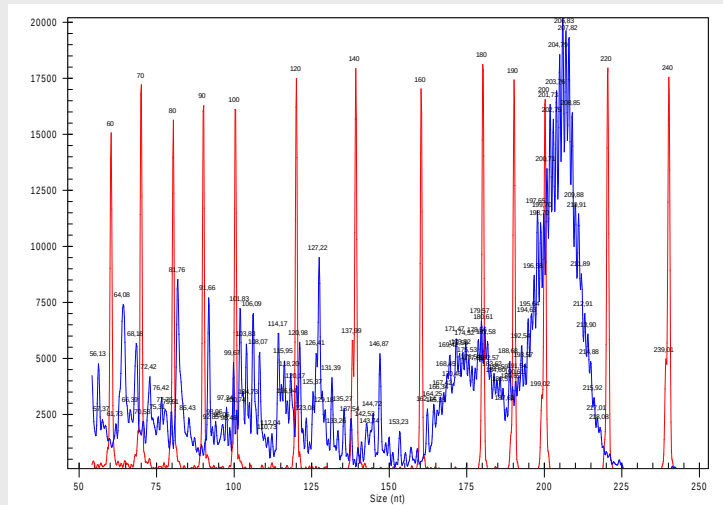
- CD20
- CD8
- CD5
- CD56
- Granzyma
- CD138
- *ALK* (anaplastic large cell lymphoma kinase)



ANALISIS DE REORDENAMIENTO DE TCR-Gamma

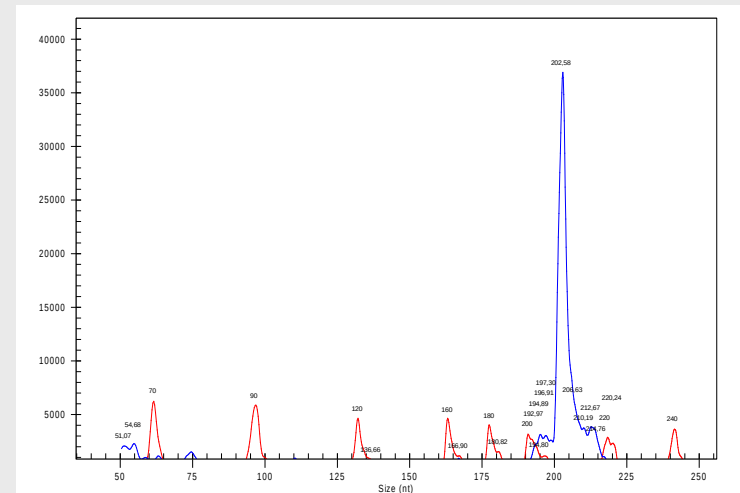


Control Positivo.



Control Negativo

207 pb



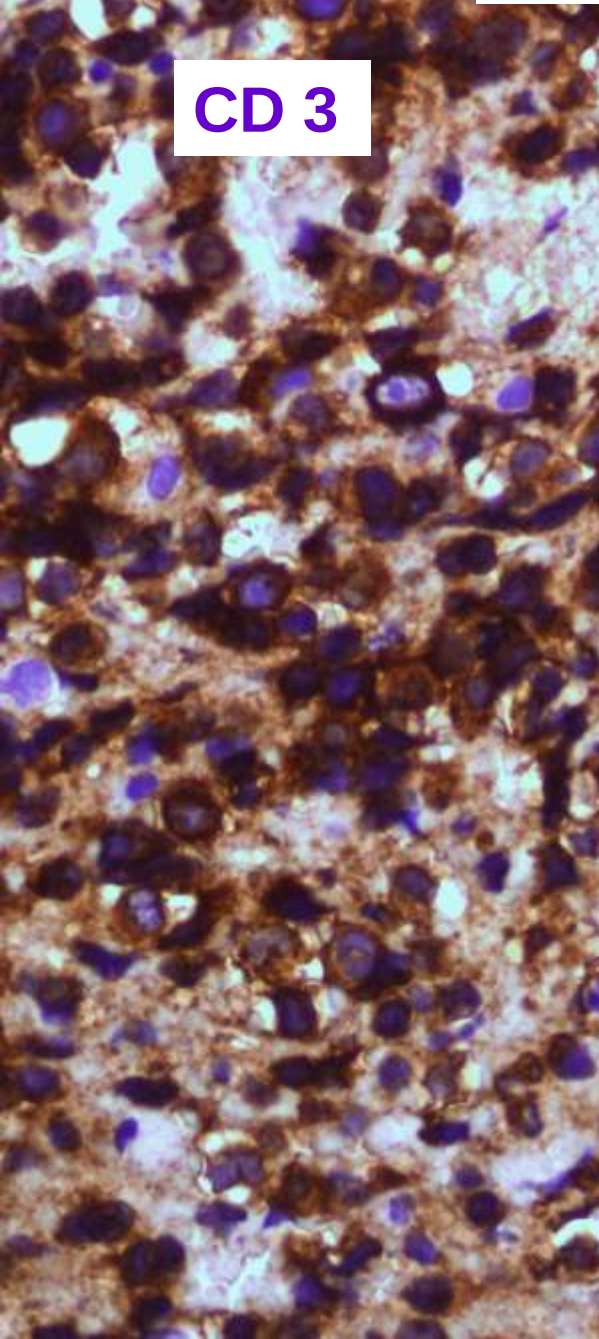
Caso Problema = Clonal

DIAGNÓSTICO HISTOLÓGICO

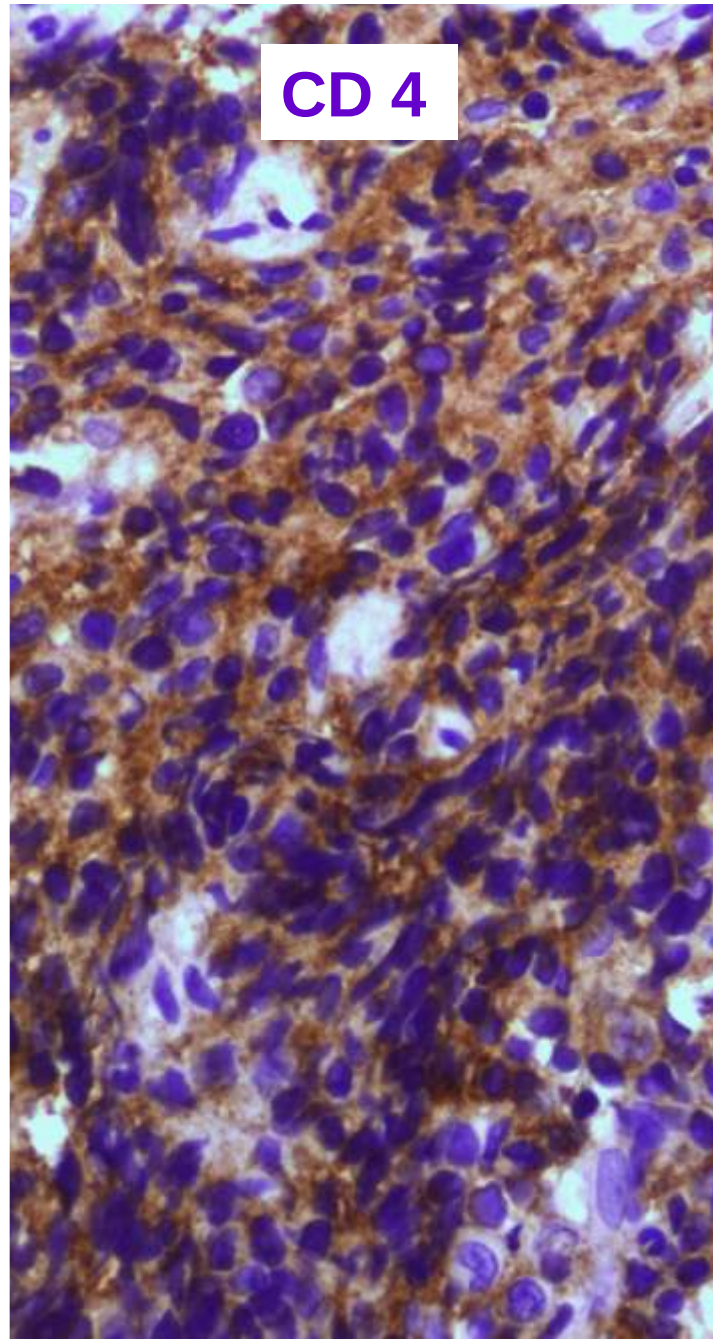
**INFILTRACIÓN CUTÁNEA POR LINFOMA
ANAPLÁSICO DE CÉLULAS GRANDES
ALK NEGATIVO (*ALCL*, *ALK*-)**

... y volvimos al bloque celular

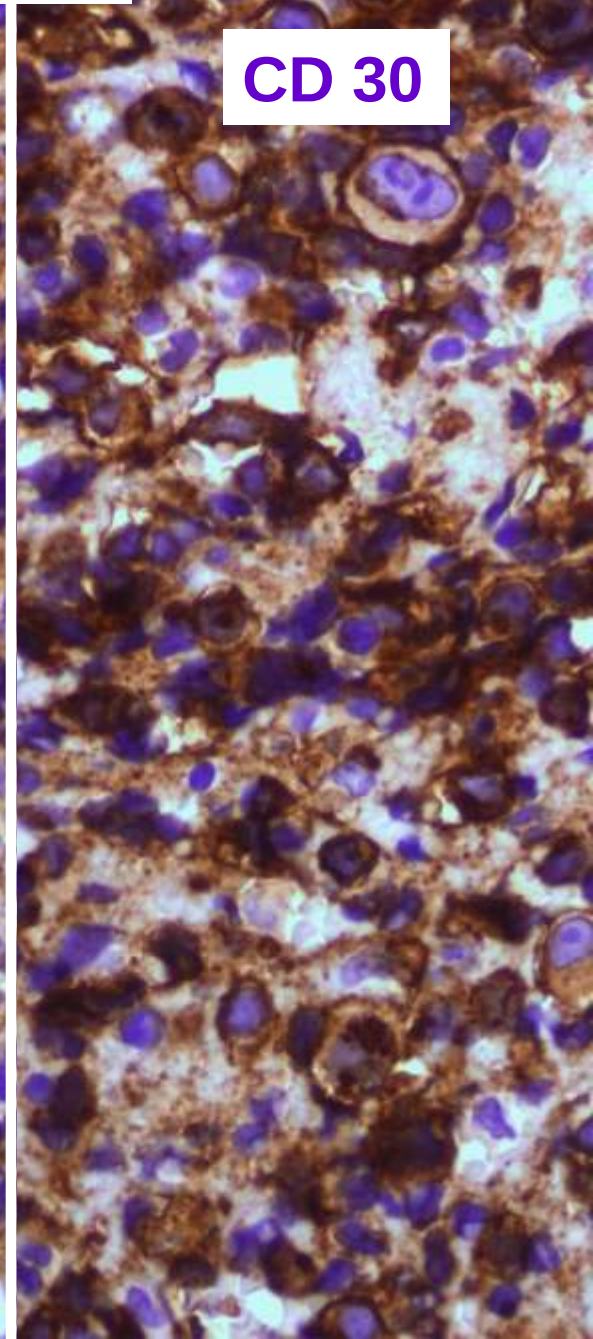
CD 3



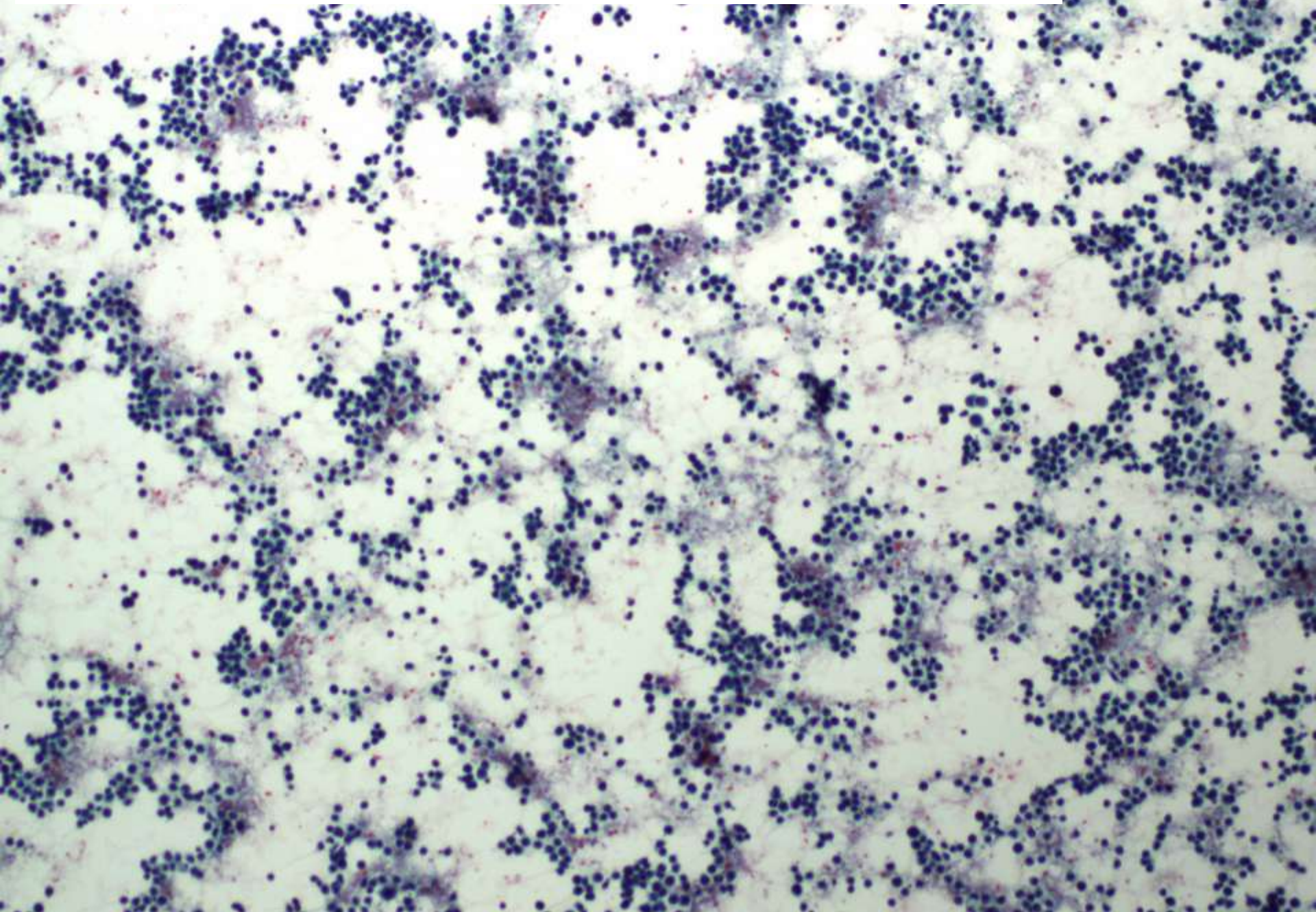
CD 4

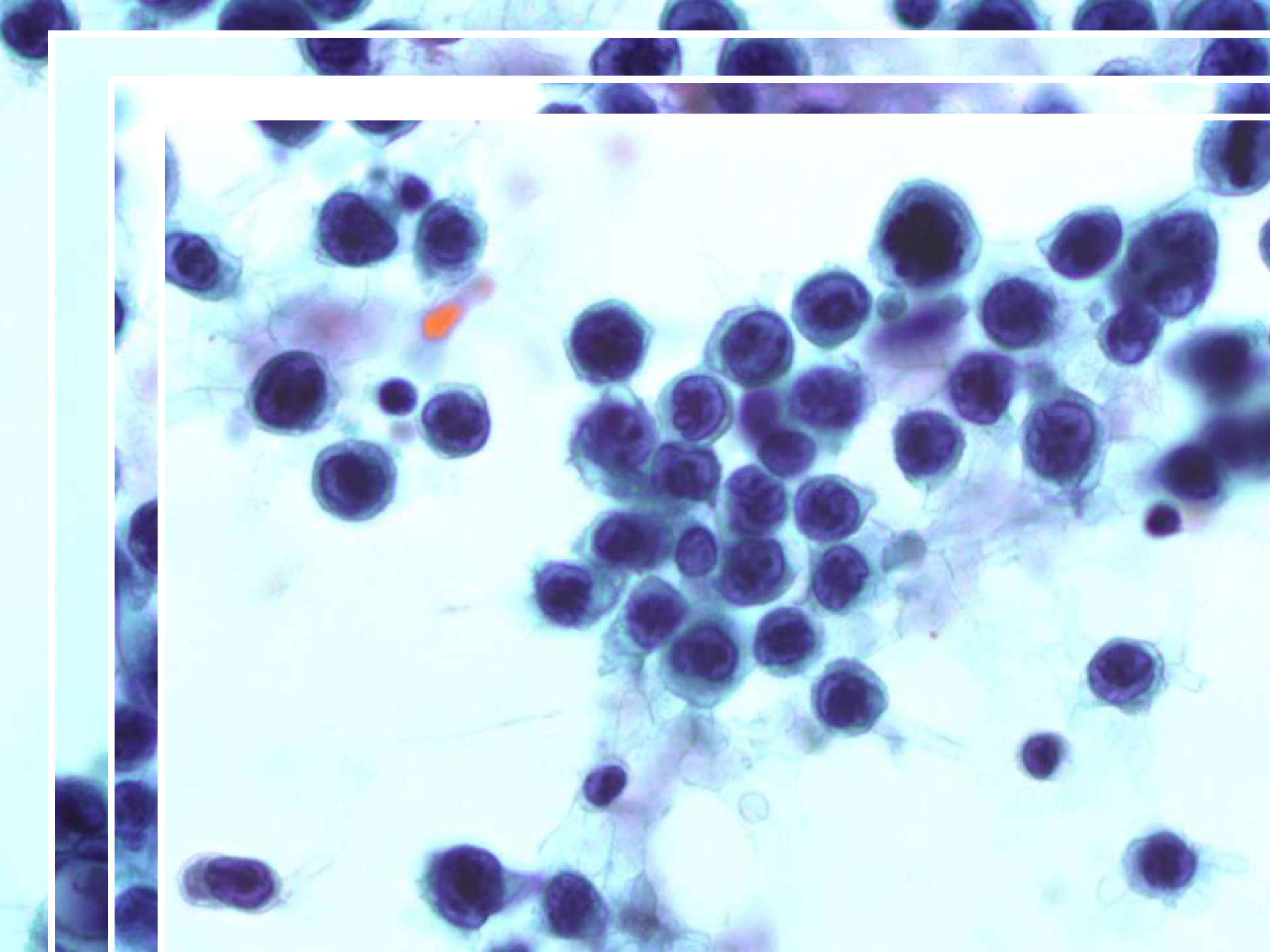


CD 30



10 meses después de la PAAF inguinal: derrame pleural masivo





LINFOMA ANAPLÁSICO DE CÉLULAS GRANDES ALK NEGATIVO (ALCL, ALK-)

- ✓ Incluido como una *entidad provisional* en la 4a edición de la Clasificación de la OMS, dentro del grupo de las *Neoplasias de células T y NK maduras*
- ✓ Se define como una *neoplasia T CD30+*, indistinguible de su contrapartida ALK+ excepto por la ausencia de dicha proteína
- ✓ Suele expresar marcadores T y/o proteínas asociadas a gránulos citotóxicos
- ✓ La mayoría reordena el receptor de células T
- ✓ *Diagnóstico diferencial*: ALCL primario cutáneo, otros linfomas B o T CD30+ con rasgos anaplásicos y con el linfoma de Hodgkin clásico

LINFOMA ANAPLÁSICO DE CÉLULAS GRANDES ALK NEGATIVO (ALCL, ALK-)

- ✓ Muestra un pico de incidencia entre *40-65 años* (superior a ALCL, ALK+), siendo algo más frecuente en el sexo masculino
- ✓ Es de afectación *nodal y extranodal* (hueso, partes blandas, piel...)
- ✓ Suele debutar en *estadio avanzado* y el pronóstico es desfavorable, con mala respuesta a la QT
- ✓ La histología ganglionar puede *simular* también afectación por *carcinoma*
- ✓ Se distinguen *cuatro patrones histológicos* (común, linfohistiocítico, tipo Hodgkin y compuesto)
- ✓ Es característico el pleomorfismo celular, con elementos multinucleados (en “corona”), *células “hallmark”* (de núcleo excéntrico, arriñonado o en herradura) y mitosis

ALCL, ALK-: INMUNOFENOTIPO

1. **CD30+** intenso en todas las células tumorales, con refuerzo de membrana y Golgi
2. **ALK-**

- Marcadores T en 50% de los casos (los más constantes son CD43, CD2, CD3 y CD4; CD8 suele ser negativo)
- Marcadores citotóxicos +/-
- EMA -/+
- CD45 +/-

DXD CON OTROS LINFOMAS

	ALCL, ALK- Y ALCL PRIMARIO CUTÁNEO	LINFOMA T PERIFÉRICO (PTCL), NOS	LINFOMA DE HODGKIN CLÁSICO
CD30	<i>intenso y homogéneo con refuerzo de Golgi y membrana</i>	focal y de intensidad variable	<i>positivo intenso</i>
marcadoresT	negativo/positivo	positivo/negativo	<i>negativos</i>
PAX5	negativo	negativo	<i>positivo</i>
CD15	negativo	negativo/ <i>positivo</i>	<i>positivo</i> /negativo
CD20	negativo	negativo	negativo/ <i>positivo</i>
CD45	positivo/negativo	positivo/negativo	negativo
EMA	negativo/positivo	negativo/positivo	negativo
EBV (EBER y LMP1)	negativo	<i>positivo en las células B</i>	<i>positivo</i>
clusterina	<i>positiva</i>	negativa	negativa (?)

Cytology of Anaplastic Large Cell Lymphoma

Dear Dr. Bedrossian:

I read with interest a recent article by Gatter et al.¹ entitled "Fine-needle aspiration biopsy of anaplastic large cell lymphoma, small cell variant with prominent plasmacytoid features: case report," in which an anaplastic large cell lymphoma (ALCL) with plasmacytoid features, intranuclear inclusions, and absence of lymphoglandular bodies was cytologically misinterpreted as suggestive of melanoma in a fine-needle aspiration (FNA). I wish to report a case of ALCL in which the FNA smears and the biopsy specimens were misinterpreted as metastatic carcinoma.

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Cytomorphologic Examination of Anaplastic Large Cell Lymphoma by Fine-Needle Aspiration Cytology

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Hannah Wen, MD
Filiz Sen, MD
Kasturi Das, MD

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BACKGROUND. The cytomorphology of anaplastic large cell lymphoma (ALCL) is distinctive yet variable. To the authors' knowledge, to date only small case series have described the cytologic findings noted in patients with ALCL. The current series is the largest case series presented to date to retrospectively review the cytomorphologic findings noted in patients with ALCL, with specific attention paid to those with anaplastic lymphoma kinase (ALK)-negative ALCL.

METHODS. Over a 13-year period, the available Diff-Quik cytology smears and surgical excision specimens taken from patients with ALCL were evaluated. Different clinical and morphologic parameters were evaluated, including ALK status.

RESULTS. A total of 37 cases were retrieved and evaluated, 19 of which had both cytology and surgical pathology specimens available for review. ALK-negative ALCL cytology smears were found to have a high number of anaplastic cells compared with ALK-positive cases. The hallmark cells in the ALK-negative cases were not classic.

CONCLUSIONS. ALCL can be diagnosed accurately by fine-needle aspiration cytology (FNAC) alone when aided by immunocytochemistry in ALK-positive cases. Ancillary studies should be anticipated such that material for cell block preparation and molecular studies is taken at the time of FNAC. The results of the current study demonstrate the varied FNAC morphology of ALCL. The presence of severe pleomorphism and anaplasia was found to correlate with ALK-negative status. *Cancer (Cancer Cytopathol)* 2007;111:499–507. © 2007 American Cancer Society.

KEYWORDS: anaplastic large cell lymphoma, anaplastic lymphoma kinase (ALK), fine-needle aspiration cytology, lymphoma.

TABLE 1
Demographic Data and Cytomorphologic Features of the Samples

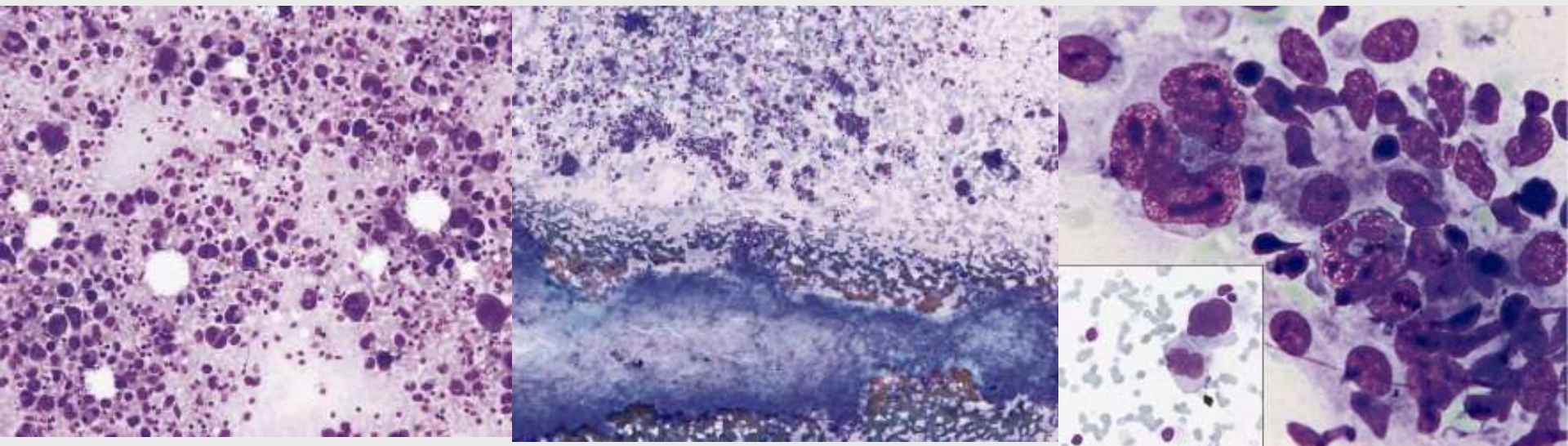
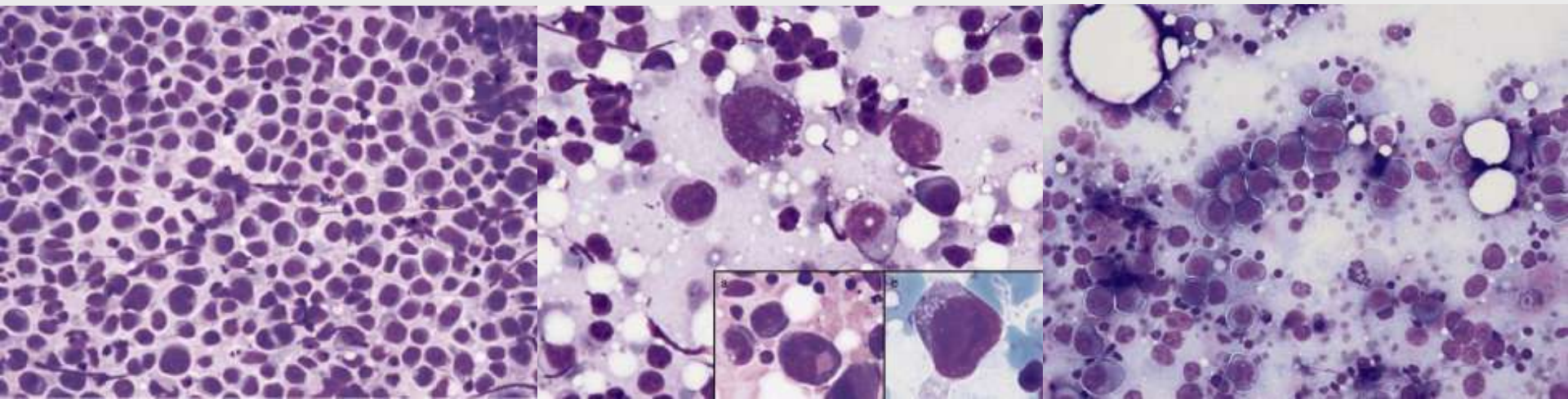
Cytology	Location	Age, years	Sex	Small cells	Plasmacytoid	MNGC/Wreath	Hallmark	Pleomorphism	Vacuoles	Nuceoli	Mitotic	Background	Anaplastic cells	Monotony	ALK
NHL*	Inguinal LN	45	Man	+	–	–	–	Mild	–	Multiple, single	0	+	0	+	–
Atypical lymphocytes, favor reactive	Inguinal LN	25	Woman	+	+	–	+	Mild	–	Multiple, single	3	+	3	–	+
ALCL*	Cervical LN	81	Woman	–	–	+	+	Severe	+	Single, prominent	6	+	6	–	–
Consistent with T-cell lymphoma	Paraspinal mass	91	Man	–	–	–	+	Severe	+	Multiple	0	+	16	–	
NHL, large cell	Ascites, cecum	61	Man	–	–	+	+	Severe	–	Multiple	0	–	10	–	
NHL, large cell	Supradavicular LN	63	Man	–	+	–	–	Mild	+	Single, prominent	0	+	6	–	
NSGCT vs ALCL	Axillary LN	27	Man	–	+	–	–	None	+	Multiple, prominent	0	+	0	+	+
Malignant neoplasm*	Supradavicular LN	65	Man	–	–	–	+	Severe	+	Multiple	0	+	9	–	–
ALCL	Axillary LN	55	Man	–	–	+	+	Severe	+	None	0	+	58	–	
NHL, large cell	Inguinal LN	49	Woman	–	–	–	–	None	+	Single, prominent	0	–	0	+	
ALCL	Retropéritoneum	60	Woman	–	–	–	–	None	–	Single, prominent	0	+	1	–	
ALCL*	Axillary LN	70	Man	–	–	+	+	Severe	+	Single, prominent	1	+	15	–	–
NHL, large cell	Mediastinum	30	Man	–	–	–	–	None	–	Multiple	1	+	0	+	
GCT vs ALCL*	Mediastinum	22	Woman	+	+	+	–	Mild	+	Single, prominent	0	+	5	–	–
Lymphoma, favor Hodgkin lymphoma	Supradavicular LN	29	Man	–	–	–	–	None	+	Single, prominent	0	+	0	+	
Hodgkin lymphoma	Cervical LN	45	Man	–	–	+	+	Severe	+	Multiple	1	+	17	–	
ALCL	Inguinal LN	27	Man	–	–	+	+	Mild	+	Multiple, prominent	1	+	3	–	+
NHL, large cell	Cervical LN	39	Man	+	–	+	+	Severe	+	Multiple, prominent	1	–	28	–	
ALCL	Cervical LN	87	Woman	–	–	+	–	Mild	+	Single, prominent	1	+	9	–	
ALCL*	Retropéritoneum	72	Woman	–	–	+	+	Severe	+	Multiple	0	+	21	–	–

MNGC indicates multinucleated giant cell; ALK, anaplastic lymphoma kinase; NHL, non-Hodgkin lymphoma; LN, lymph nodes; +, positive; –, negative; ALCL, anaplastic large cell lymphoma; NSGCT, nonseminomatous germ cell tumor; GCT, germ cell tumor.

* Samples with known ALK status.

Tumor maligno Ganglio inguinal 69 Hombre + + - + Marcado + Prominente + +/- Abundantes - -

pleomórfico



Cytologic and Immunocytochemical Findings of Anaplastic Large Cell Lymphoma

Analysis of Ten Fine-Needle Aspiration Specimens over a 9-Year Period

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BACKGROUND. Anaplastic large cell lymphoma (ALCL) has raised much controversy in the field of hematolymphoid pathology. Its nature is becoming better characterized with recent advances in molecular genetics. However, to the authors' knowledge, a detailed description of the fine-needle aspiration (FNA) cytology of ALCL is lacking and the application of immunocytochemical study, including immunostaining for anaplastic lymphoma kinase (ALK) protein, to cytology samples has not been studied to date.

METHODS. The authors reviewed 10 FNA specimens of ALCL from 8 patients encountered at Pamela Youde Nethersole Eastern Hospital and Queen Mary Hospital in Hong Kong over a 9-year period from early 1993 to the end of 2001. The cytologic and immunocytochemical findings (including ALK protein overexpression) of the specimens were correlated with histologic and immunohistochemical findings of surgical biopsy specimens.

RESULTS. Six of the eight patients had ALCL of the common variant, whereas the remaining two patients had ALCL of the small cell variant. FNA specimens of ALCL of the common variant yielded many loosely dispersed "hallmark" cells that contained eccentric kidney-shaped or embryo-like nuclei, several prominent rod-shaped or angulated basophilic nucleoli, and abundant amphophilic cytoplasm. "Doughnut" cells, tumor cells with multilobated nuclei, and multinucleated giant cells with a wreath-like arrangement of nuclei occasionally were found. A small number of "plasmacytoid" tumor cells, nondescript small round tumor cells, and reactive polymorphs also was present. In contrast, "plasmacytoid" cells and nondescript small to medium-sized tumor cells represented the predominant cell population in ALCL of the small cell variant. The "plasmacytoid" appearance was exaggerated further in air-dried smears. In air-dried smears, small intracytoplasmic vacuoles and scanty azurophilic granules also were noted. On immunocytochemical study performed using the cell block materials, the majority of tumor cells demonstrated membranous and paranuclear "dot-like" positivity for CD30. The staining for epithelial membrane antigen, leukocyte common antigen, and T-cell markers was variable. Positive staining for ALK protein was demonstrated beautifully in two of the cases.

CONCLUSIONS. Despite the wide morphologic spectrum of ALCL, a definitive diagnosis on the basis of FNA cytology is possible on careful interpretation of the cytologic features and a high index of suspicion. The cytologic diagnosis can be confirmed further with proper application of immunostaining to cell block sections. Immunocytochemical study for ALK protein, which provides useful prognostic information, also can be demonstrated satisfactorily using cytology samples. *Cancer (Cancer Cytopathol)*, *Cancer (Cancer Cytopathol)* 2003;99:33-43.

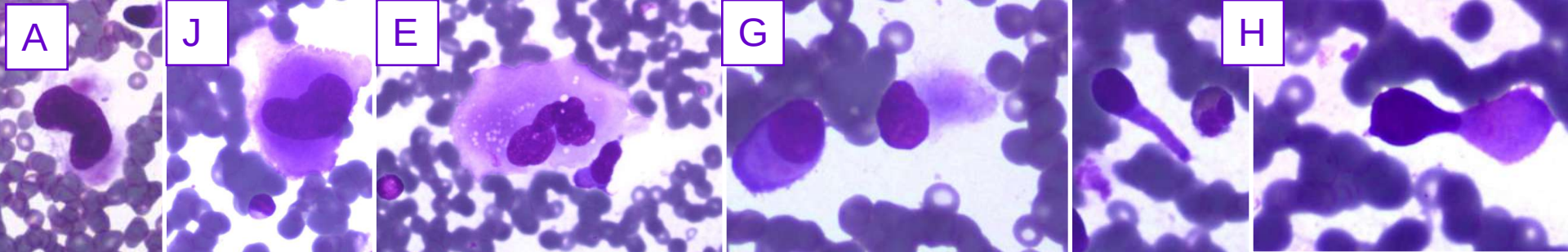
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KEYWORDS: anaplastic large cell lymphoma (ALCL), CD30, ALK, cytology.

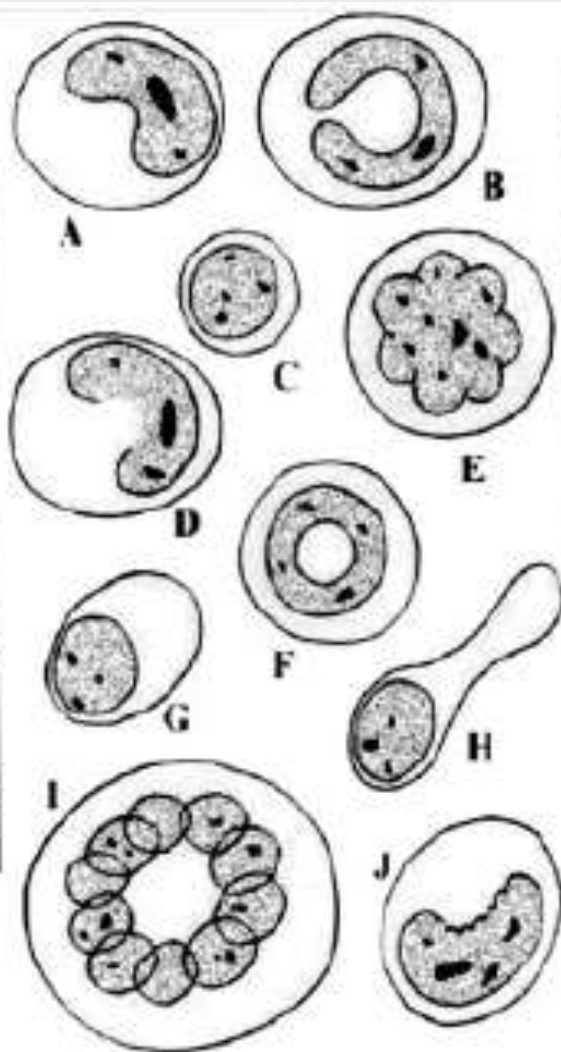
TABLE 2
FNA Cytology Findings for the 10 Reported Cases

FNA Case No.	Patient 1		Patient 2	Patient 3	Patient 4	Patient 5		Patient 6	Patient 7	Patient 8
	1a	1b	2	3	4	5a	5b	6	7	8
"Hallmark" cells with kidney-shaped or embryo-like nuclei	++	+	+	+	+	+	+	+	+/-	+/-
"Doughnut" cells	+	+	++	+	+	-	-	-	-	-
Multinucleated cells with a wreath-like arrangement of nuclei	-	+/-	-	-	-	+	-	+	-	-
Cells with multilobated nuclei	-	-	+/-	-	-	+/-	-	+/-	-	-
Small to medium-sized "plasmacytoid" cells	+	+	+	+	+	+	+	+	++	++
Nondescript, small to medium-sized cells	+	+	+	+	++	+	+	+	++	++
Prominent rod-shaped/angulated basophilic nucleoli	+	+	+	+	-	+	-	+	-	-
Paranuclear cytoplasmic accentuation	+	+	-	-	-	-	-	+	+	+
Mitotic figures	-	-	+	+	-	+	-	-	+	+
Apoptotic bodies	+	+	+	+/-	+	+/-	-	+	+/-	+
Background polymorphs	+/-	+	+	+	+/-	+	+	+	+	+
Final histologic diagnosis (based on surgical biopsies)	Anaplastic large cell lymphoma, common variant								Anaplastic large cell lymphoma, small cell variant	

FNA: fine-needle aspiration; +: positive; +/-: occasionally present; -: negative.



Cytology of Tumor Cells



A, D & J: "Hallmark" cells with kidney / embryo-like nuclei

B & F: "Doughnut" cells

C: Non-descript small round cell

E: Cell with Multilobated nucleus

G: "Plasmacytoid" cell

H: "Hand mirror" cell (seen in air-dried direct smears only)

I: Multinucleated cell with wreath-like arrangement of nuclei

Immunocytochemistry

CD30 +
LCA + (often)
CD45RO + (often)
CD3 + (sometimes)
EMA + (sometimes)
CD20 / CD79a -
CD15 -
Cytokeratin -
S-100 protein -

Anaplastic Large Cell Lymphoma

ALK +
(Good prognosis)

ALK -
(Poor prognosis)

HVH: BX DE ALCL CON CITOLOGÍA PREVIA

LOCALIZACIÓN	EDAD	SEXO	DIAGNÓSTICO CITOLÓGICO	DIAGNÓSTICO HISTOLÓGICO
Nódulo submaxilar izquierdo	63	H	Linfoma, sugestivo de ALCL (CD45+; CD30+; Vim+; CD3+)	ALCL, ALK-
Ganglio laterocervical izquierdo	35	H	Linfoma de Hodgkin (CD30+; CD15+)	ALCL, ALK- (CD15 + focal)
Ganglio supraclavicular izquierdo	29	M	Linfoma de alto grado (Vim+; CD45+; CK-)	ALCL, ALK+
Masa supraclavicular izquierda	61	M	ALCL, ALK+	ALCL, ALK+
Hueso iliaco	38	H	ALCL, ALK+	ALCL, ALK+
Ganglio supraclavicular izquierdo	19	M	ALCL, ALK+	ALCL, ALK+

***ALCL, ALK-* en citología: qué debemos recordar...**

- ¡Que existe!
- El aspecto citológico de las células anaplásicas/pleomórficas no es específico de línea, ayudando poco al diagnóstico
- Las células plasmocitoides simulan neoplasias de células plasmáticas o tumores NE
- Ir a la búsqueda de las células *hallmark*, a menudo escasas, que son la claves morfológicas del diagnóstico

***ALCL, ALK-* en citología: qué debemos recordar...**

- Dificultades añadidas a la interpretación de la entidad como de estirpe linfoide:
 - ❖ Cohesividad celular (en citología y biopsia)
 - ❖ Citoplasma anfófilo evidente
 - ❖ Ausencia de cuerpos linfoglandulares en afectación extraganglionar
 - ❖ Escasa presencia de *c. hallmark* en ALK- (donde la propia pérdida de ALK supone un handicap al dx)
 - ❖ Pérdida ocasional de CD45 (ALC) y/o marcadores T
 - ❖ Citometría de flujo a menudo sin alteraciones

ALCL, ALK- en citología: un consejo...

- Incluir al **CD30** en el panel IHQ complementario de un tumor maligno pleomórfico **CKs- / CD45- / Vimentina+**...
- ...sin olvidar que:

➤ *DLBCL*

➤ carcinoma embrionario

➤ melanoma

➤ sarcoma de alto grado



Pueden expresar CD30