



Adapting to technological change in haematopathology

Fine-needle aspiration and haematopathology

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Issues

- Haematopathologists in the UK are generally tissue biased.
- The 'Google effect'
- Rapid *mimimally invasive diagnosis* in 'lumps & bumps' clinics.
- Imaging (US/CT, esp PET) are now picking up incidental / post therapy **visceral** lesions.
- PET – not entirely specific, often picks up macrophage response avidly in post therapy lesions (Deauville score: >3) – requiring sampling to determine if residual disease is present.
- Morbidity / mortality associated with surgical access usually precludes utility of this modality.
- Often lesions are at sites where interventional radiologists would not dare to go.
- Therapy controls disease better, so more relapses encountered in routine clinical practise – do we have to biopsy all of them?

Cytologic material & haematolymphoid malignancy diagnosis

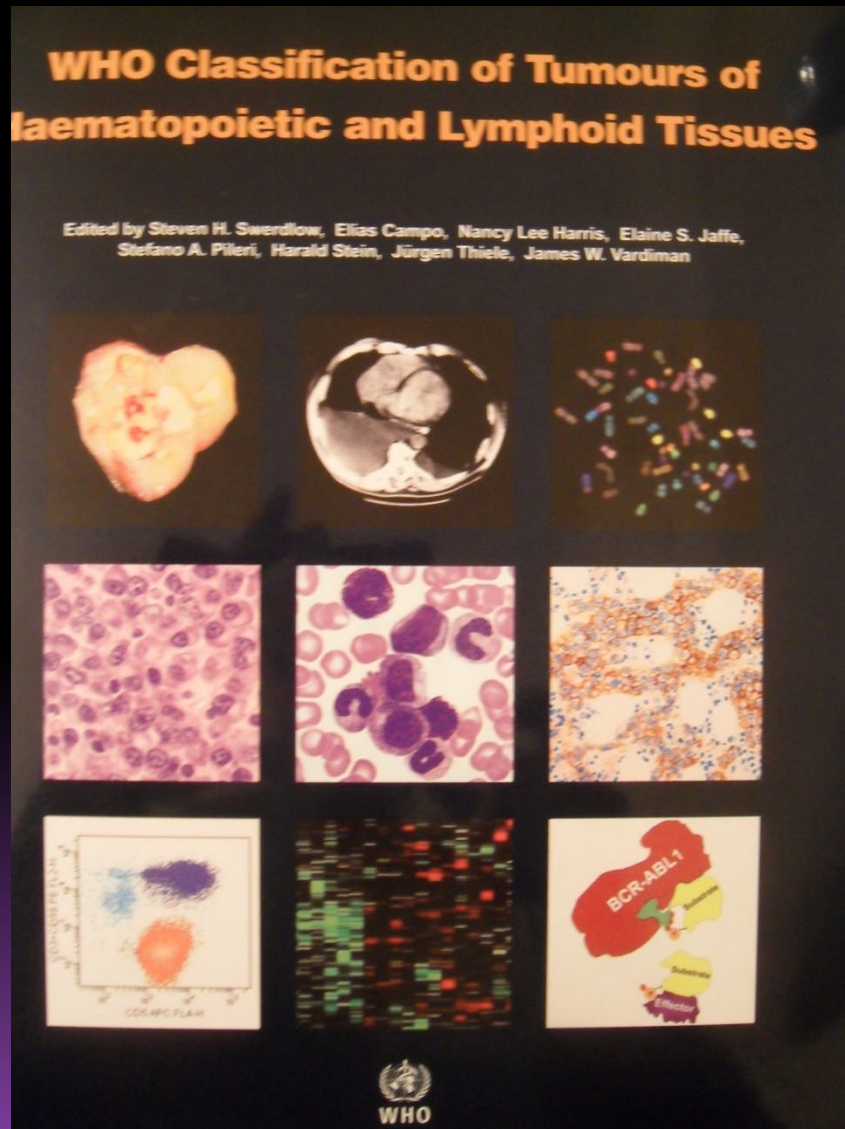
■ Clinical

- Can one use cytologic material to make a diagnosis of lymphoma / leukemia (akin to using a blood sample)?
- Is this diagnosis reliable?
- Are we able to get enough material out to do the same workup that we would be able to do on biopsy material?
- Is the extent of sampling adequate?

■ Laboratory

- Is it possible to perform ancillary testing on such samples – immunohistochemistry, flow cytometry, FISH, B & T-cell clonality studies?
- Can one convert cytologic material to histologic material?

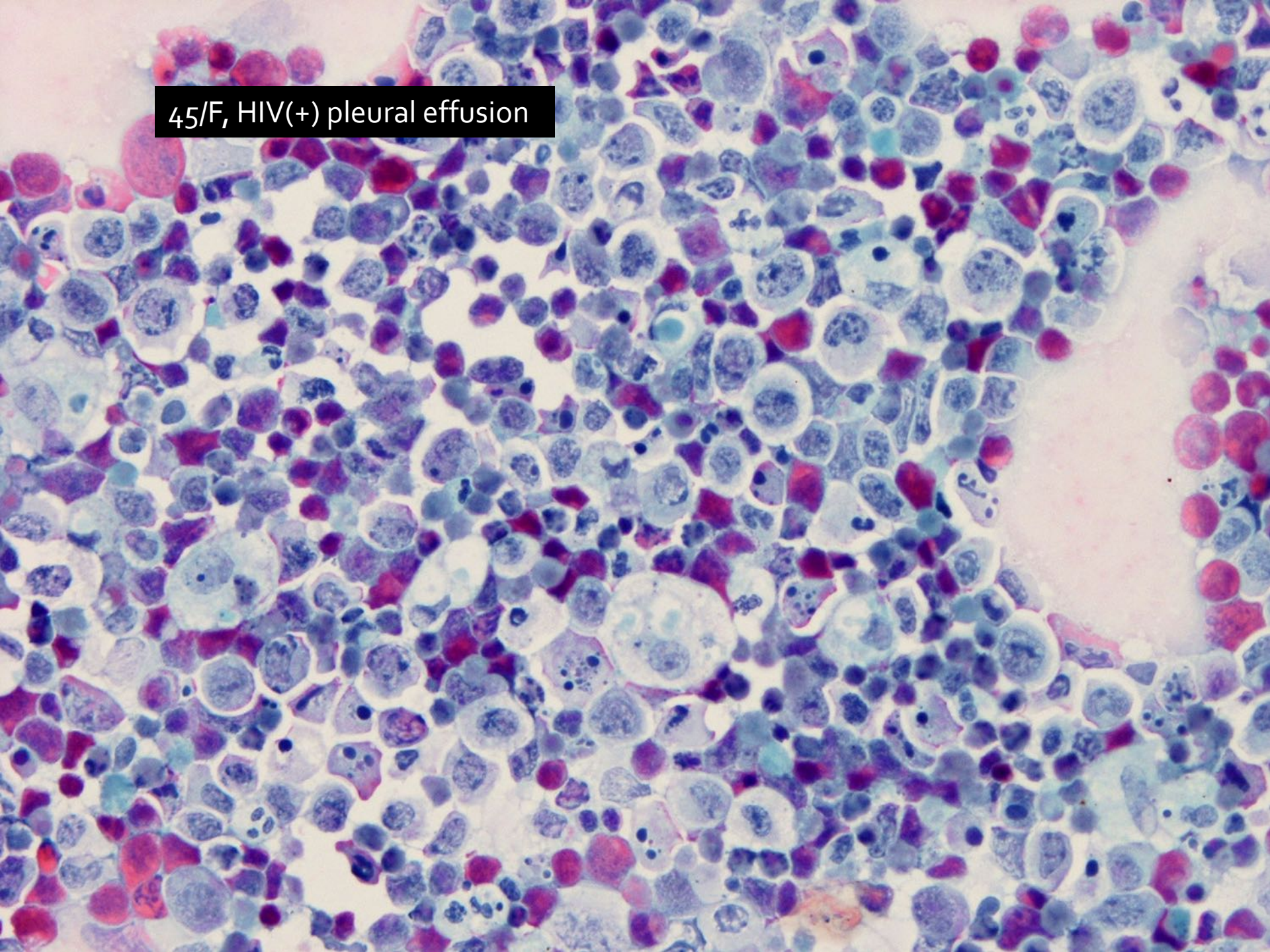
Can one use cytologic material to make a diagnosis of lymphoma / leukemia (akin to using a blood sample)?

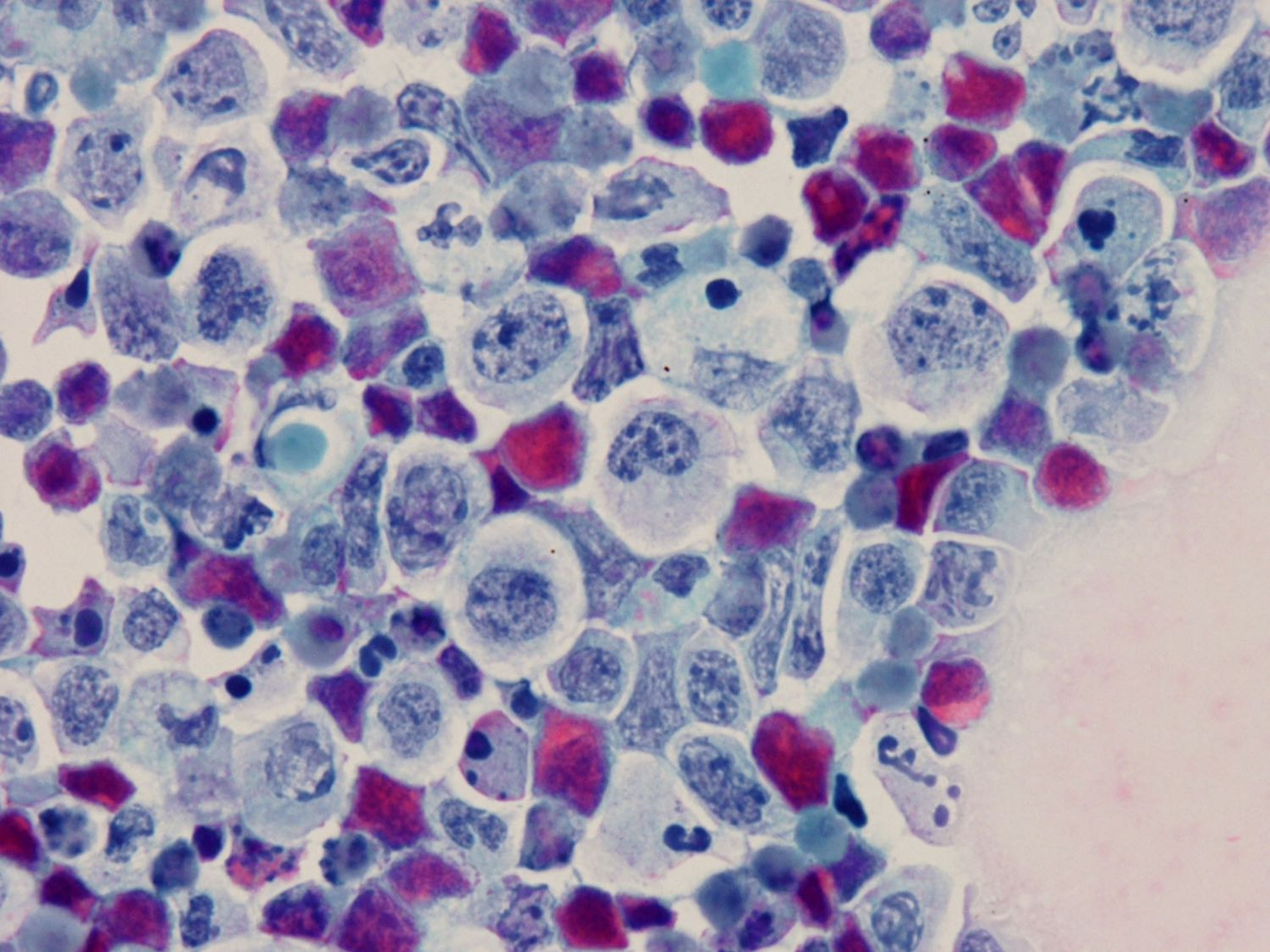


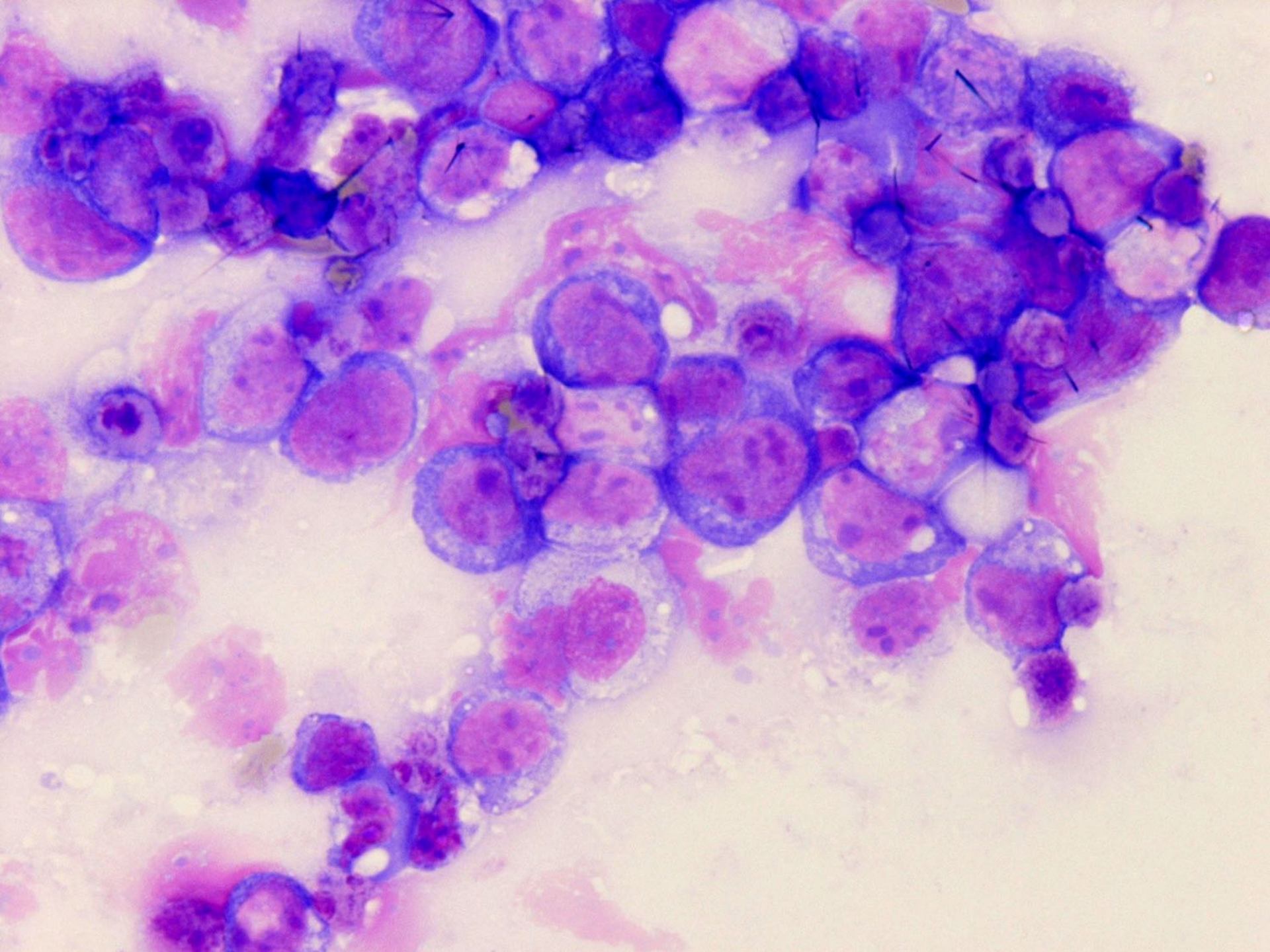
WHO 2008 – diagnosis based on:

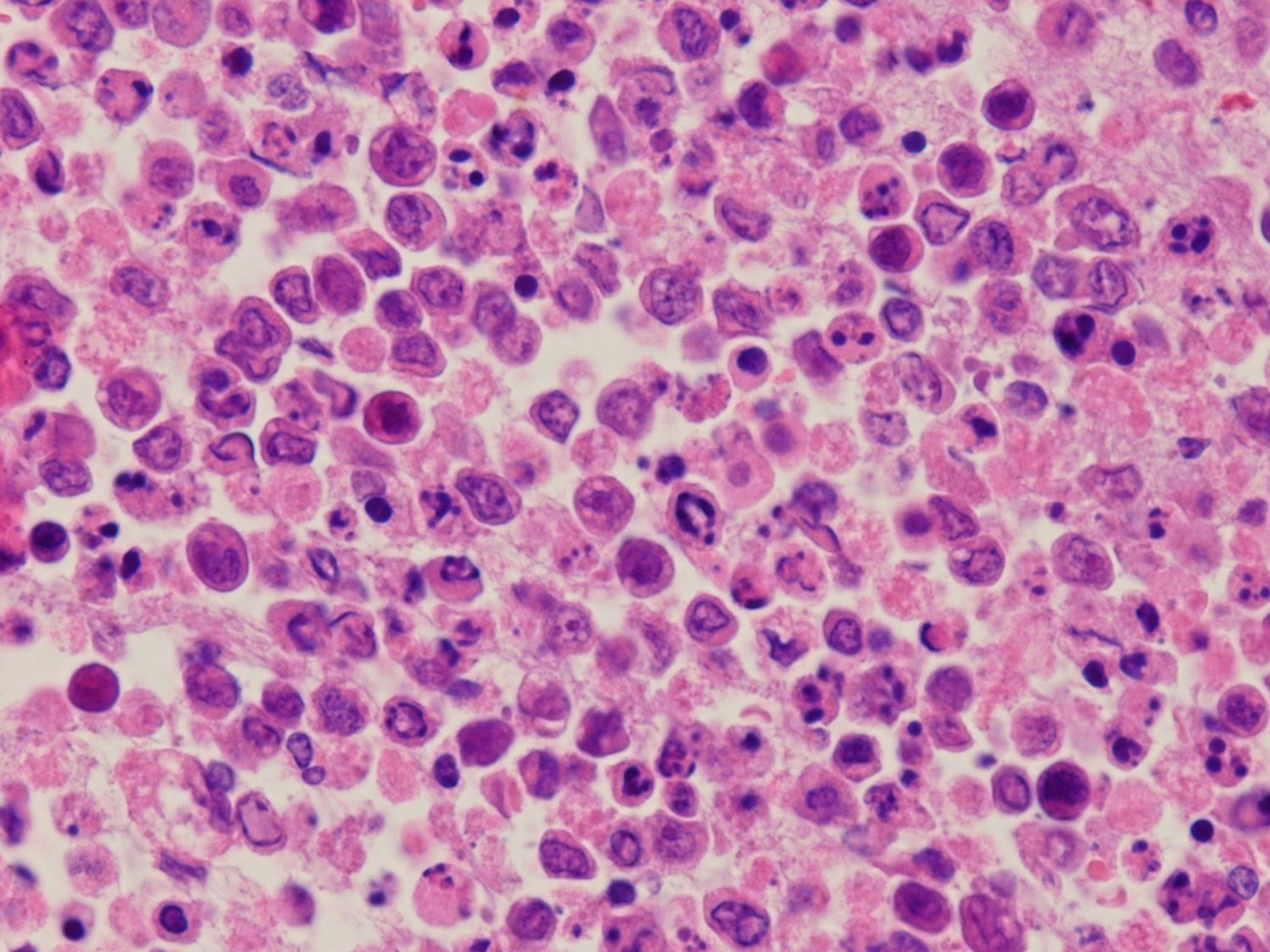
- Cytologic
- Immunophenotypic
- Genetic
- Molecular data

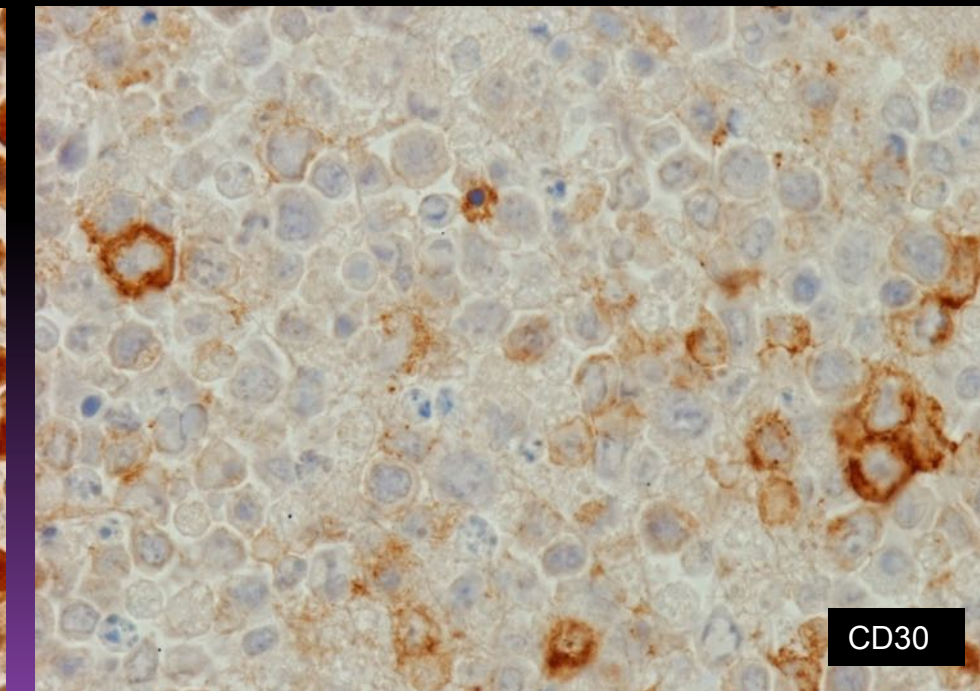
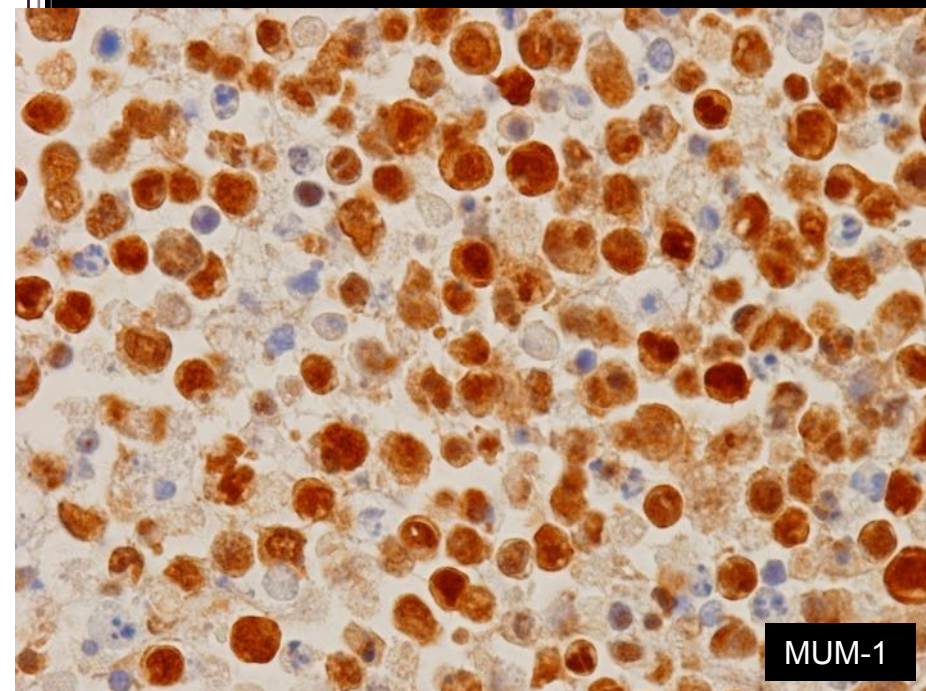
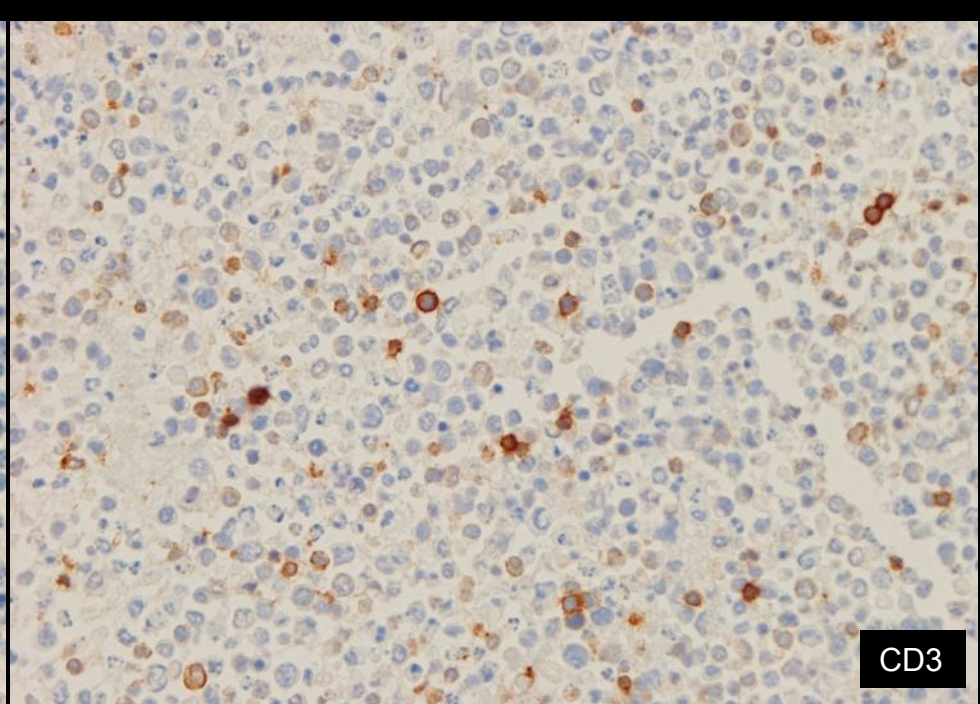
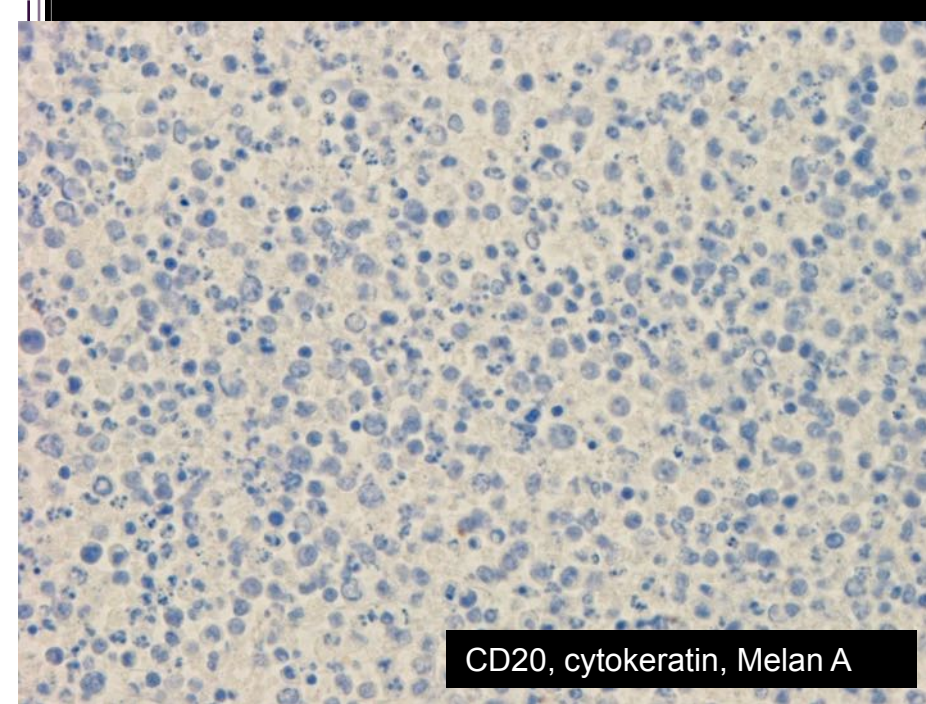
45/F, HIV(+) pleural effusion

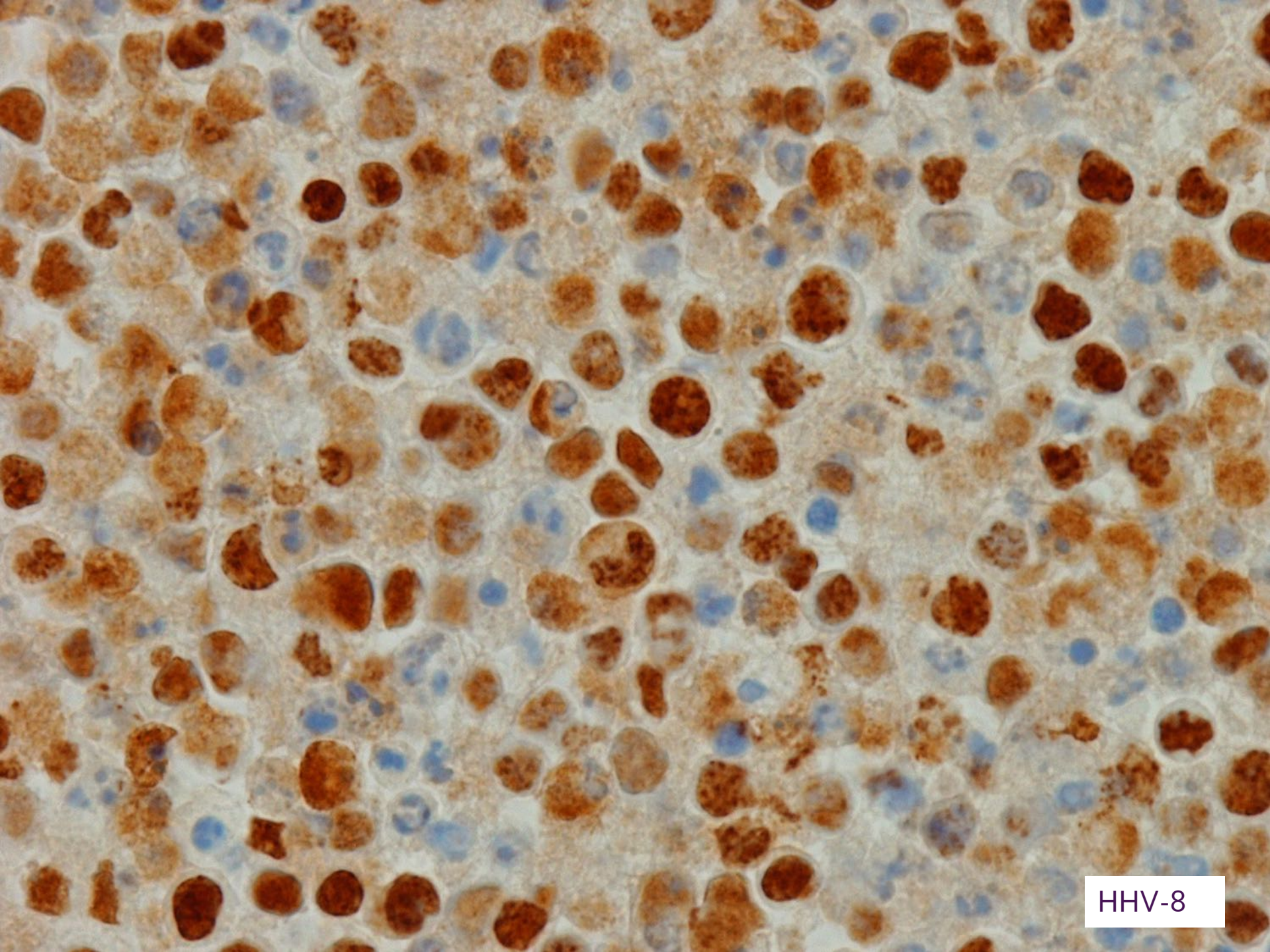












HHV-8

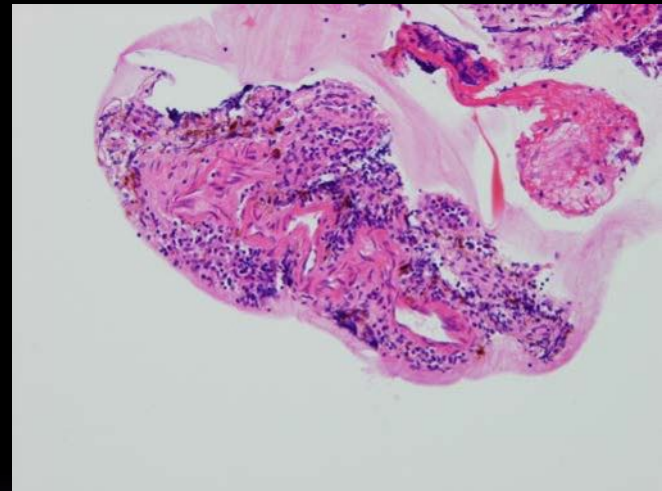
Diagnosis:

Primary Effusion Lymphoma

Cell block technology

- Conversion of cytology specimens → histologic specimen
- Most cytology specimens if appropriately collected.
- Needle washings / additional passes
- Normal saline (2 ml)
- Centrifuged
- Supernatant discarded
- Sediment mixed with plasma
- Thrombin added
- Clot prepared
- Clot transferred to cassettes
- Fixed & routine processing

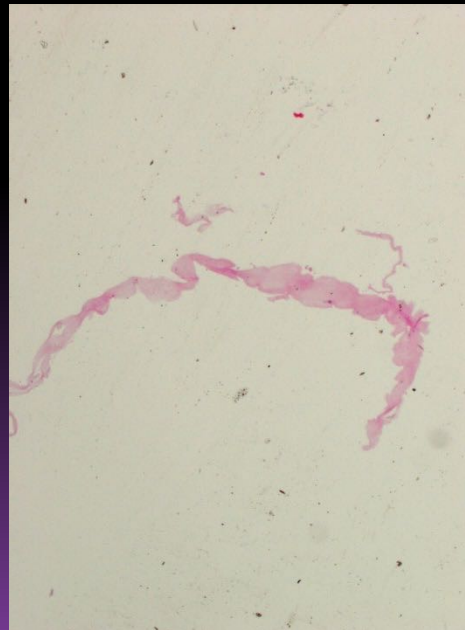
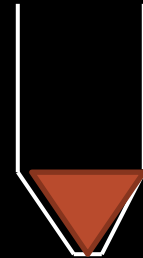
- Allows evaluation of architecture



- Allows
 - Special stains
 - Immunohistochemistry
 - FISH
 - Molecular (PCR / NGS)

Cell blocks – technical issues

- ▣ Type of container used



Cell blocks - technical issues



Double the material

EBUS: **E**ndoscopic **B**ronchial **U**ltra**S**ound

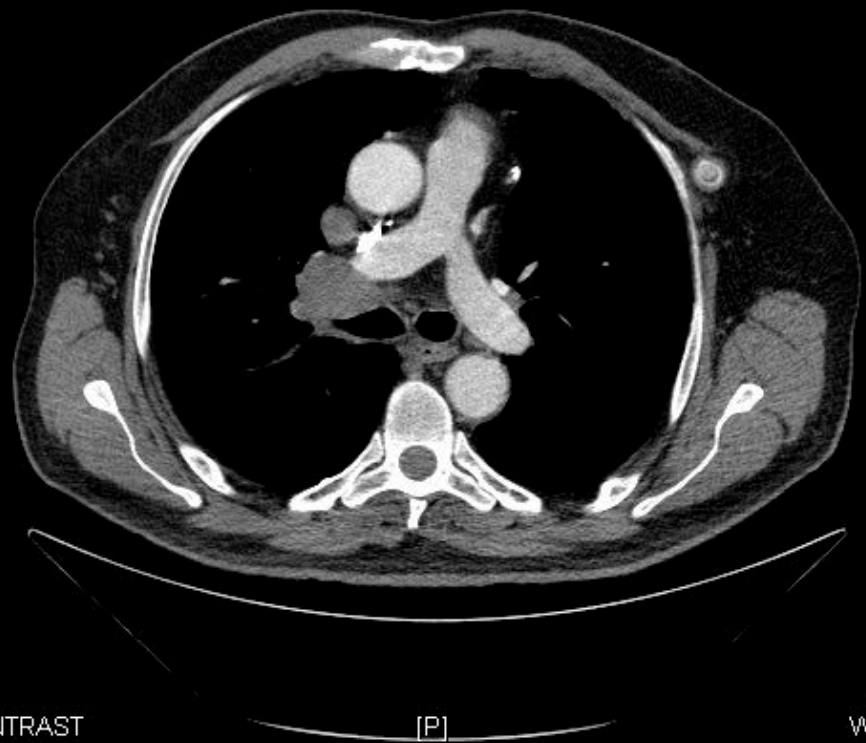
EUS: Endoscopic ultrasound



Visceral lymph node / mass sampling

The EBUS story

[R]



[L]

CONTRAST

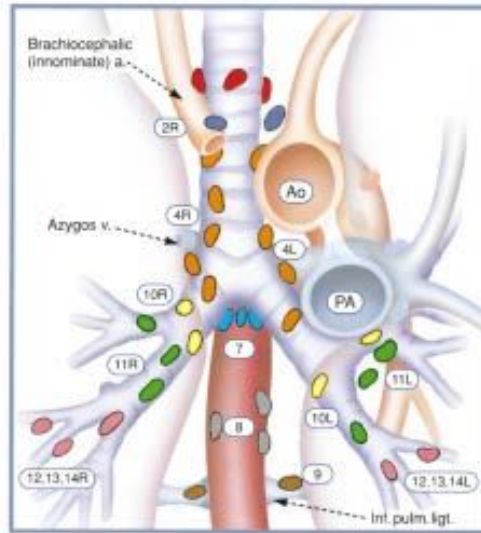
C60
W400

[P]

TABLE 3
Procedures Used to Sample Lymph Nodes, by Lymph Node Level

Lymph node level	Mediastinoscopy	Thoracotomy	Chamberlain/VATS	Esophageal sonography
2L, 2R	✓	✓		
4L, 4R	✓	✓		
5, 6		✓	✓	✓
7	✓	✓		✓
8, 9		✓		✓
10L, 10R		✓	✓	
11-14		✓		

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Superior Mediastinal Nodes

- 1 Highest Mediastinal
 - 2 Upper Paratracheal
 - 3 Pre-vascular and Retrotracheal
 - 4 Lower Paratracheal (including Azygos Nodes)
- N₁ = single digit, ipsilateral
N₂ = single digit, contralateral or supraclavicular

Aortic Nodes

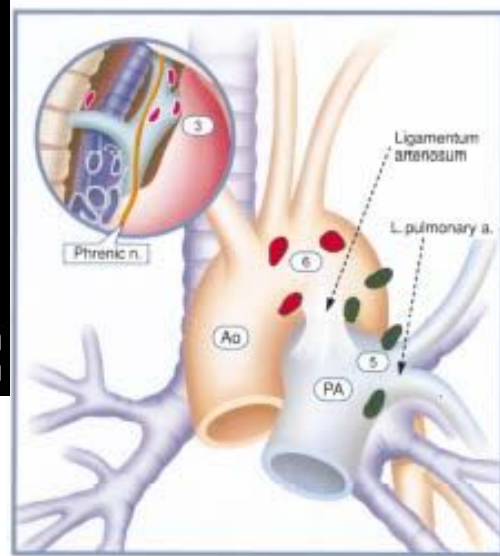
- 5 Subaortic (A-P window)
- 6 Para-aortic (ascending aorta or phrenic)

Inferior Mediastinal Nodes

- 7 Subcarinal
- 8 Paraesophageal (below carina)
- 9 Pulmonary Ligament

N₁ Nodes

- 10 Hilar
- 11 Interlobar
- 12 Lobar
- 13 Segmental
- 14 Subsegmental



(Mountain/Dresler modifications from Naruke/ATS-LCSG Map)

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Where radiologists do not tread....

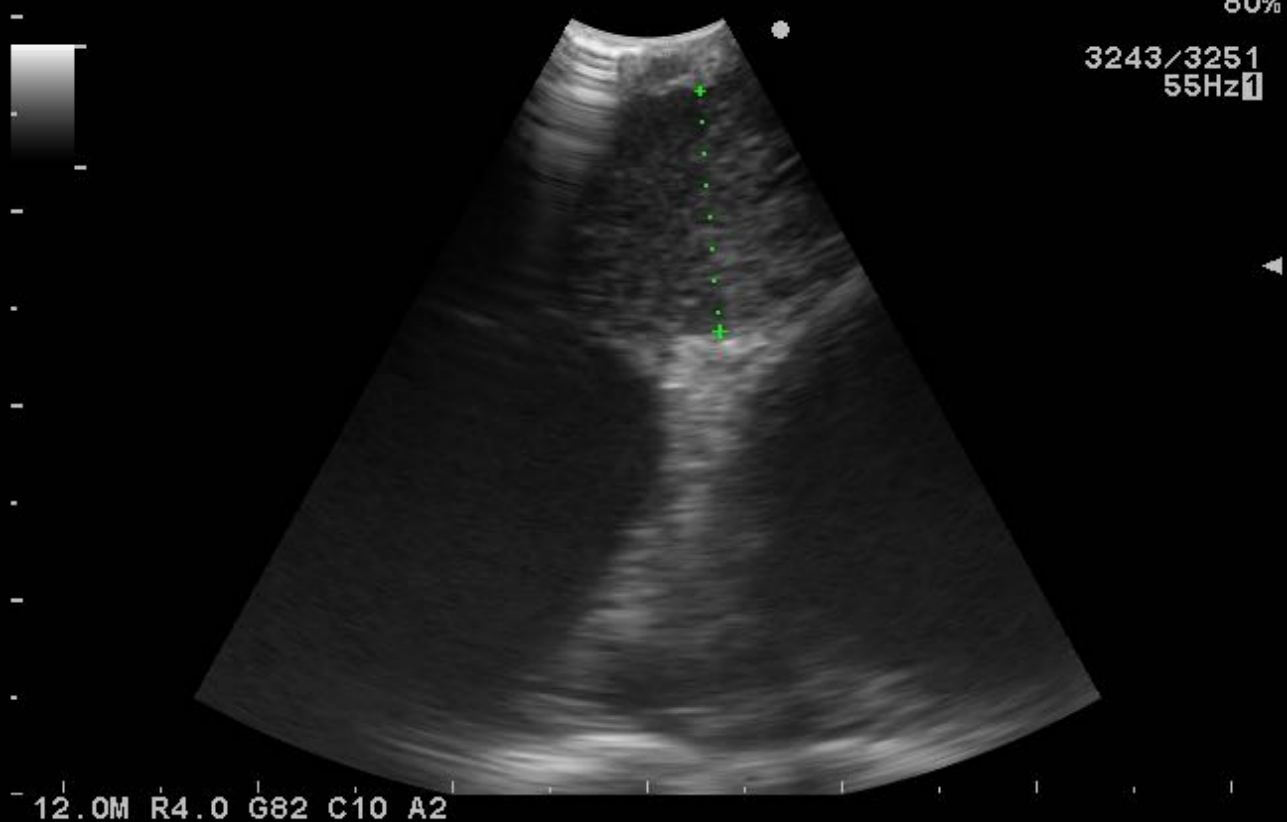
ALOKA

GSTT EBUS TEAM
PROSOUND ALPHA 10

: 78Y

:F

23-01-'14
11:26:52



Dist: 1.24cm

1:EBUS

Probe:OLY-R8C2

Endoscopic ultrasound setup

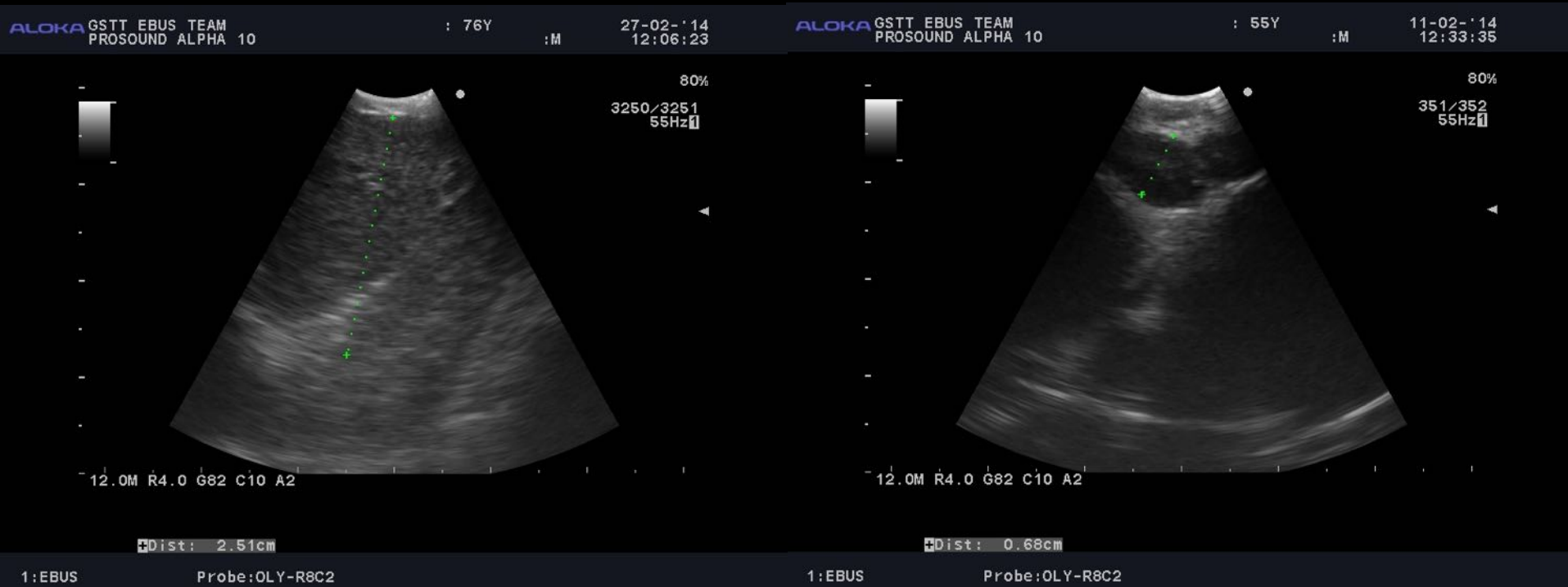


Rapid on site assessment (ROSE) setup





What can be targetted



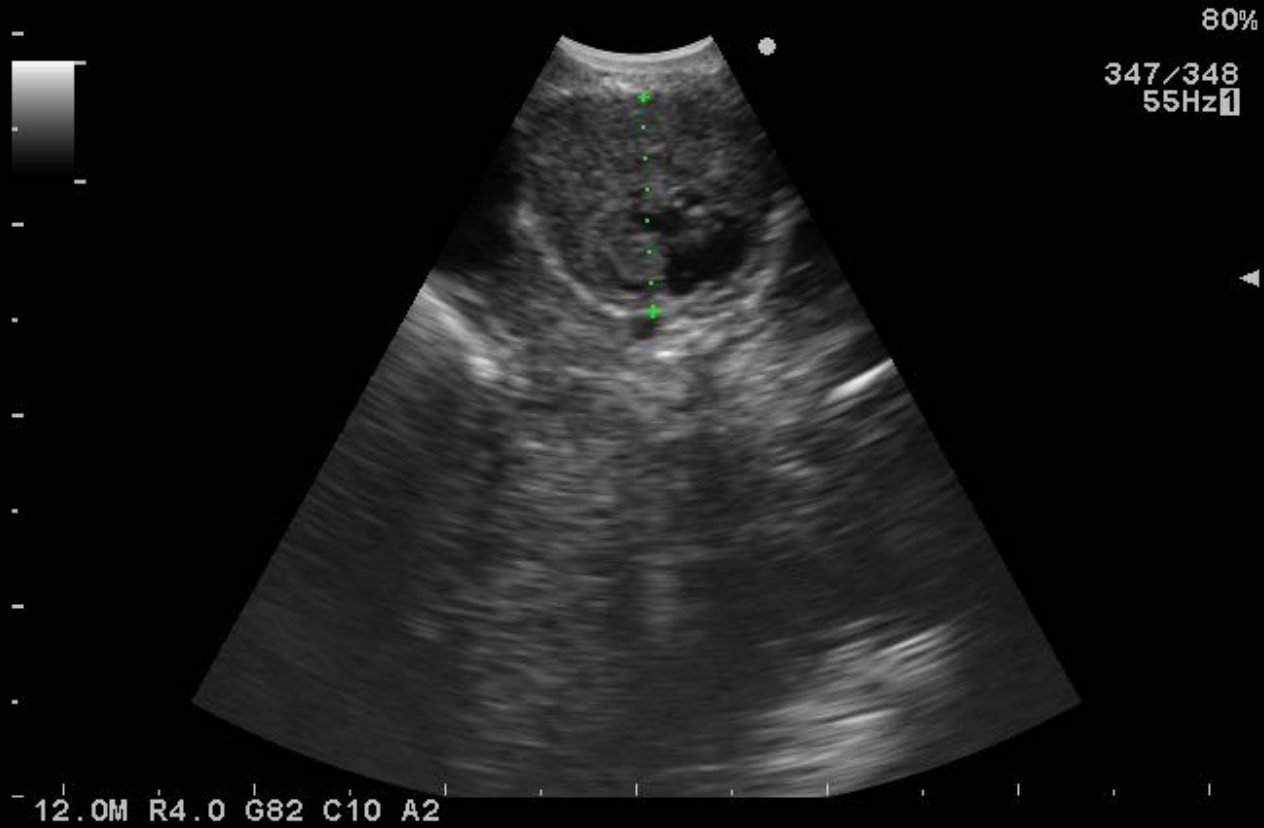
Where to sample

ALOKA GSTT EBUS TEAM
PROSOUND ALPHA 10

: 69Y

: F

21-01-'14
15:41:32

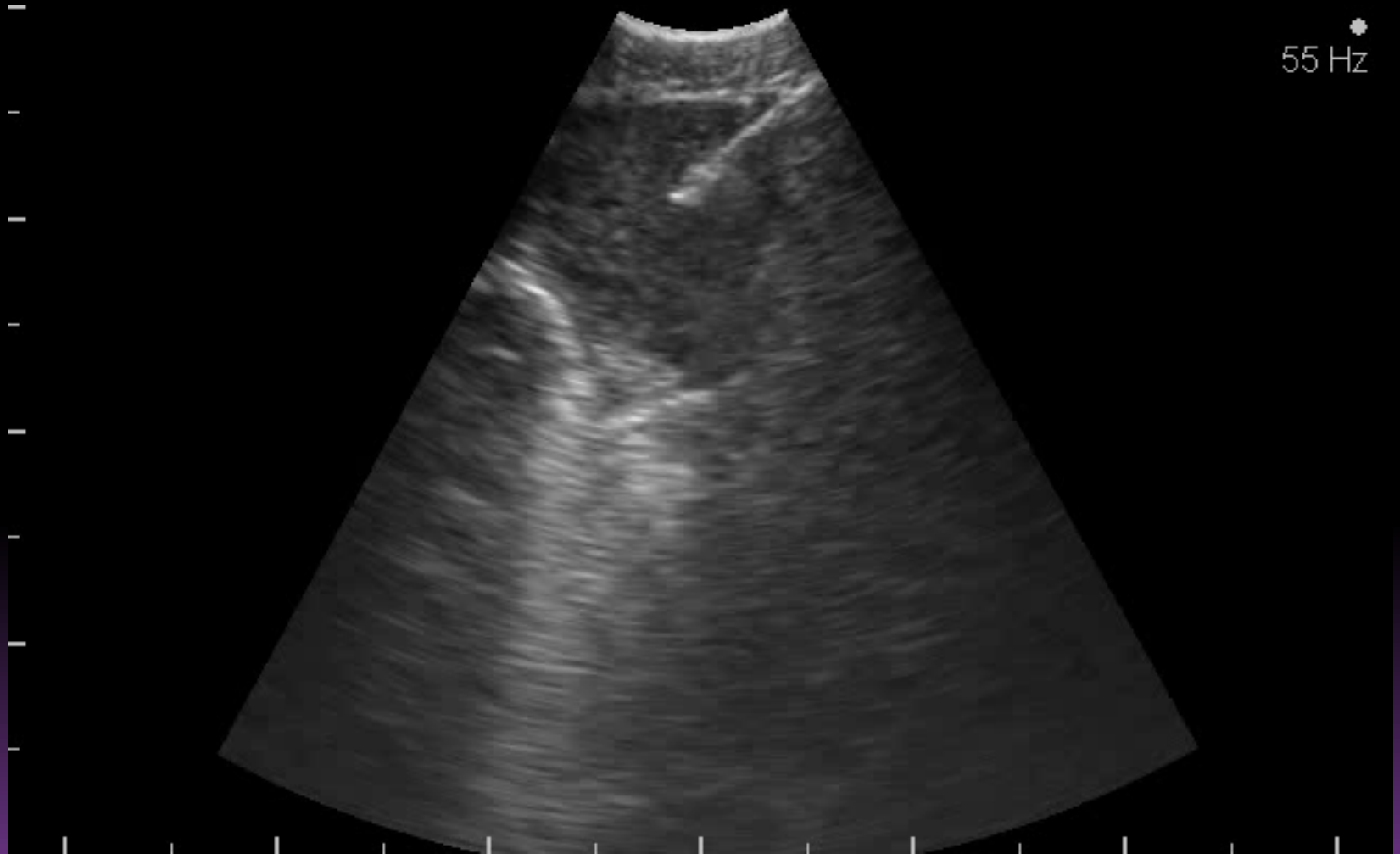


Dist: 1.12cm

1:EBUS

Probe:OLY-R8C2

Is sampling adequate?



How to deal with a lymph node aspirate

FNA

Intra-procedural assessment

- Abscess
- Granulomas

- Metastatic carcinoma
- Hodgkin lymphoma
- High grade NHL

- Reactive lymph node
- Low grade Non-Hodgkin lymphoma

Needle washings – same / additional passes

Microbiology

Cell block
IHC / FISH / Molecular

Flow cytometry

Cell block

Cytologic material & haematolymphoid malignancy diagnosis

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- ✓ Can one convert cytologic material to histologic material?

74 F Se:2
Im:143

[A]

Study Date:29/01/2010

Study Time:10:50:00

MRN:

■ Bilateral 3rd nerve palsy, deviated tongue and uvula to left

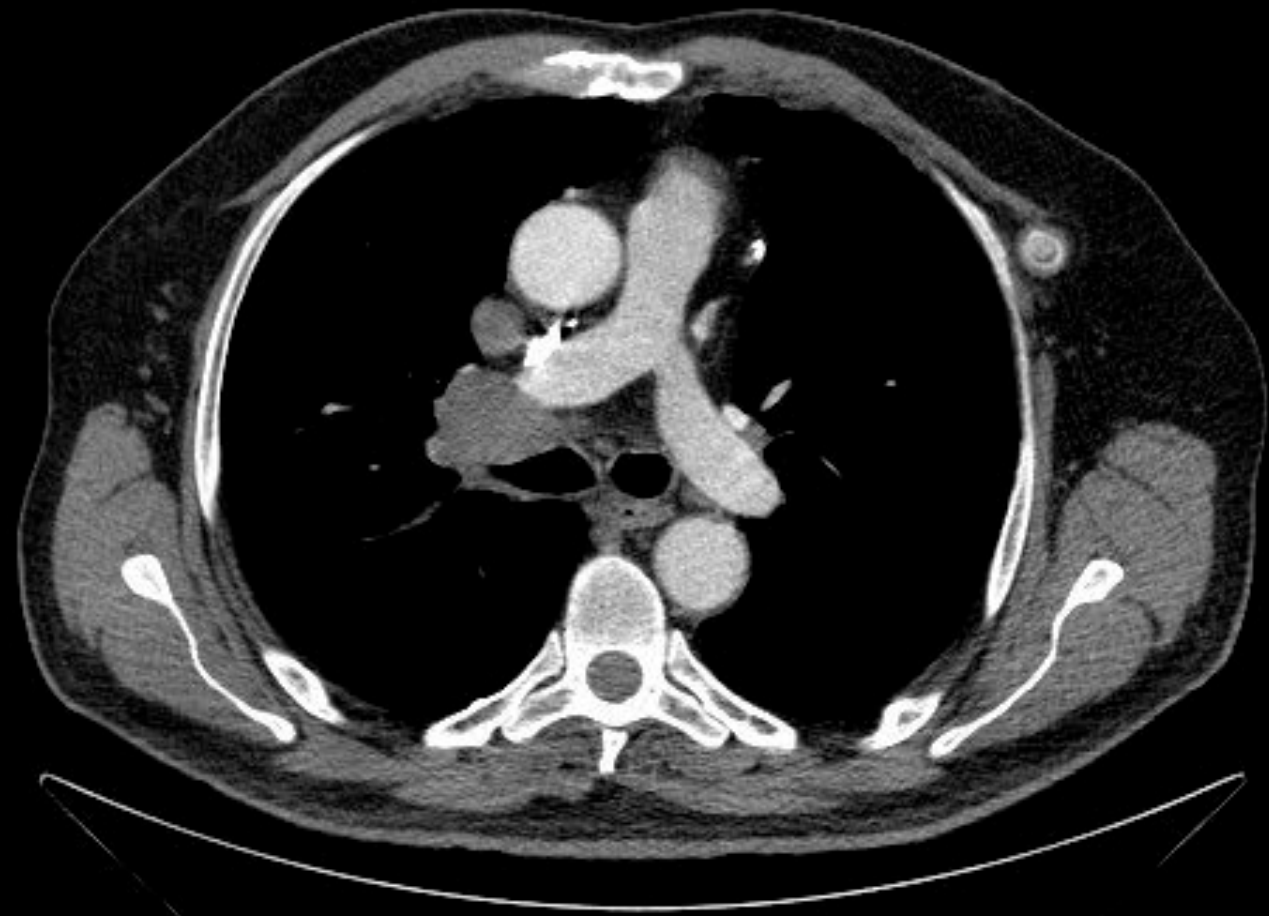
■ Soft tissue mass in the anterior and superior mediastinum anterolateral to the trachea on the right measuring 4.9 x 3.8cm.

■ Right pleural effusion

■ LDH: 2161 IU/L

[R]

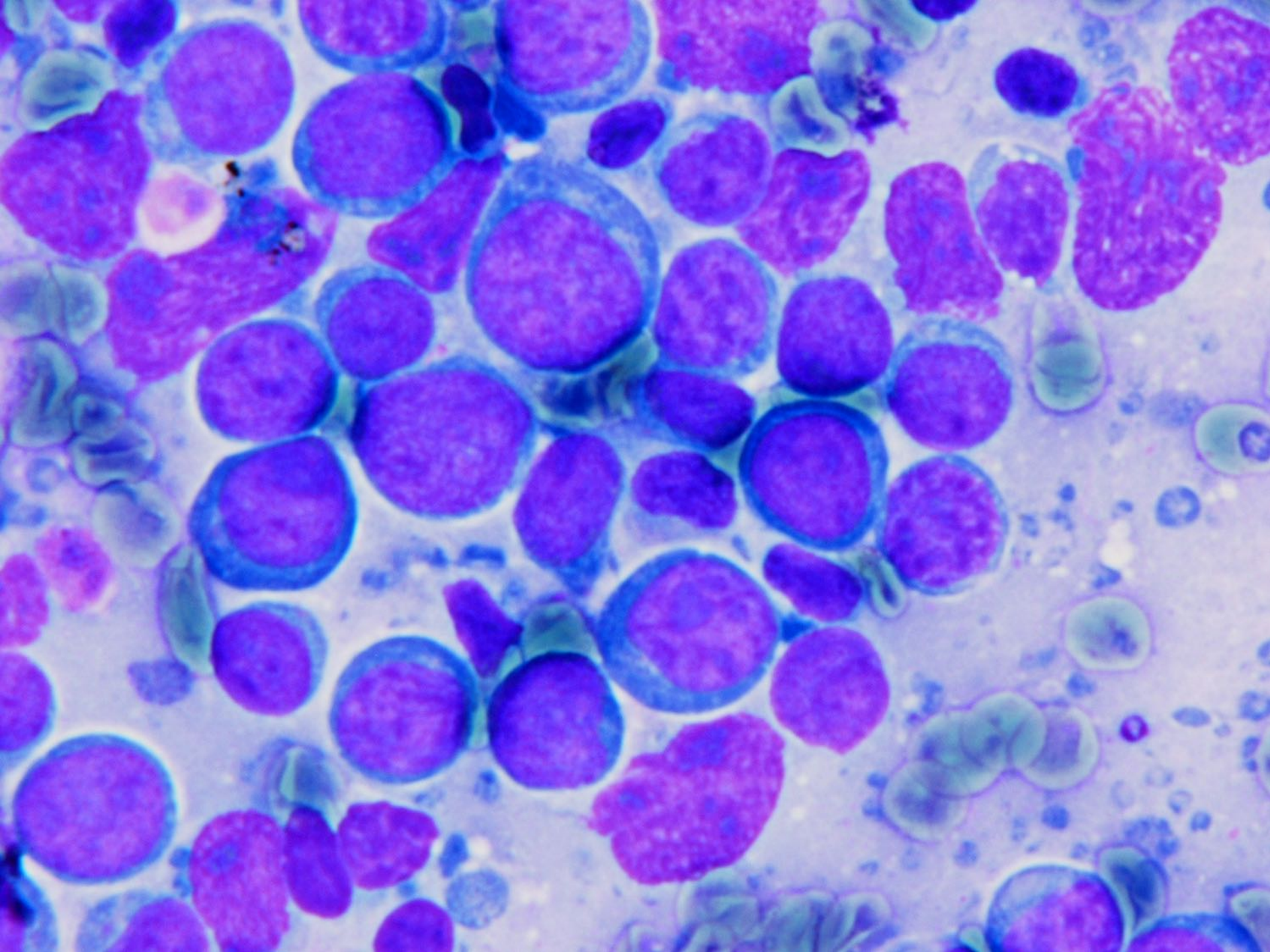
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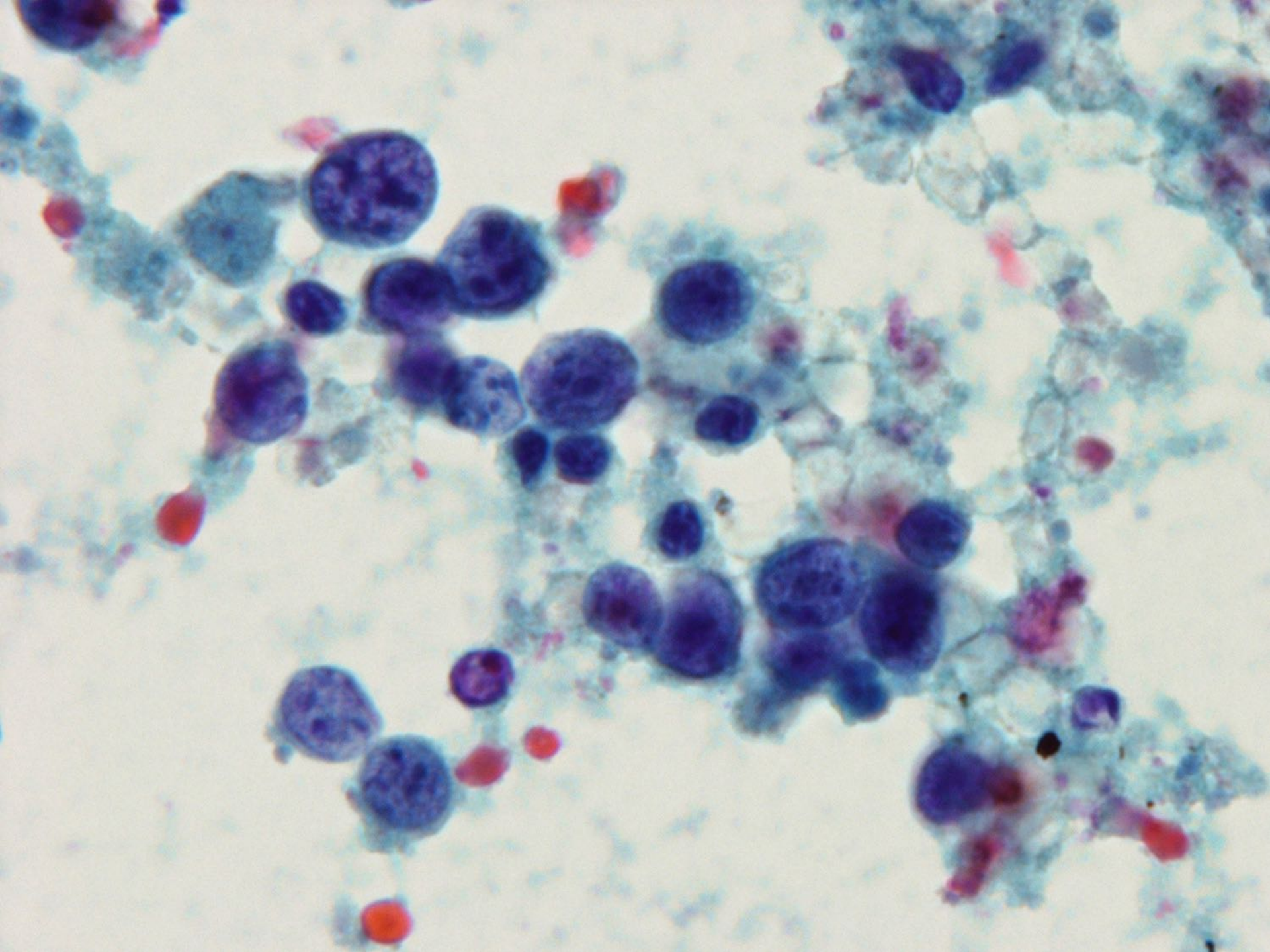


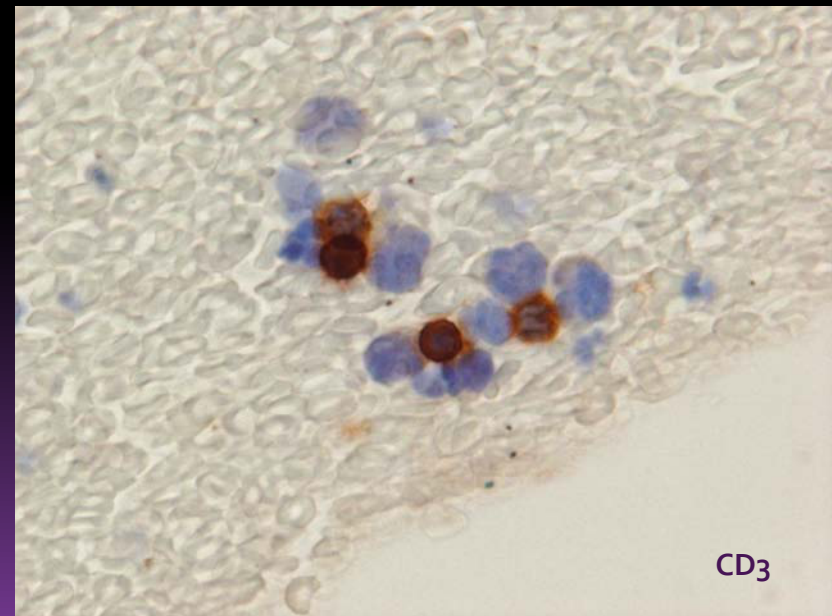
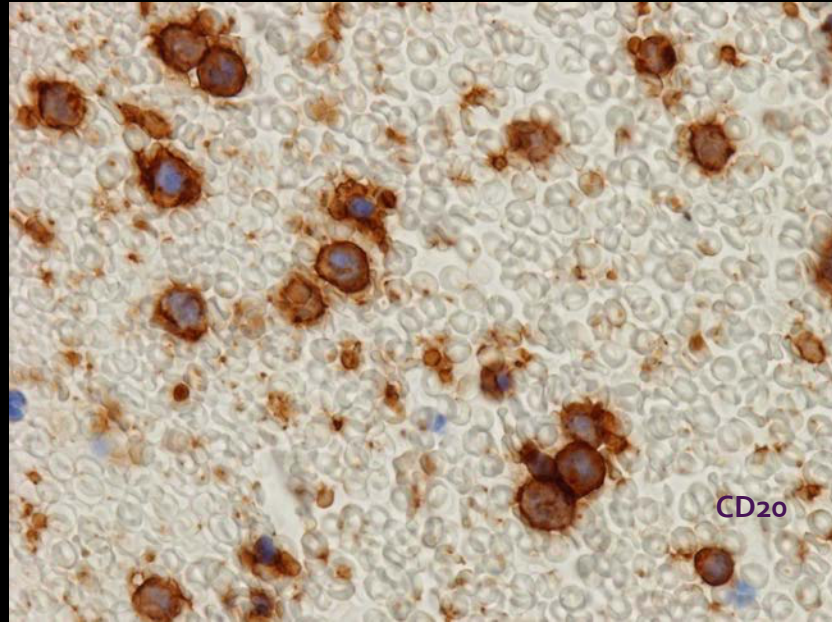
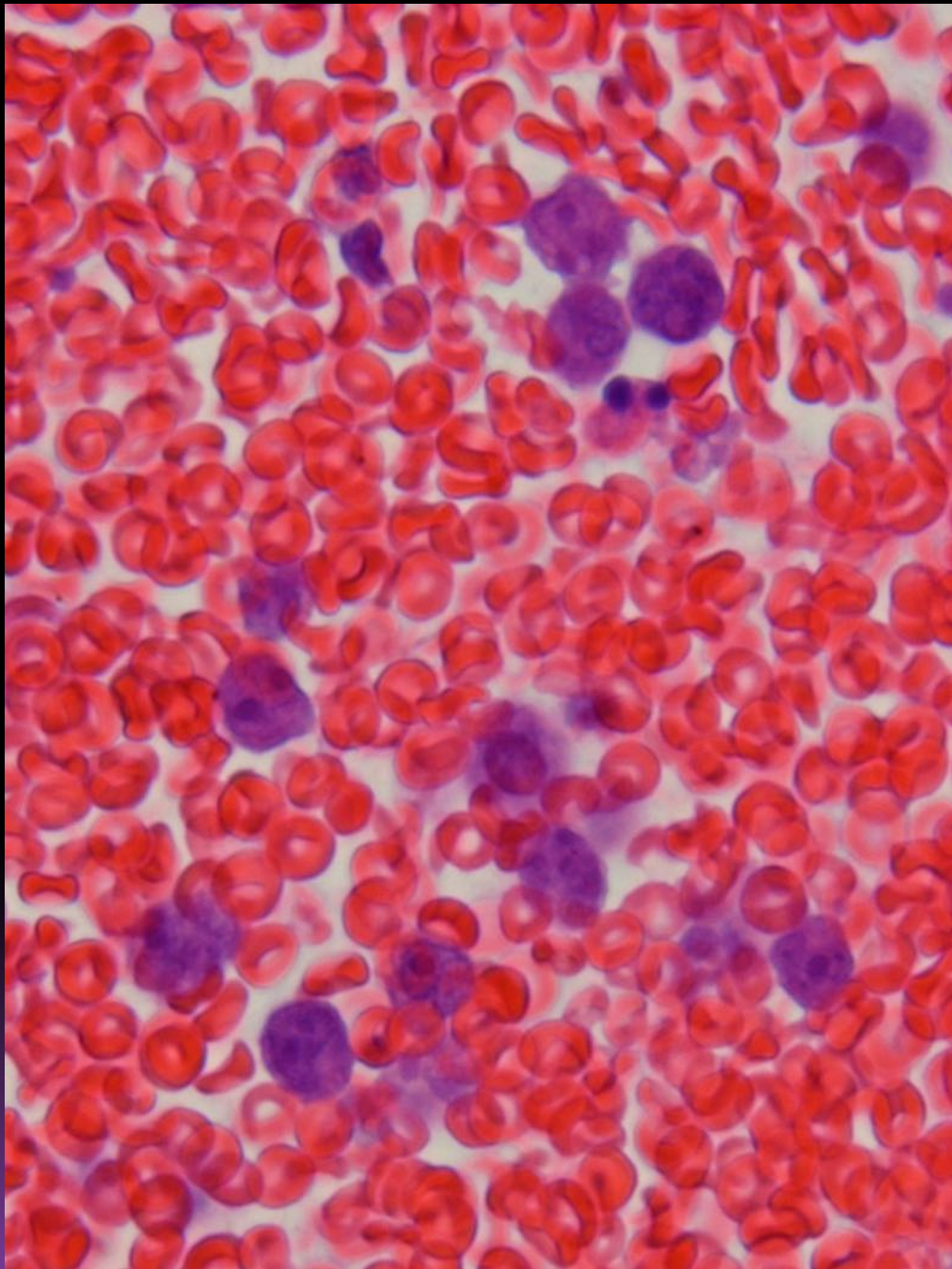
CONTRAST

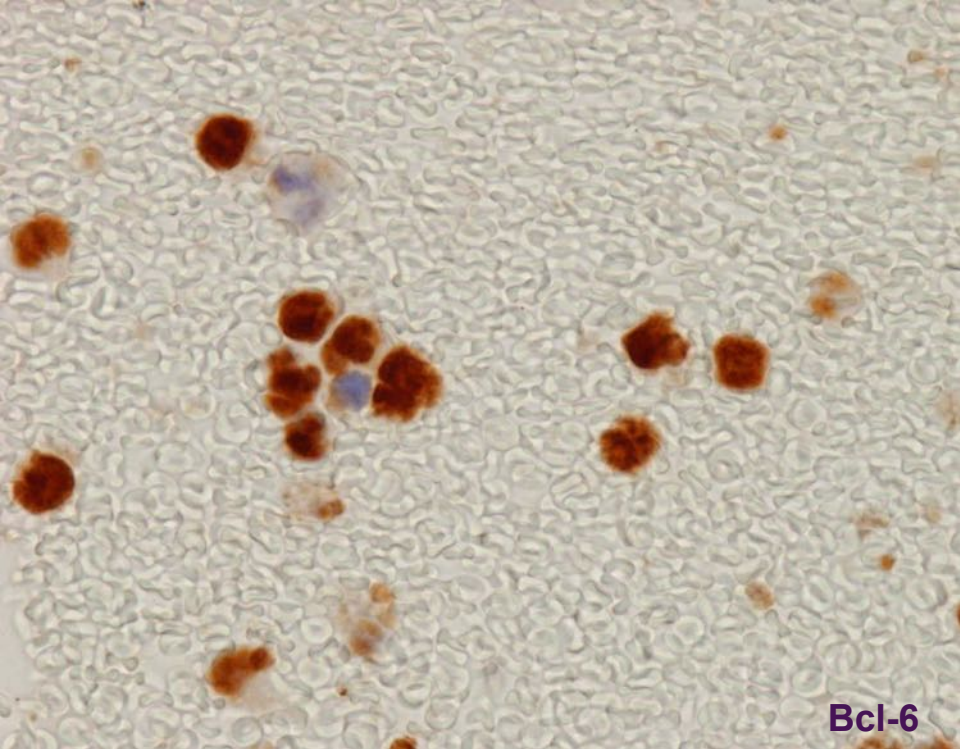
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C60
W400



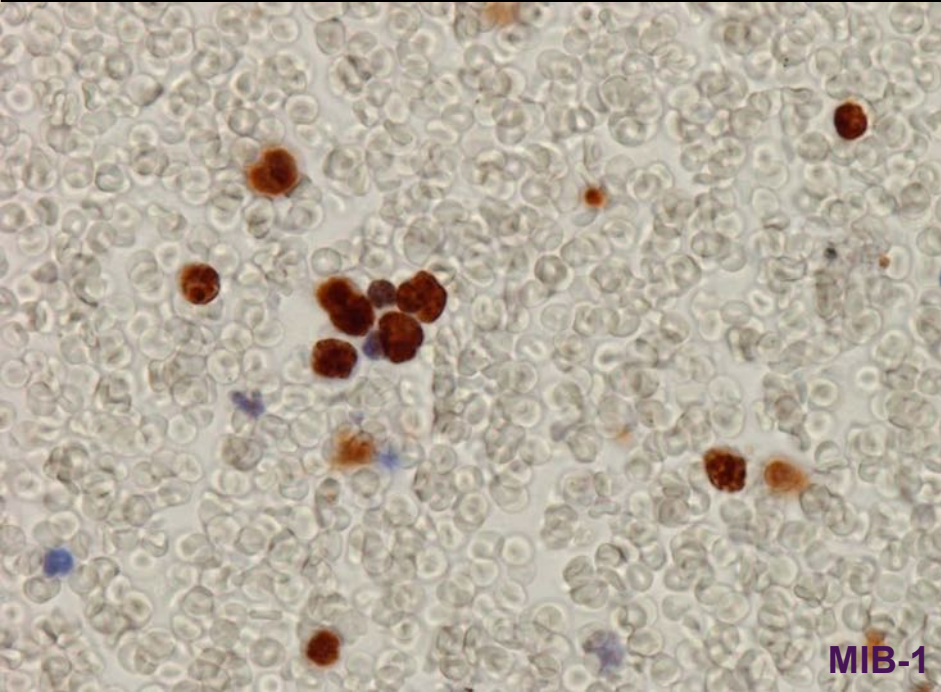
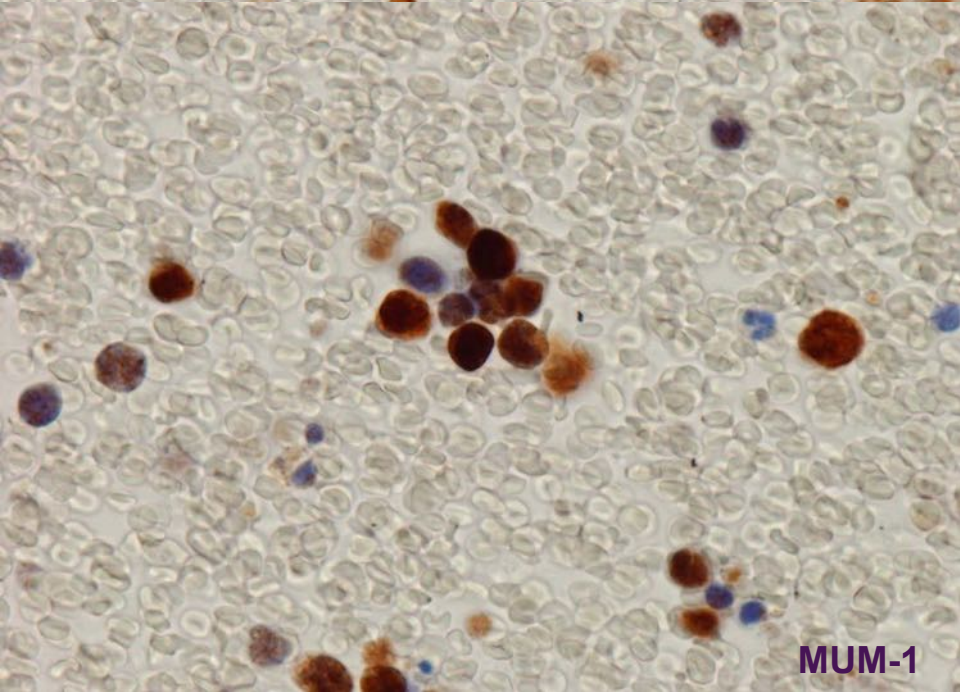






Antibody	
CD20	+
CD79a	+
Bcl-6	+
MUM-1	+
Bcl-2	+
p53	+

Antibody	
CD3	-
CD10	-
CD5	-
CD30	-
EBER	-
MIB-1	90%

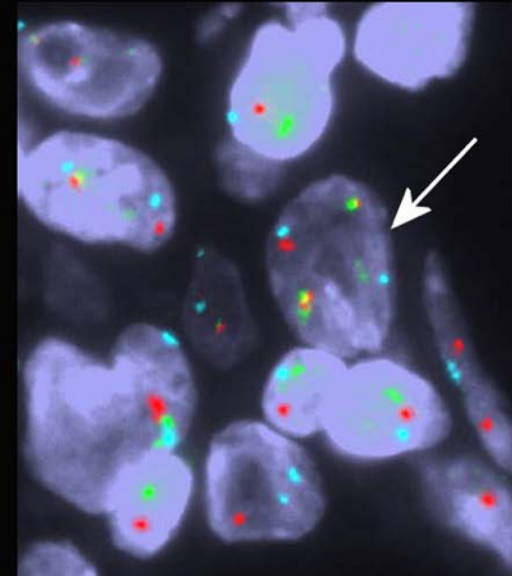
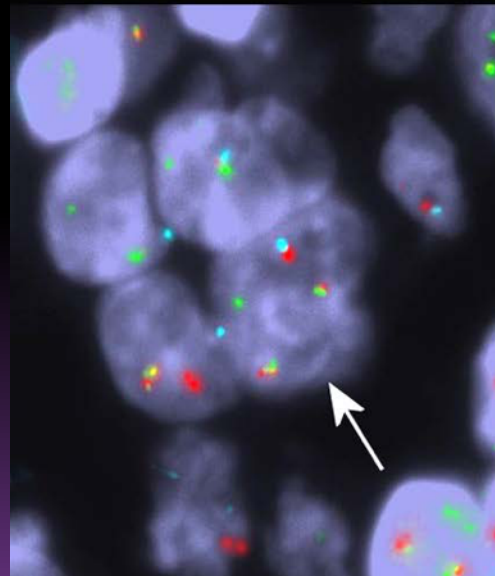
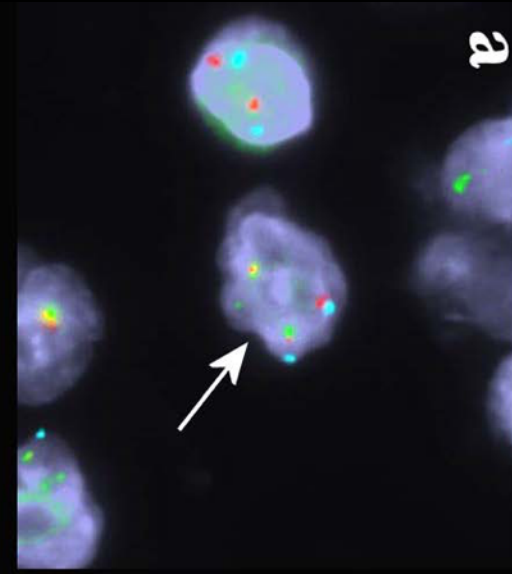
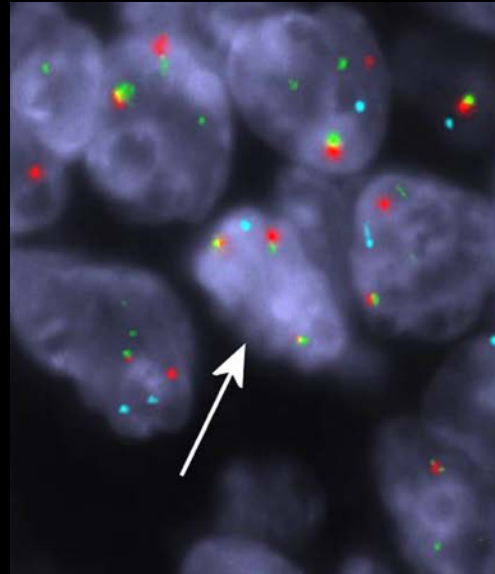


FISH

☐ MYC (+)

☐ IGH/BCL-2 negative

☐ BCL6 not rearranged



diagnosis



Non-Hodgkin lymphoma
Diffuse large B-cell lymphoma
Activated B-cell type
MYC(+)

There was still tissue left in the block if one wished to send for the REMODEL trial

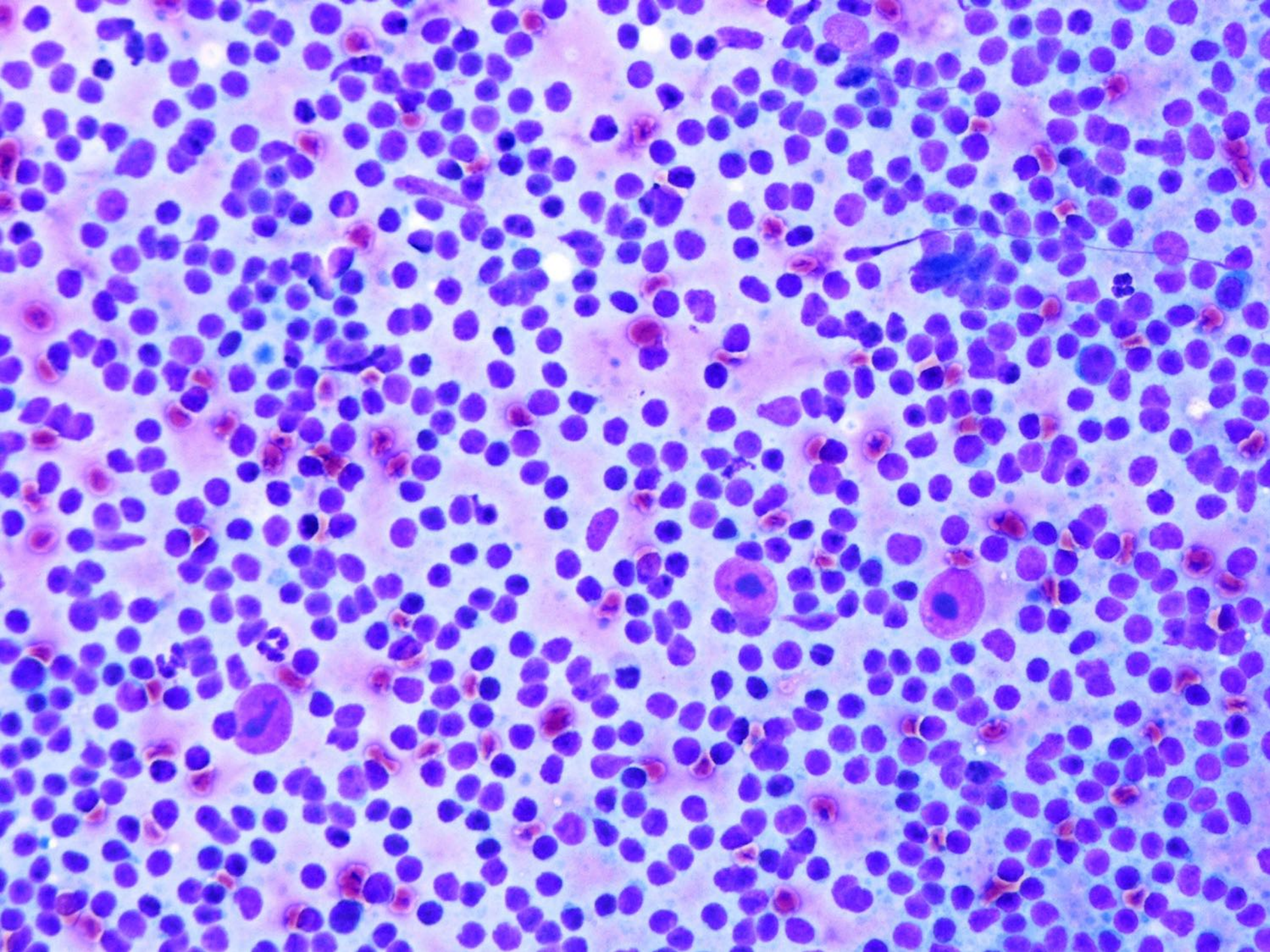
64 ♂, Fever, itching. Mediastinal mass. Subsequently, post diagnosis developed cervical masses.

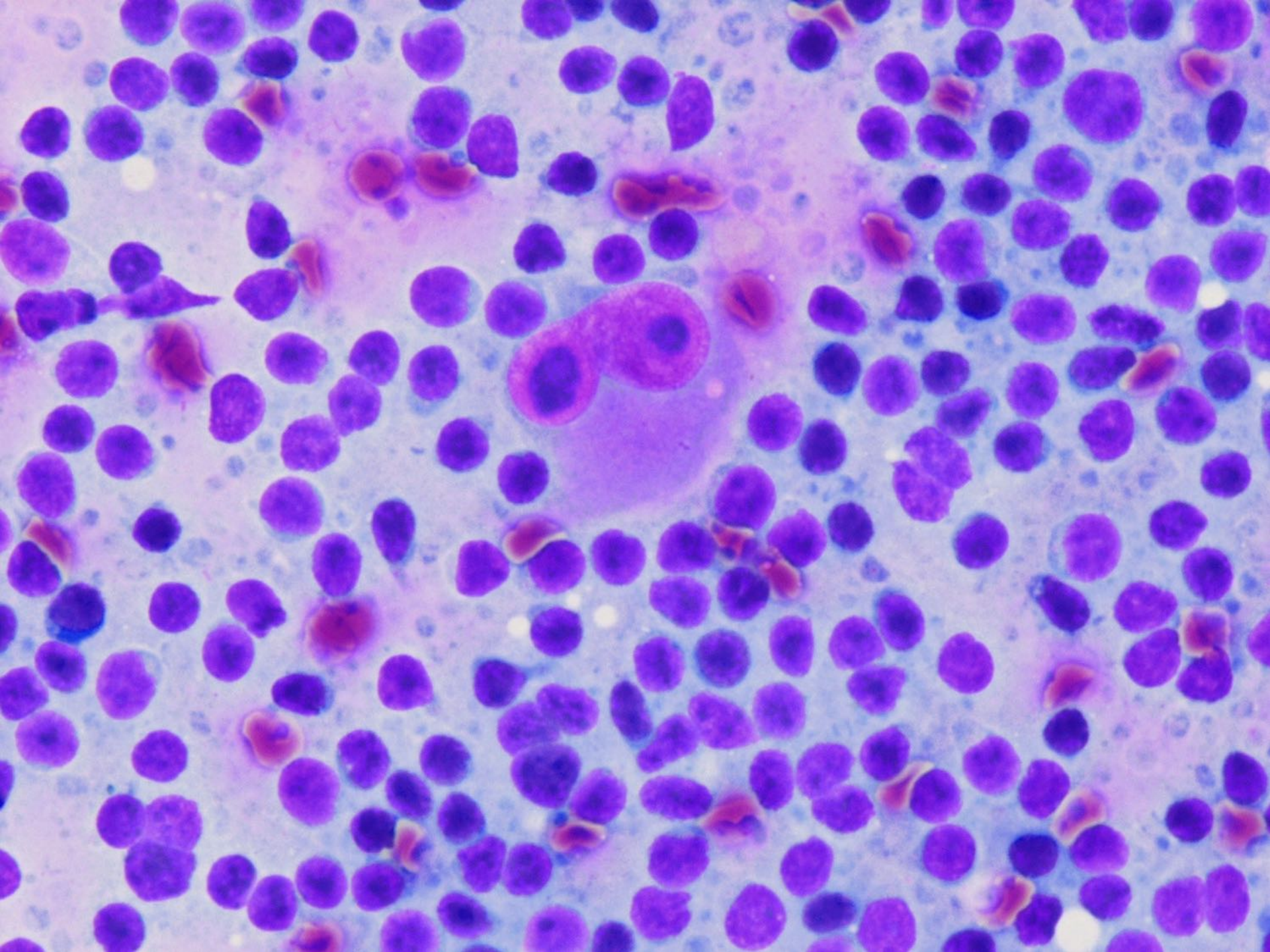
Se:2
Im:96

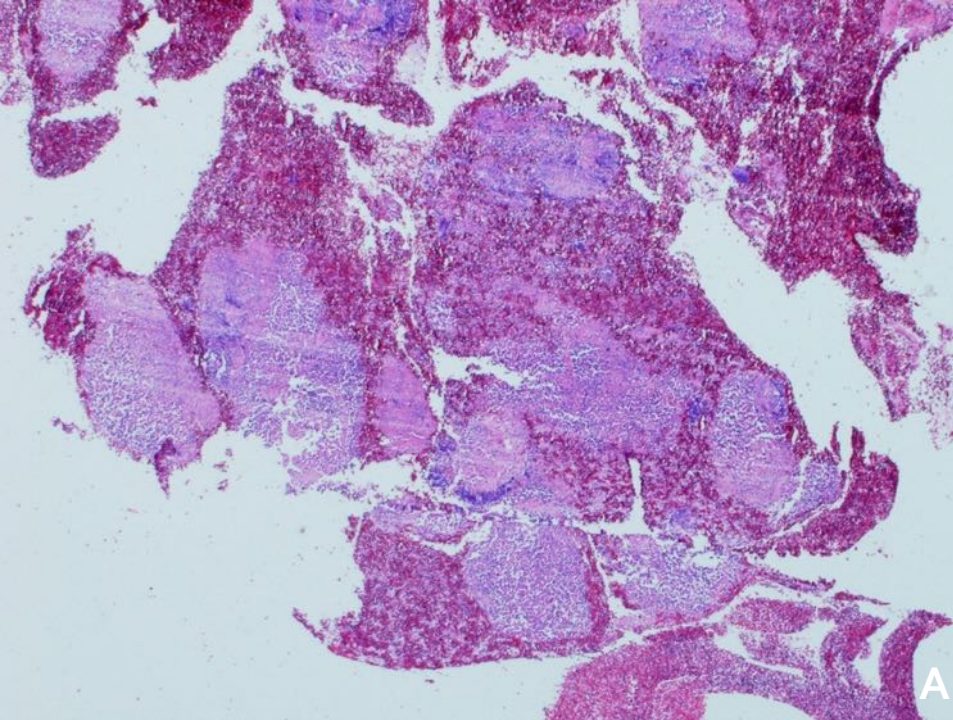
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Study Date:29/01/2010
Study Time:10:50:00
MRN:

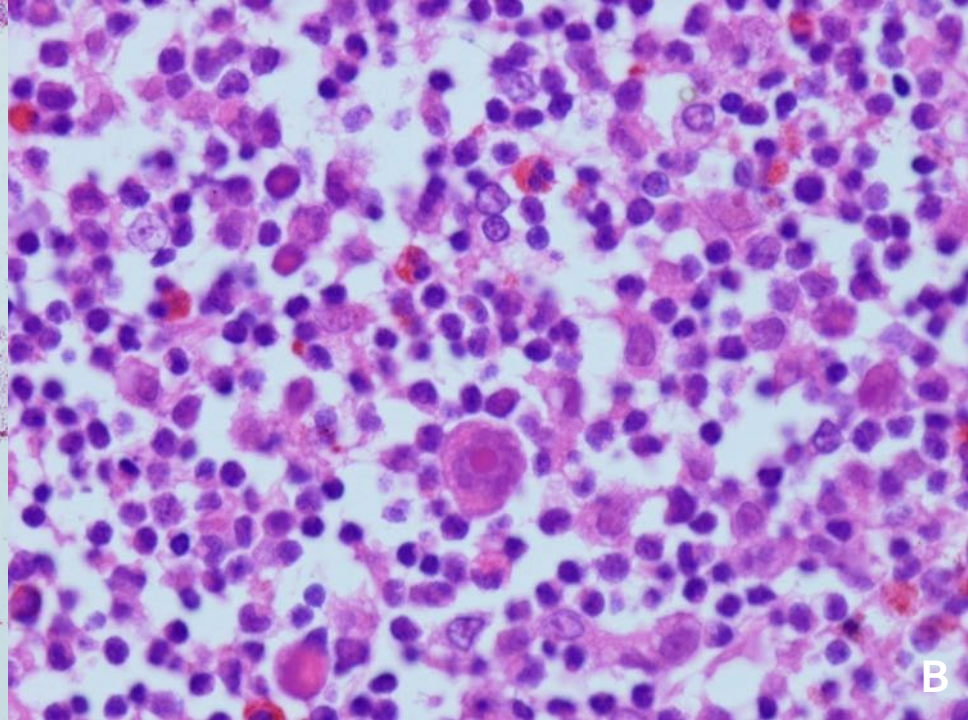




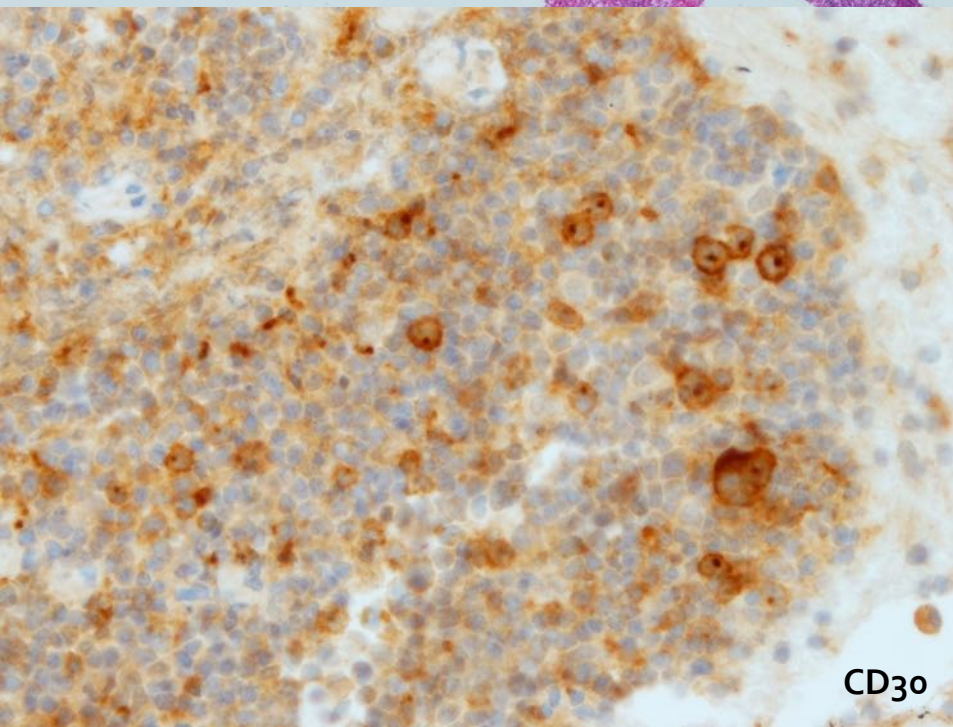




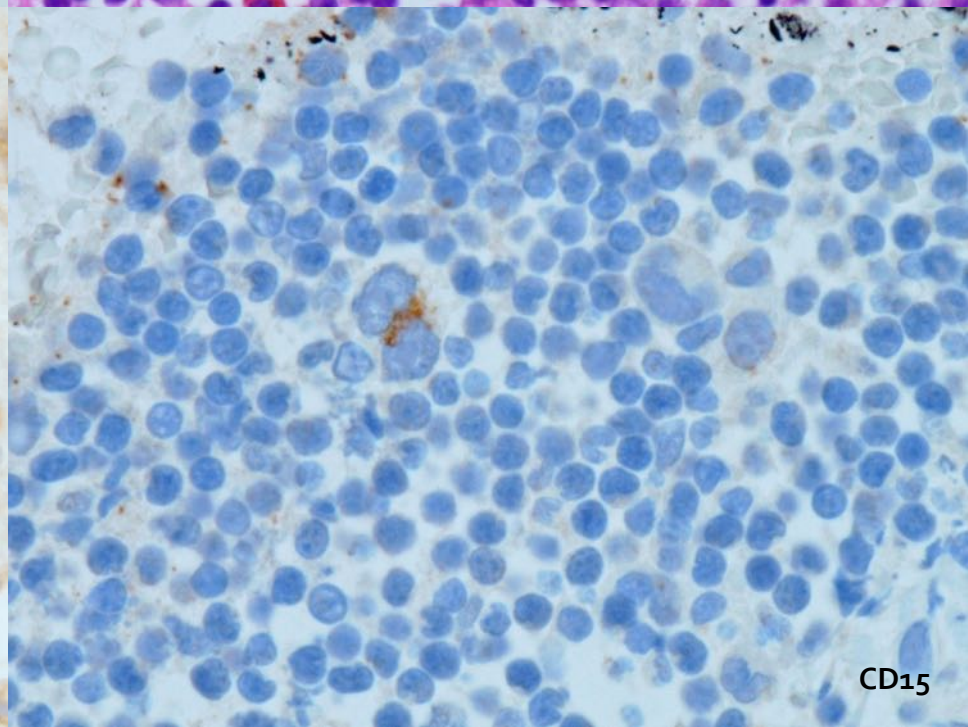
A



B



CD30

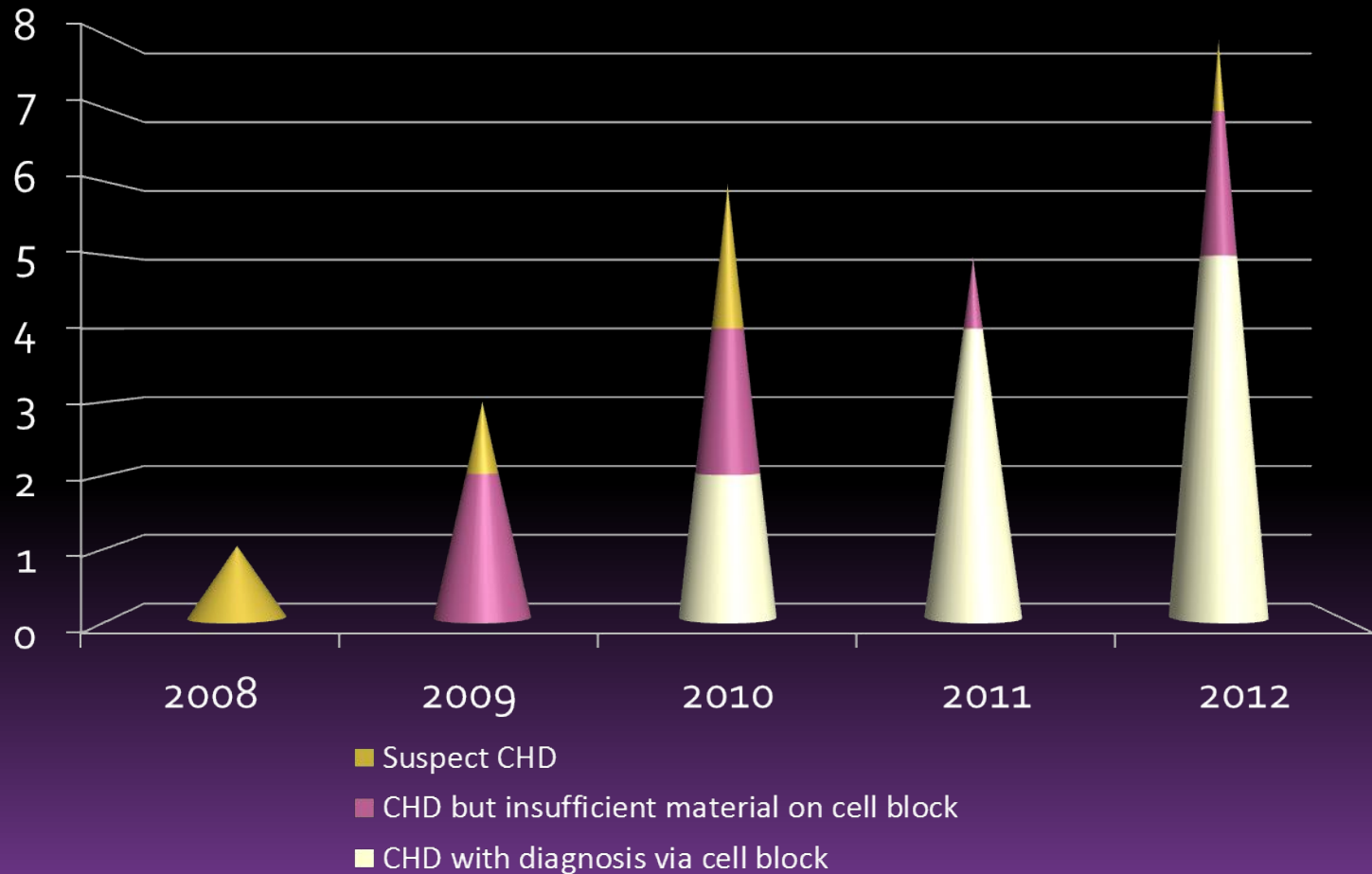


CD15

CHD diagnosed on cell blocks (11/21)

Age / Sex	EBUS site	Cell block	LCA	CD30	CD15	MUM-1	EBER	CD20	CD3
65 / F	4R, 7, 10R	Yes	-	+	+	+	+	-	-
38 / F	4R	Yes	-	+	+	+	+	-	-
39 / F	4R, 7	Yes	-	+	-	+	-	-	-
50 / M	4R	Yes	-	+	+	-	-	-	-
24 / F	4R	Yes	-	+	-	+	+	-	-
77 / M	4R, 7	Yes	-	+	+	+	+	-	-
41 / M	4l, 10	Yes	-	+	-	+	+	-	-
32 / F	4R, 4L	Yes	-	+	+	-	-	-	-
45 / F	2R, 4R	Yes	-	+	-	+	-	-	-
24 / F	4R	Yes	-	+	-	+	-	-	-
51 / F	4R+2R, 4L	Yes	-	+	+	+	+	-	-

Confidence of CHD diagnosis with development of service



How are we able to get enough out?

Single Use Aspiration Needle
 Disposable 吸引生検針 NA-201SX-4022

ViziShot Model No. **NA-201SX-4022** **OLYMPUS**

Working Length 有効長 **700mm** Minimum Channel Size 適用チャンネル径 **φ2.0mm**

Specifications 仕様

Needle Width 針径 **22G**
 Echo enhanced region 超音波反射部
 Maximum Needle Length 最大針突出長 **40mm**

This Product Contains No Natural Rubber Latex. 最大挿入部径 **φ1.8mm**

Quantity 数量 **1** Use by (Exp. date) 使用期限 **2016.11**

Single use only 再使用禁止
 Do not resterilize 再滅菌禁止

Refer to instructions. 取扱説明書参照

USA: CAUTION: Federal law restricts this device to sale by or on the order of a physician.

WARNING 警告

STORAGE 保管

Manufacturer **OLYMPUS MEDICAL SYSTEMS CORP.**
 2981 Milliken-chu, Hachioji-shi, Tokyo 192-8507, JAPAN

販売元 **オリンパス・メディカルシステムズ株式会社**
 100-8507 東京都八王子市東山町2-1-1



ViziShot Model No. **NA-201SX-4021** **OLYMPUS**

Working Length 有効長 **700mm** Minimum Channel Size 適用チャンネル径 **φ2.0mm**

Specifications 仕様

Needle Width 針径 **21G**
 Echo enhanced region 超音波反射部
 Maximum Needle Length 最大針突出長 **40mm**

This Product Contains No Natural Rubber Latex. 最大挿入部径 **φ1.9mm**

Quantity 数量 **1** Use by (Exp. date) 使用期限 **2016.07**

Single use only 再使用禁止
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Refer to instructions. 取扱説明書参照

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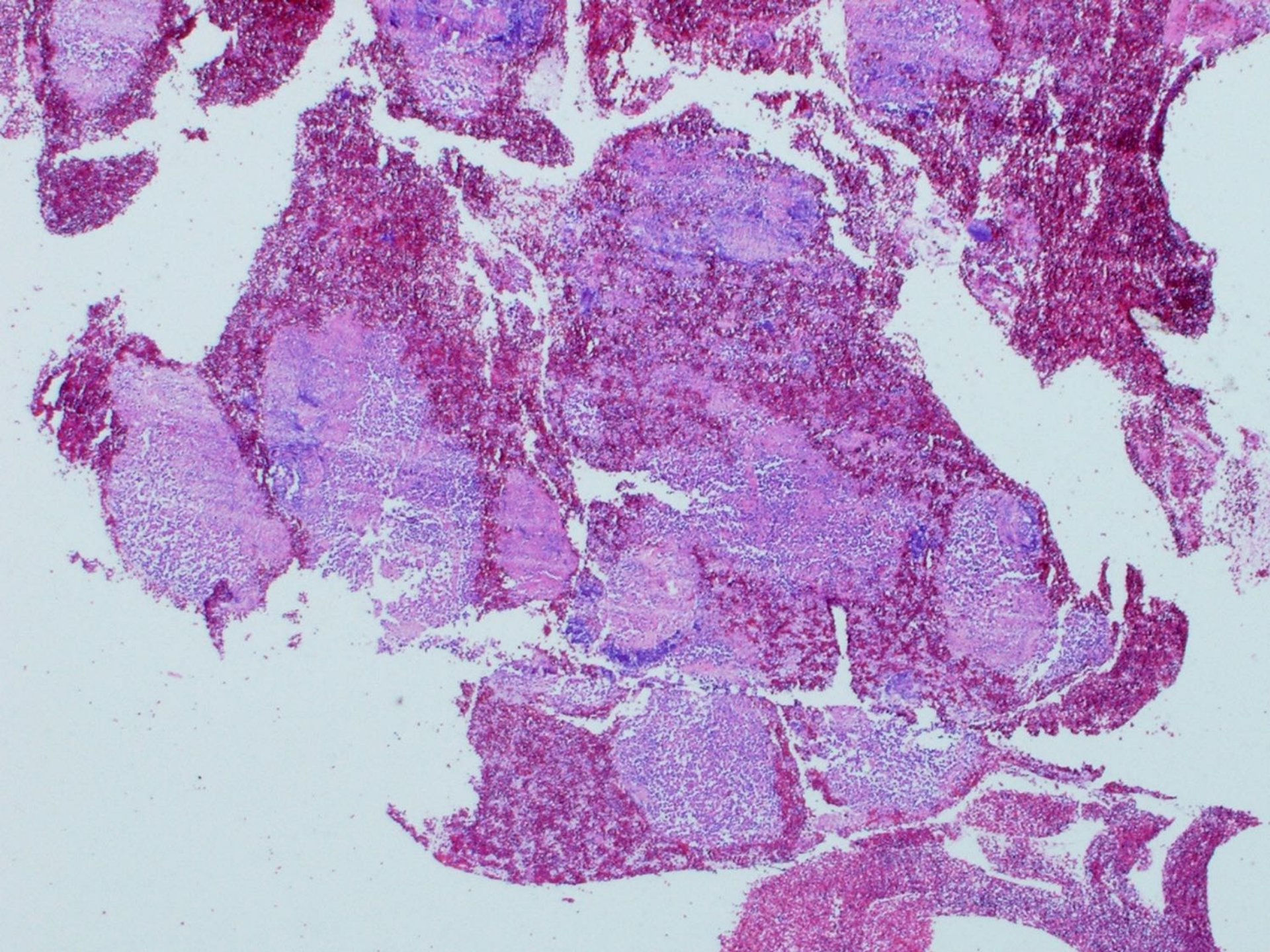
WARNING 警告

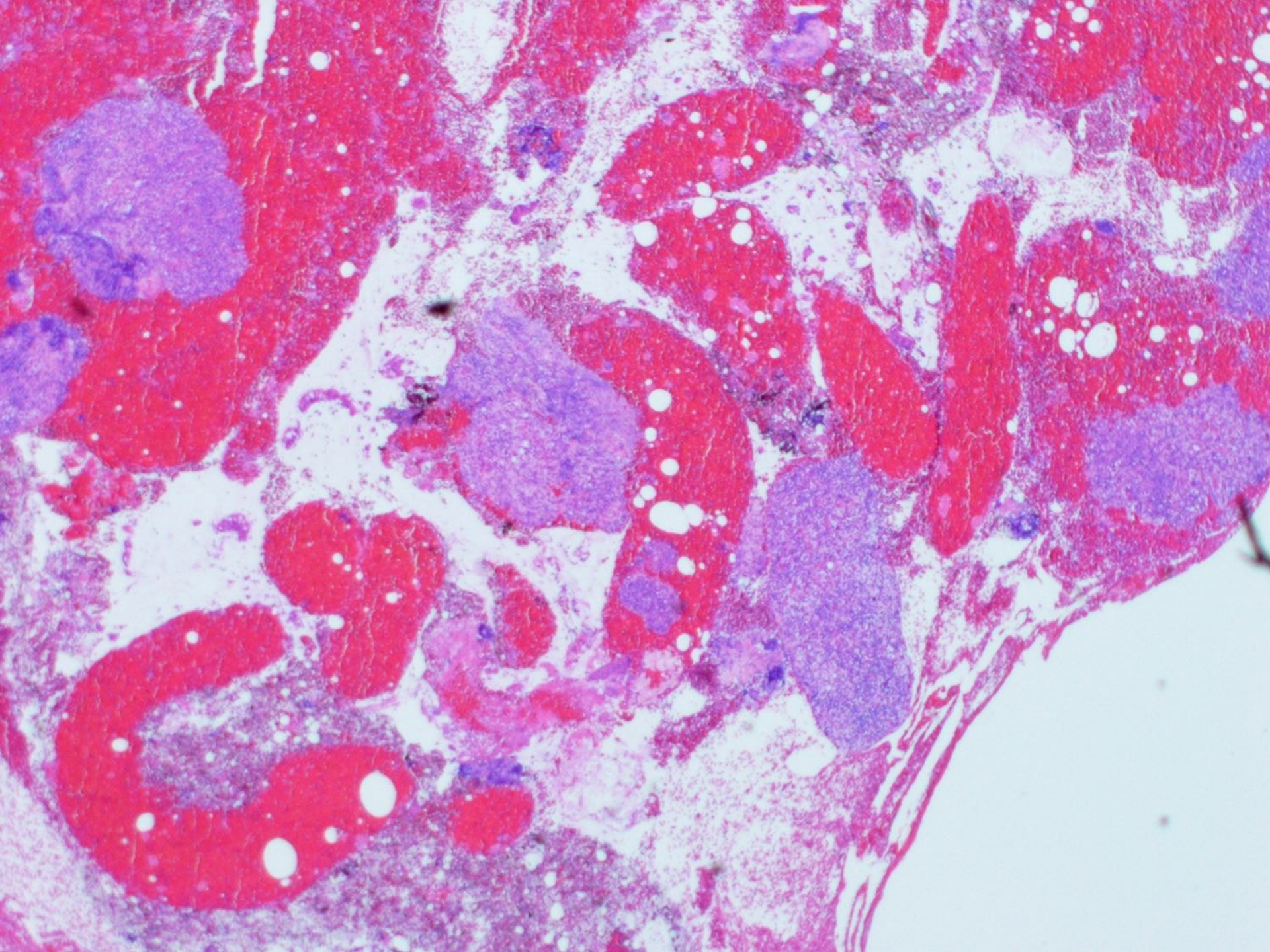
STORAGE 保管

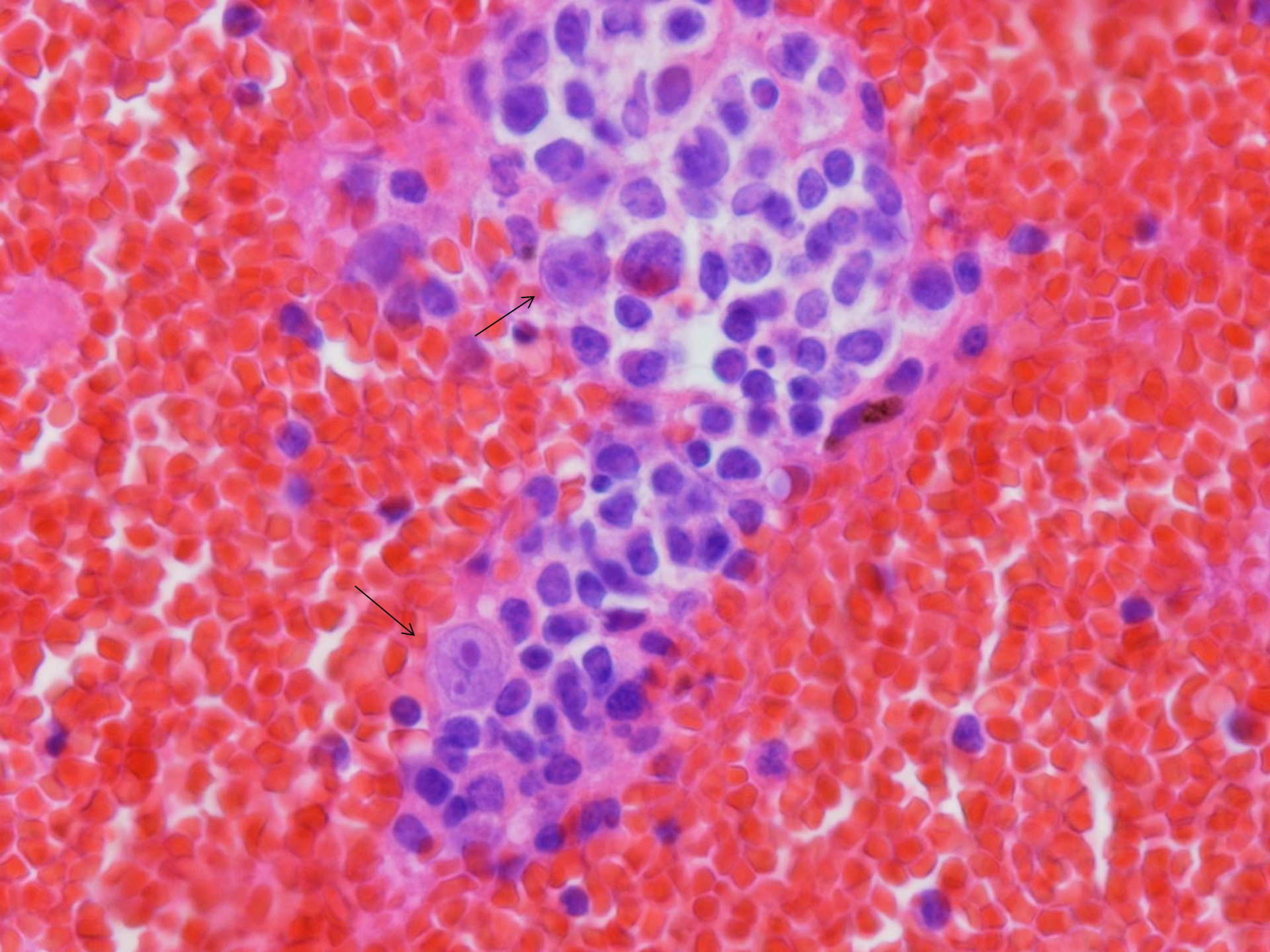
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 100-8507 東京都八王子市東山町2-1-1

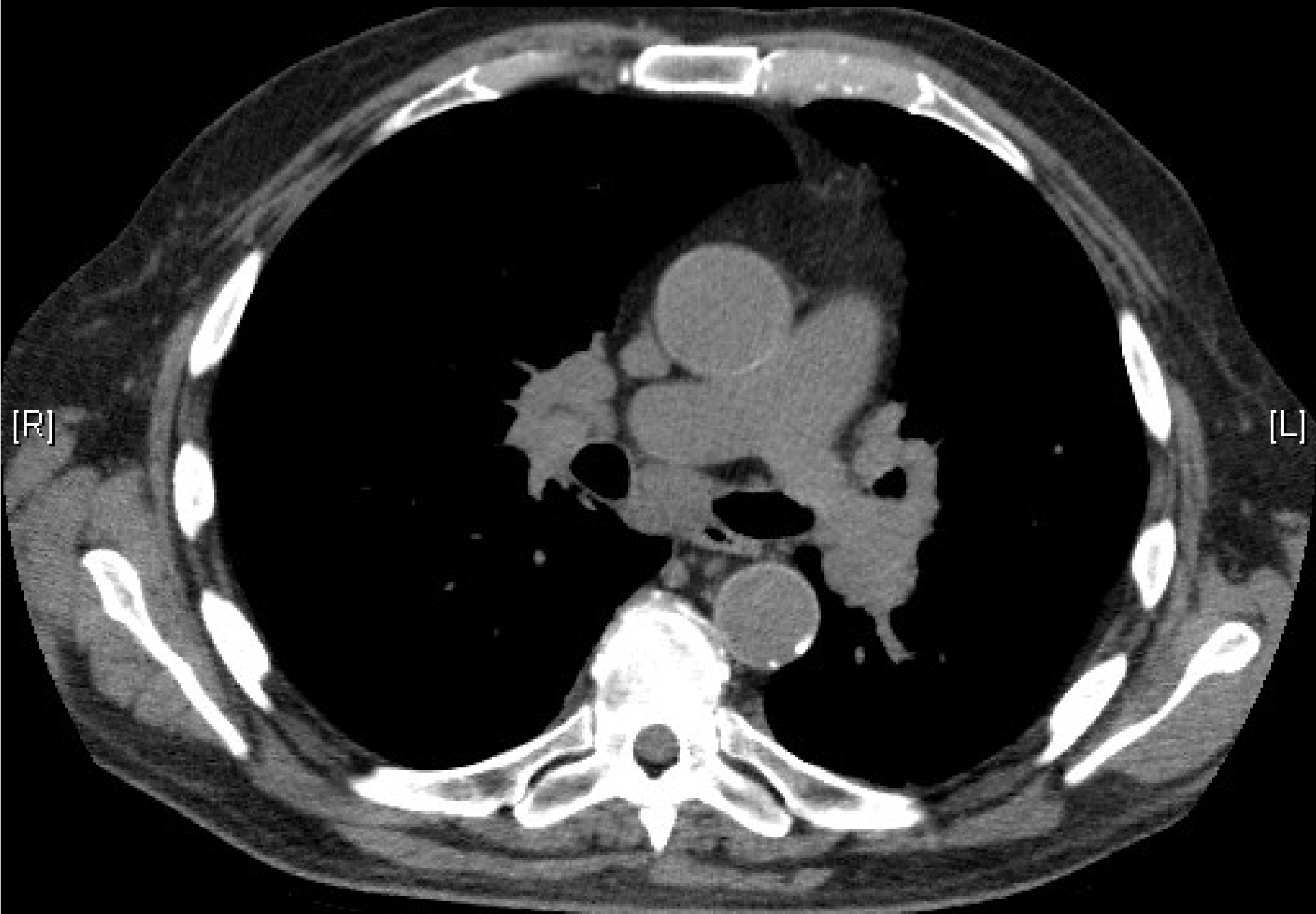


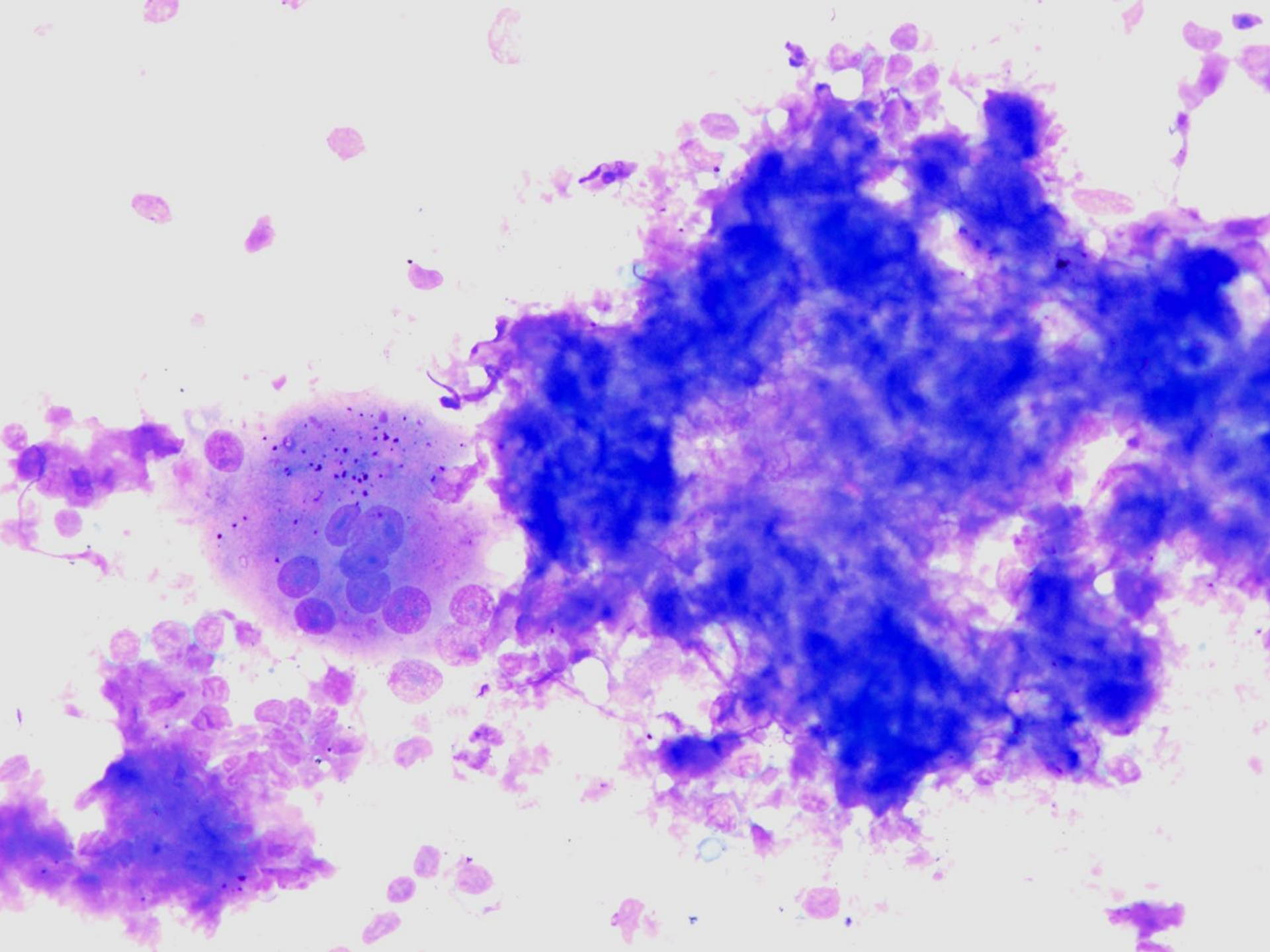


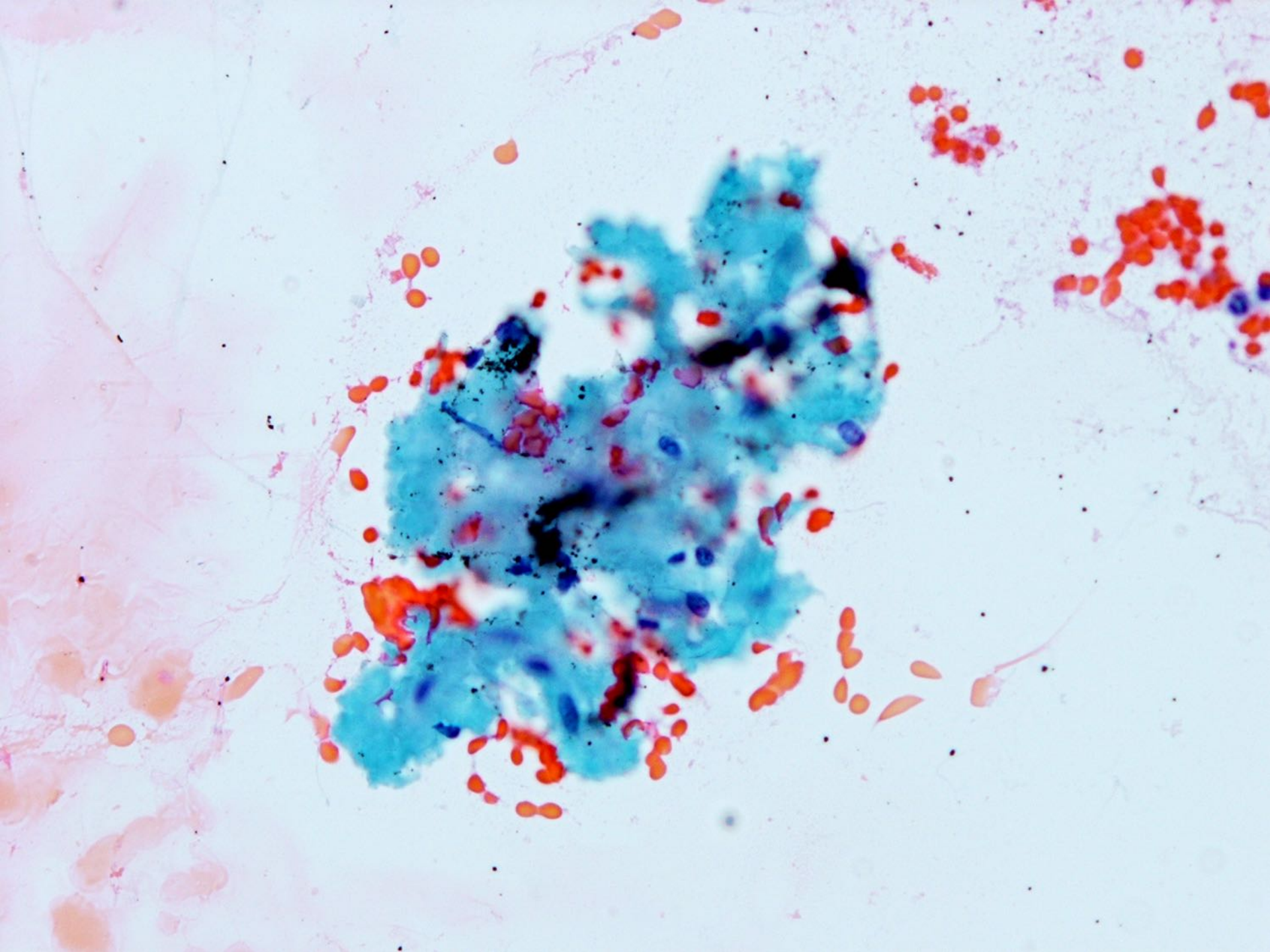


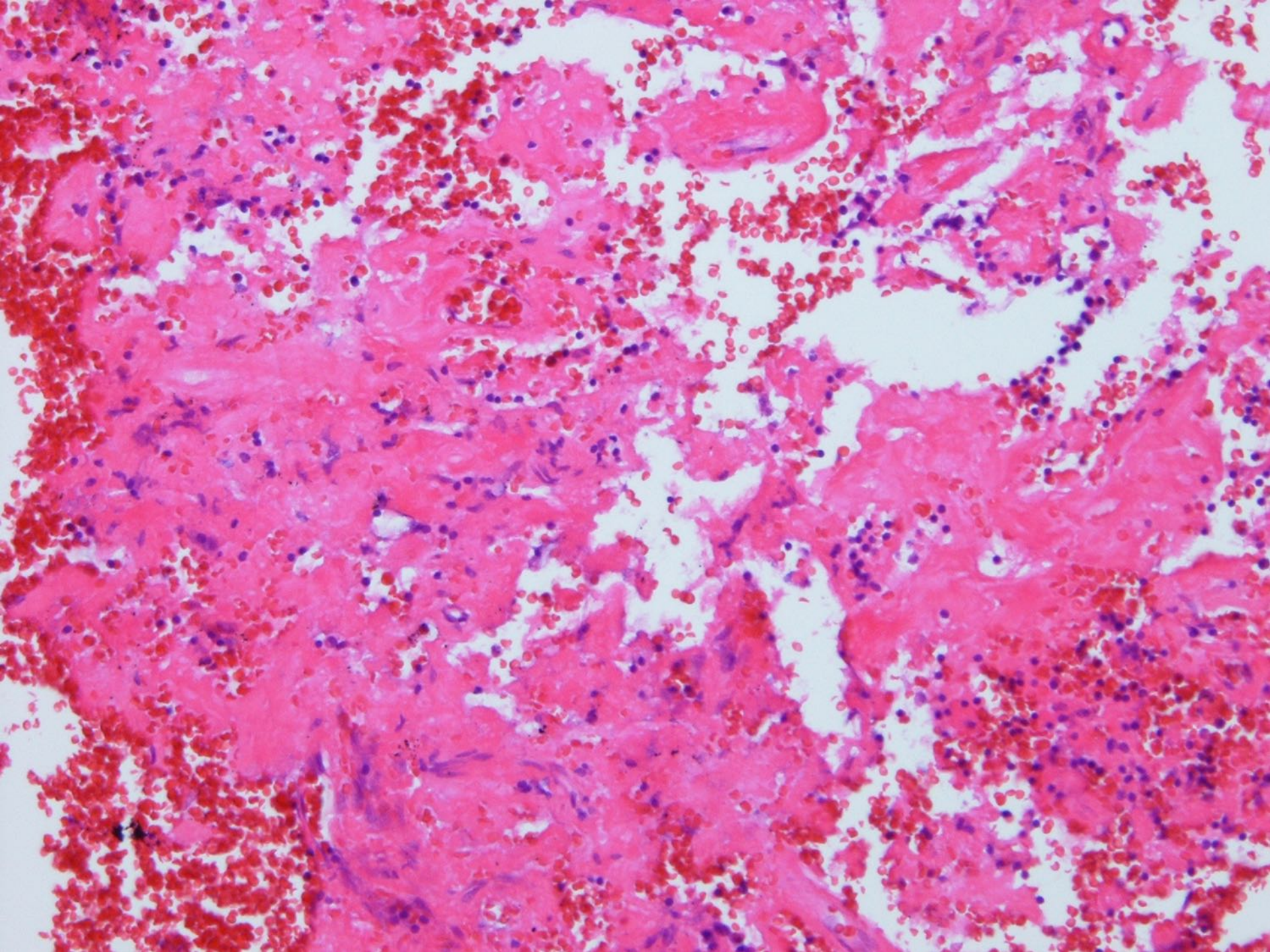


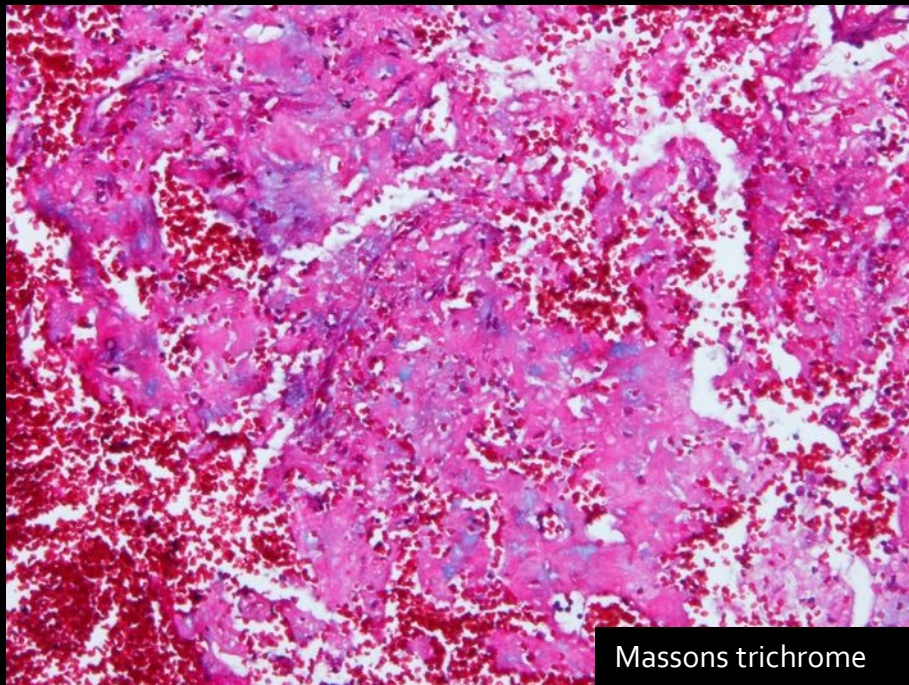
73 M, mediastinal lymphadenopathy, small volume cervical lymphadenopathy



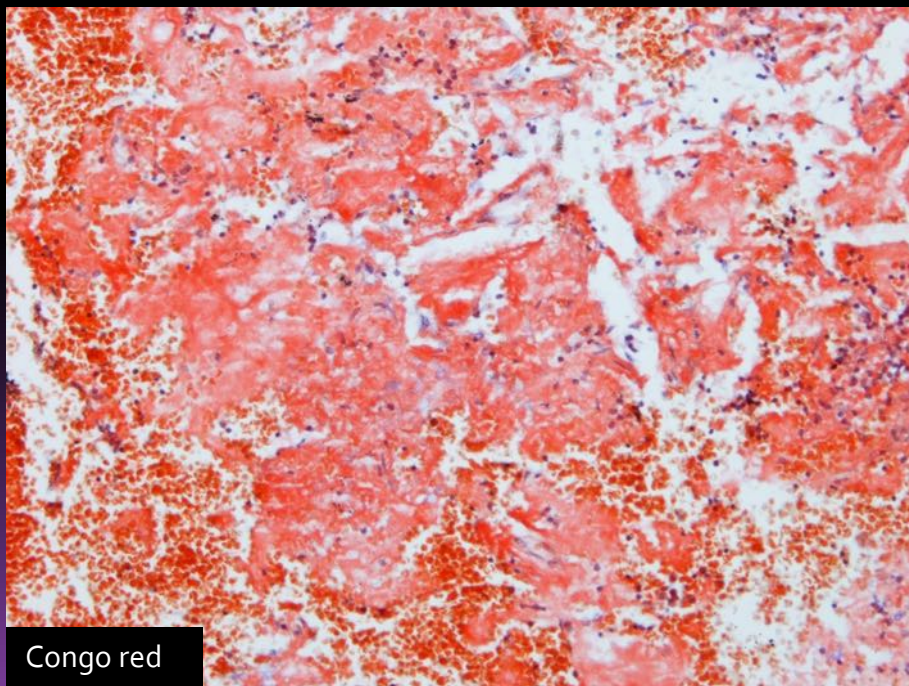




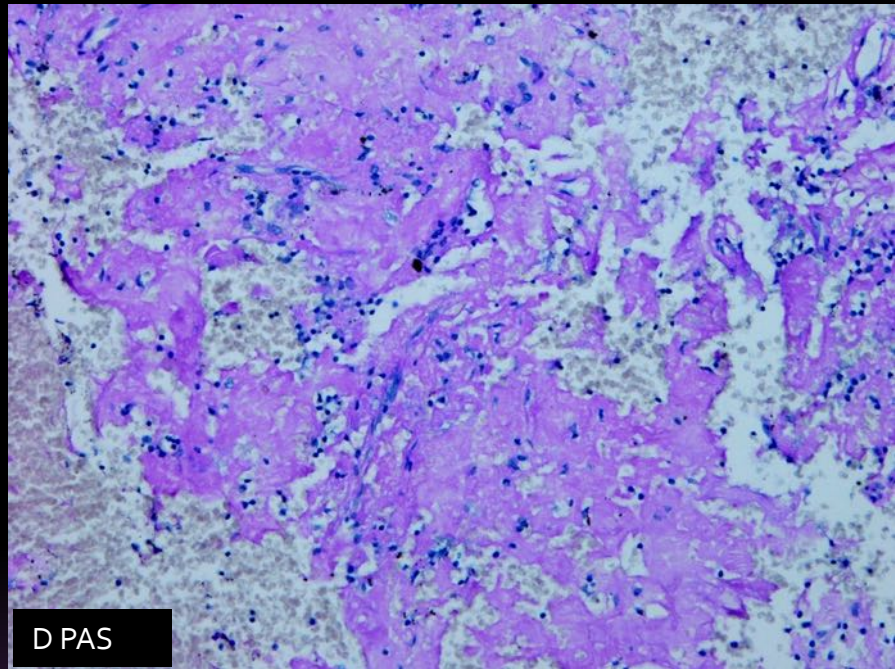




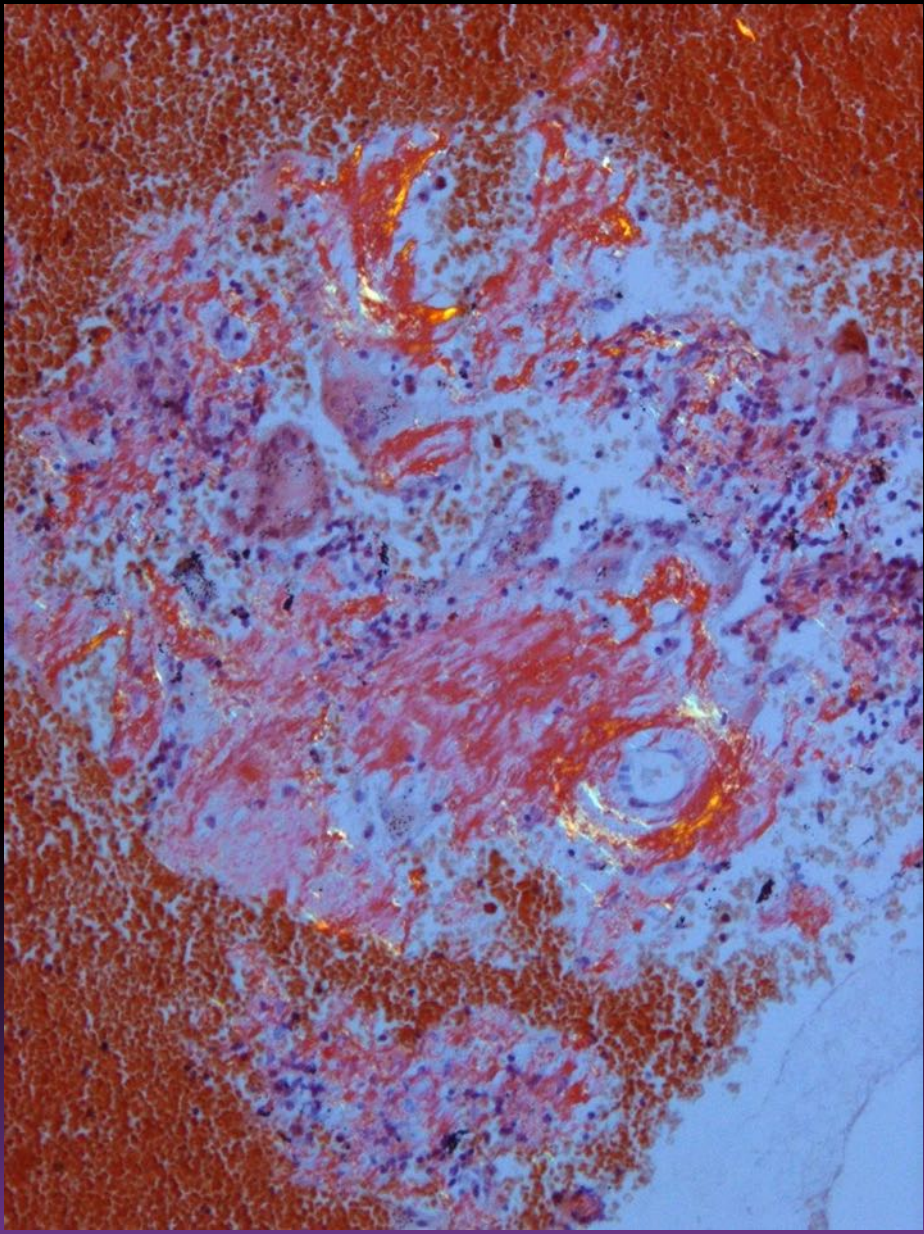
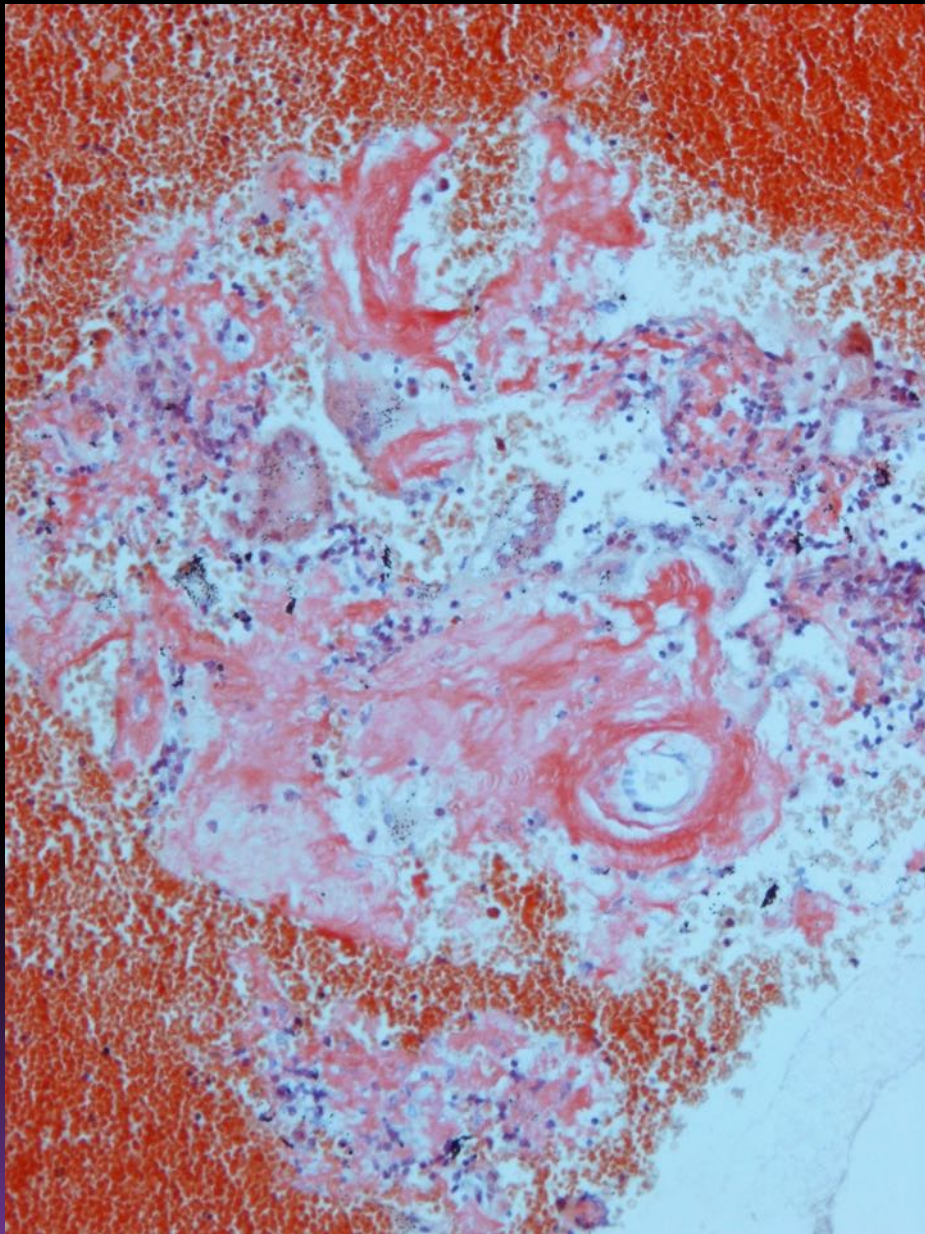
Massons trichrome



Congo red



D PAS



Negative IHC for Amyloid AA, transthyretin, kappa & lambda

Lymph node amyloidosis (amyloid of non-AA type)

Is this diagnosis reliable?

How accurate is lymphoma diagnosis on EBUS?

	Year	Nos	Sensitivity	Specificity	PPV	NPV
Moonim et al	2013	93	89	97	98	85
Marshall	2011	33	72	95		
Steinfort	2010	55	57	100		
Kennedy	2008	25	90.9	100		

Moonim MT et al. Diagnosis and subtyping of de novo and relapsed mediastinal lymphomas by endobronchial ultrasound needle aspiration. *Am J Respir Crit Care Med* 2013; 188: 216-223

Navani N, Janes S. Endobronchial ultrasound guides transbronchial needle aspiration for lymphoma: The final frontier. *Am J Respir Crit Care Med* 2013; 188: 1183-85

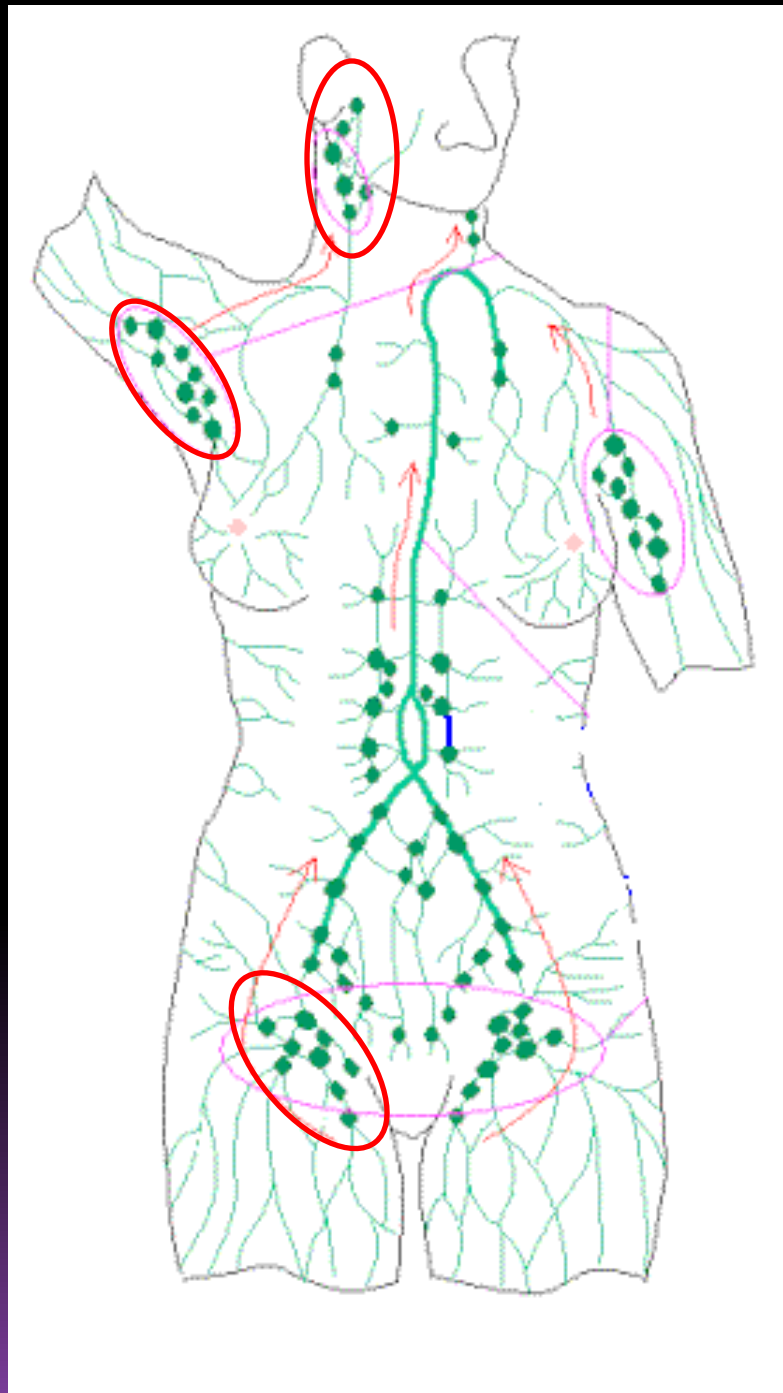
Comparison between EBUS-TBNA and final diagnoses

		Final diagnosis (n = 93)			
		High-grade NHL	Low-grade NHL	Hodgkin lymphoma	Non-lymphoma diagnosis
EBUS-TBNA diagnosis (n = 93)	High-grade B/T NHL (n=9)	9	0	0	0
	Probable high-grade NHL (n=1)	0	0	0	1
	Low-grade B-NHL (n=26)	0	26	0	0
	Hodgkin lymphoma (n=17)	0	0	17	0
	Probable Hodgkin lymphoma (n=6)	1	0	5	0
	Non- lymphoma diagnosis (n=32)	0	0	0	32
	Inadequate (n=2)	1	0	0	1

Comparison between EBUS-TBNA and final diagnoses

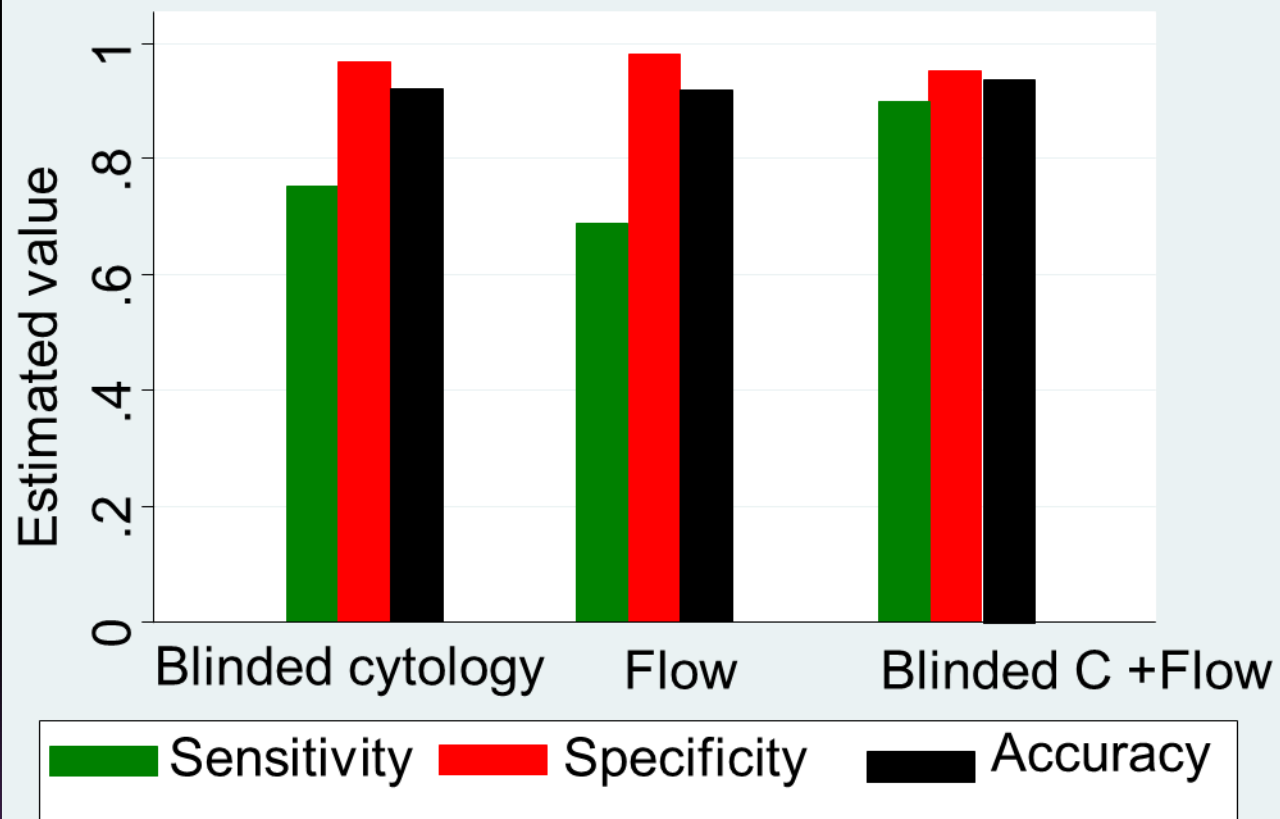
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EBUS-TBNA diagnosis (n = 93)	High-grade B/T NHL (n=9)	9	0	0	0
	Probable high-grade NHL (n=1)	0	0	0	1
	Low-grade B-NHL (n=26)	0	26	0	0
	Hodgkin lymphoma (n=17)	0	0	17	0
	Probable Hodgkin lymphoma (n=6)	1	0	5	0
	Non- lymphoma diagnosis (n=32)	0	0	0	32
	Inadequate (n=2)	1	0	0	1

Superficial lymph node / mass sampling



Do we need to biopsy every lump?

Sensitivity, Specificity & Accuracy - All Lesions



Flow cytometry of 'normal / reactive' lymph nodes

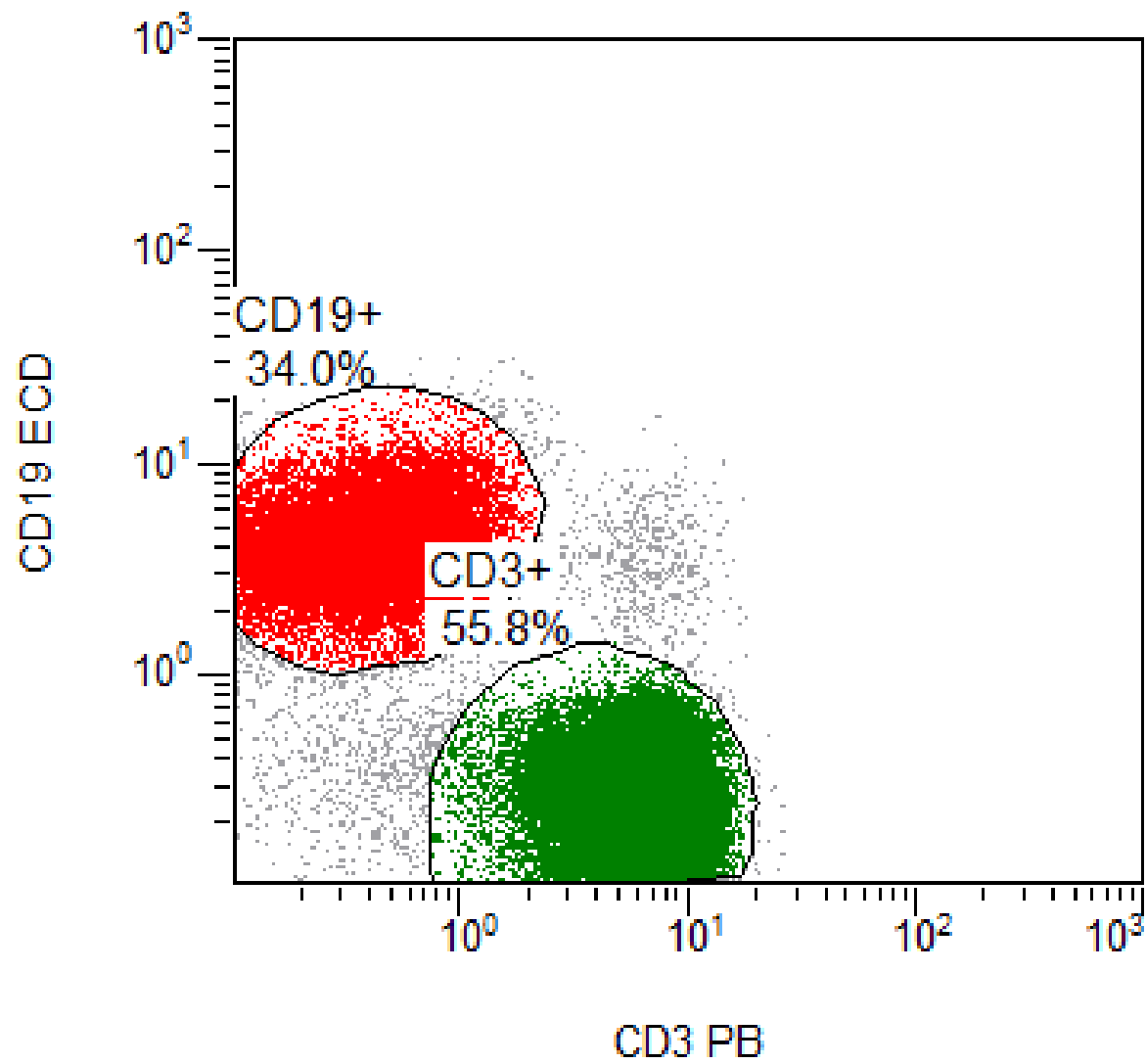
- Aspirates collected in normal saline.
- Add fetal calf serum for cell preservation
- Commercial flow cytometry transport media now available

- 4 colour vs 8 colour vs 10 colour

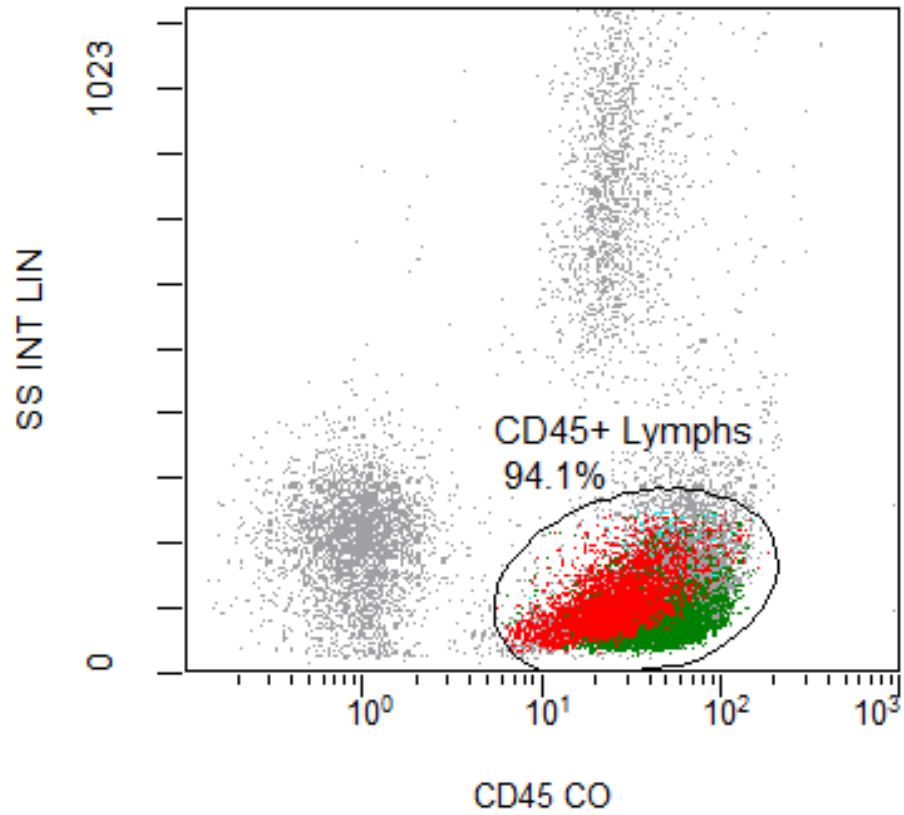
GSTT – FCM FNA panel

- 10 colour
- 2 tube
- 500 – 600 / year

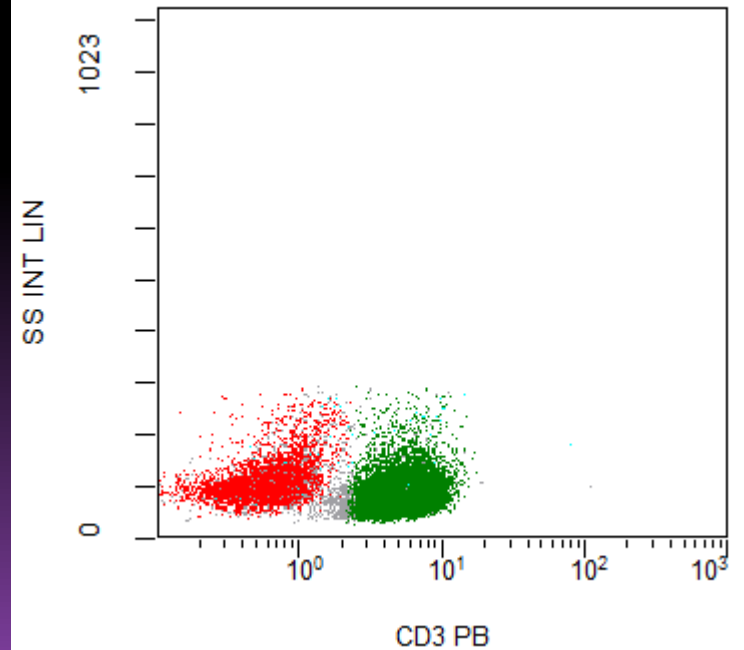
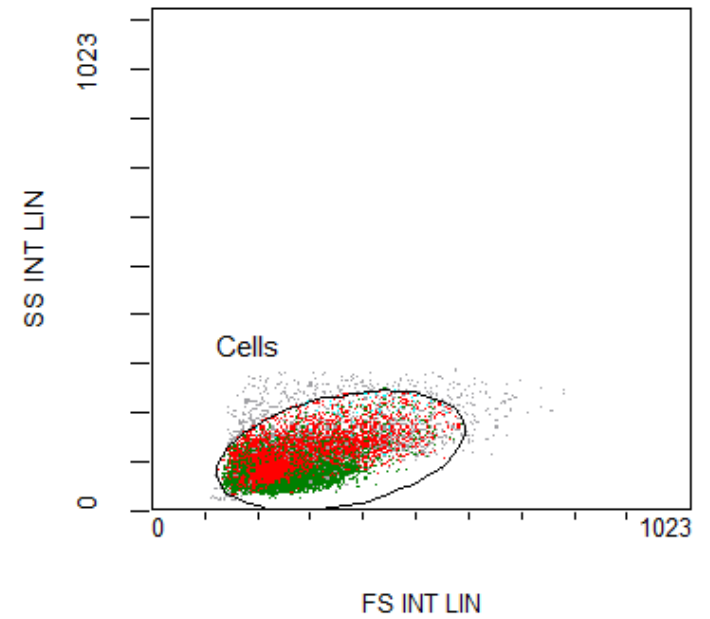
	CO	ECD	APC750	PB	PC5.5	PC7	APC	APC700	PE	FITC
Tube 1	CD45	CD19	CD20	CD3	CD5	CD7	CD4	CD8	CD14	CD43
		B-cell			T-cell					
Tube 2	CD45	CD19	CD20	FMC7	CD5	CD10	CD23		Lambda	Kappa
		B-cell			B-cell NHL				Light chains	



[Ungated] FL10 INT LOG/SS INT LIN



(20000) [CD45+ Lymphs] FS INT LIN/SS INT LIN



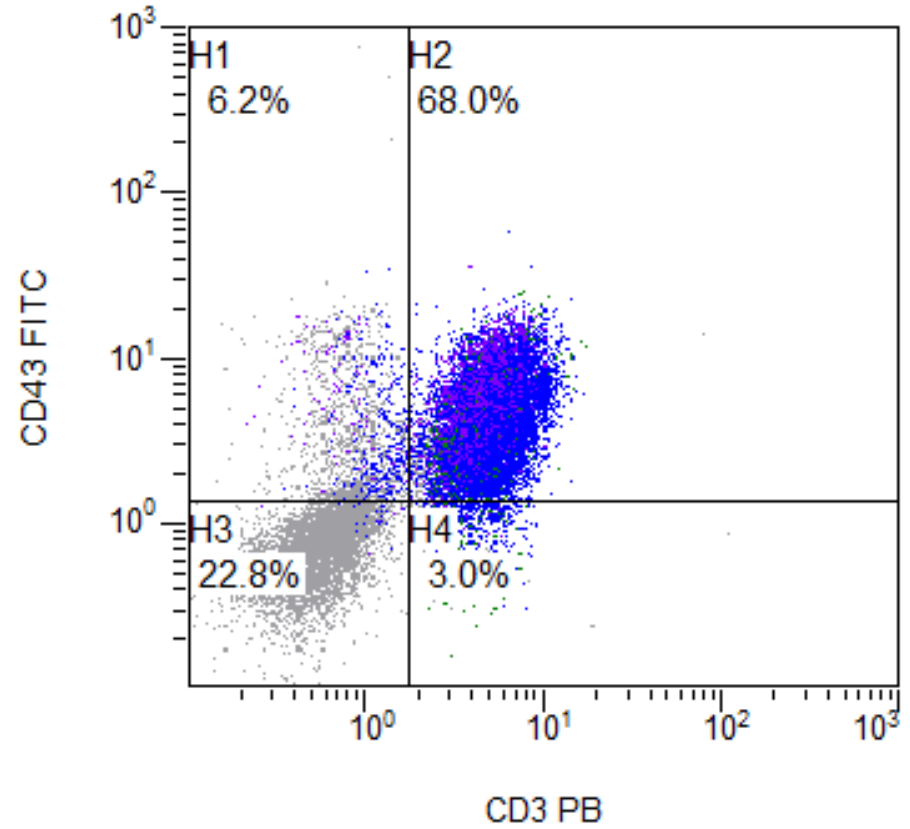
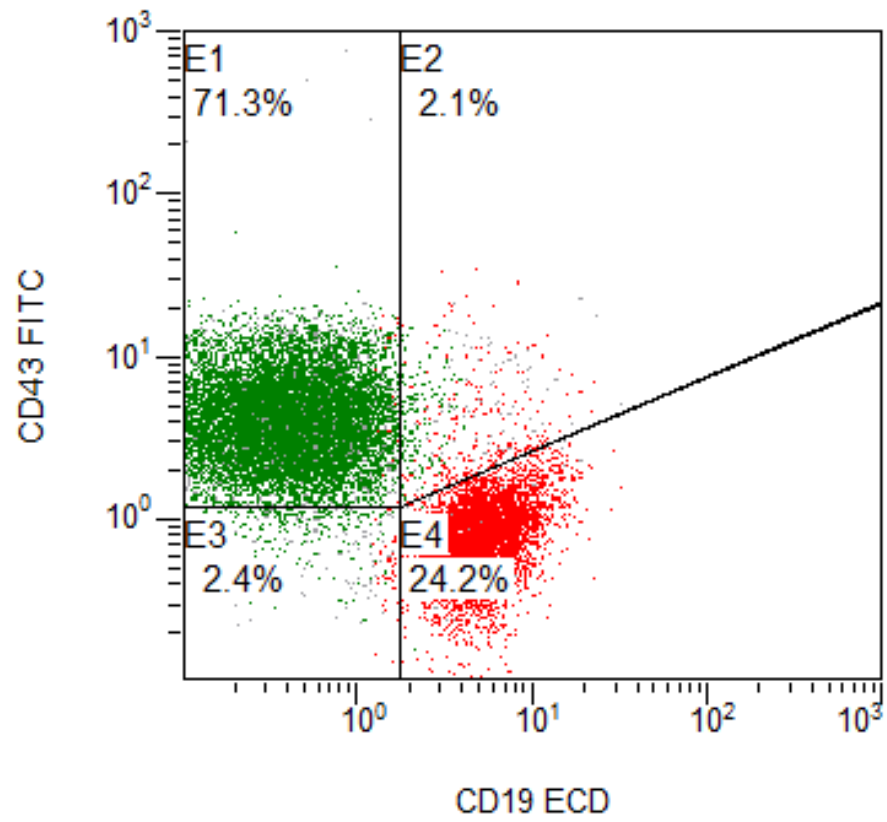
Events / tube

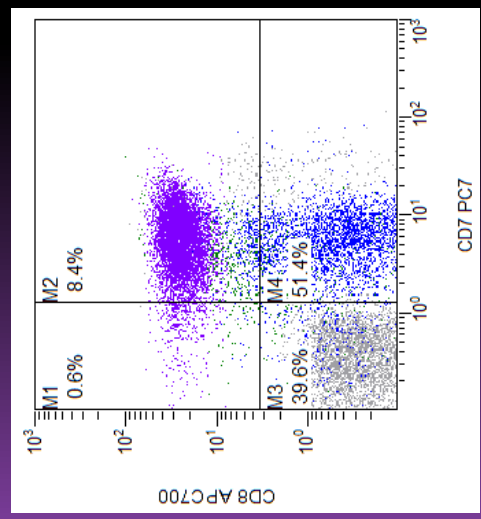
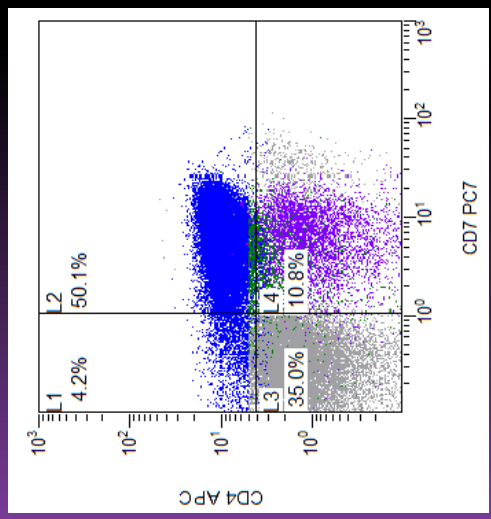
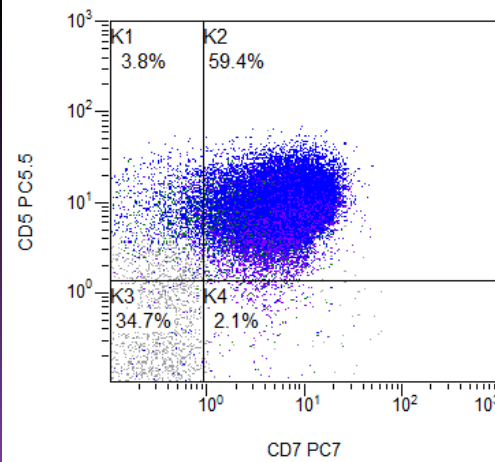
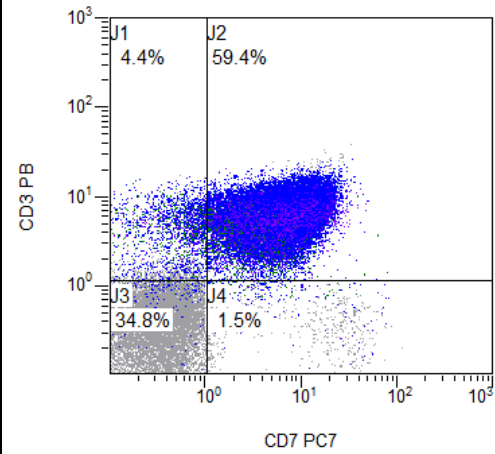
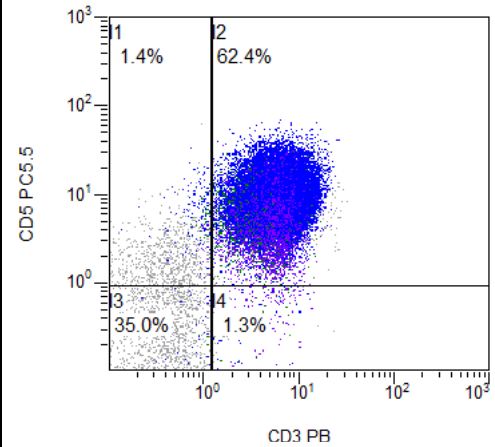
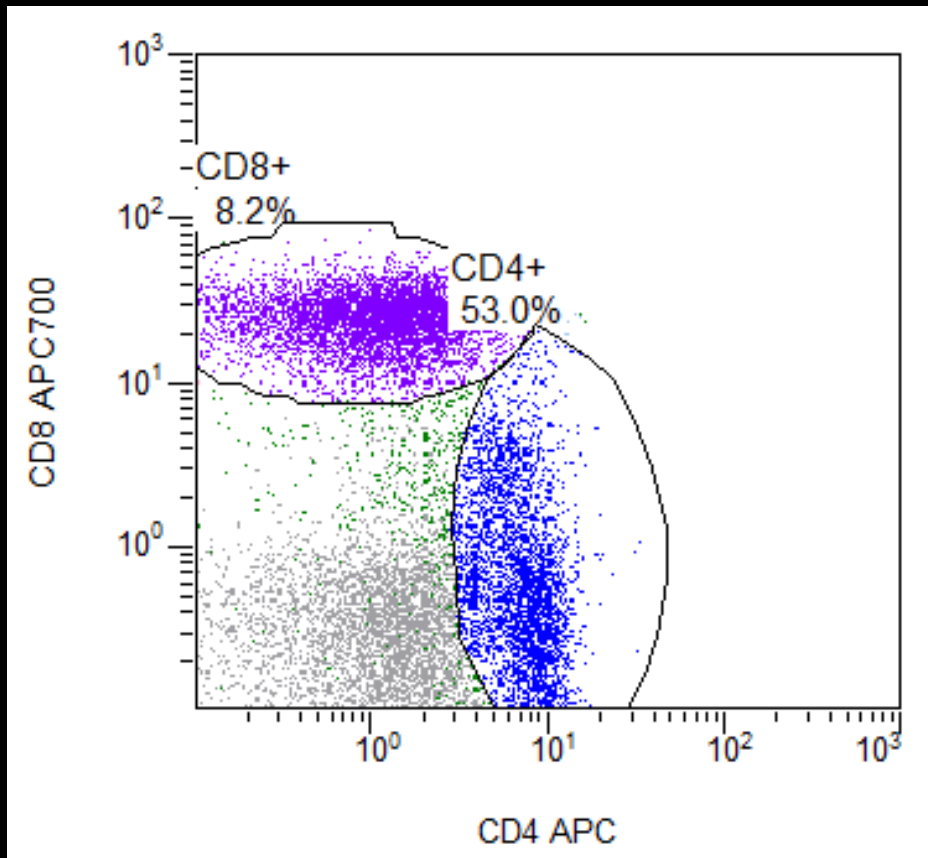
Palpable mass FNA

1000 - 10000

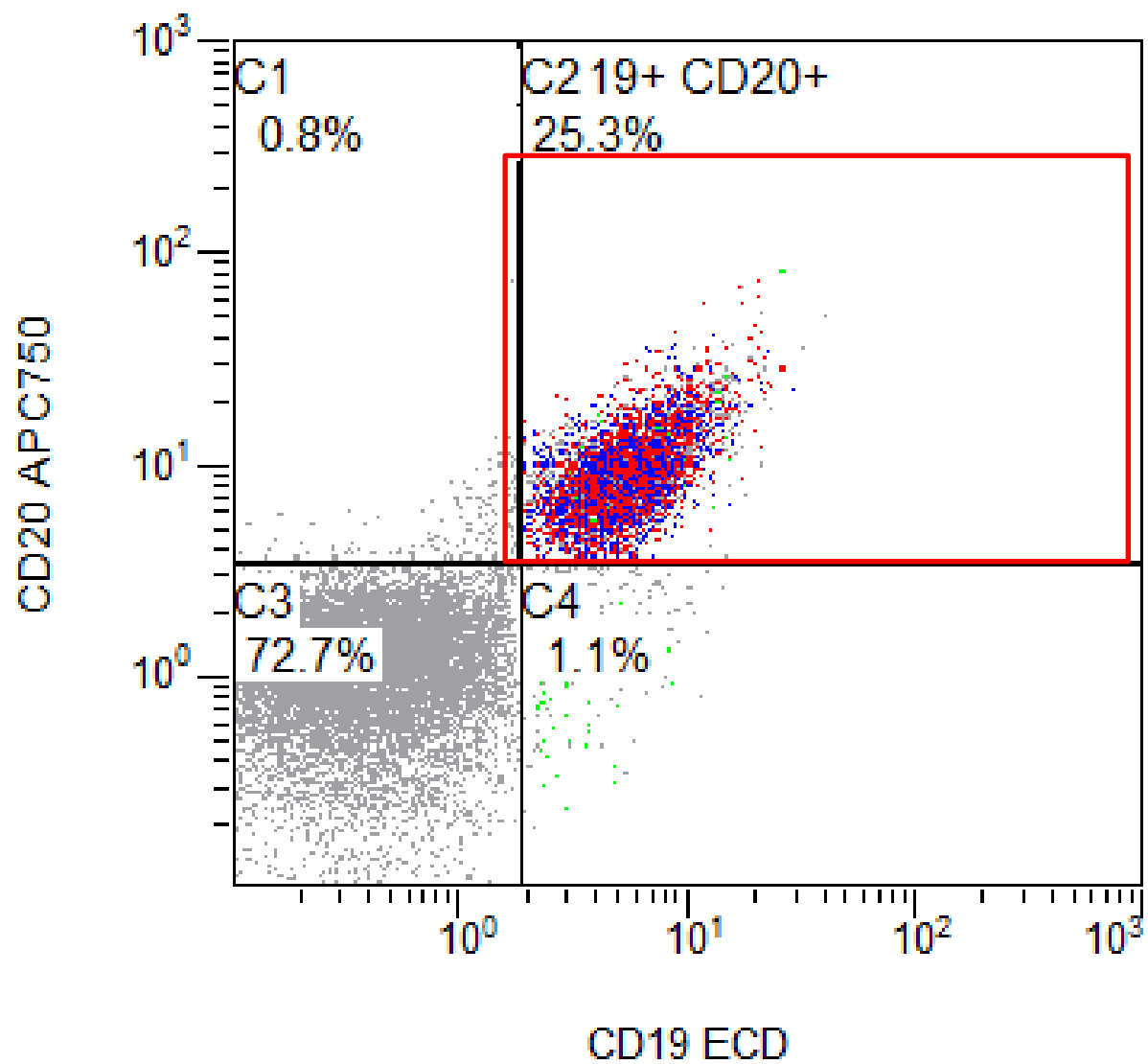
EBUS FNA

15000 - 25000

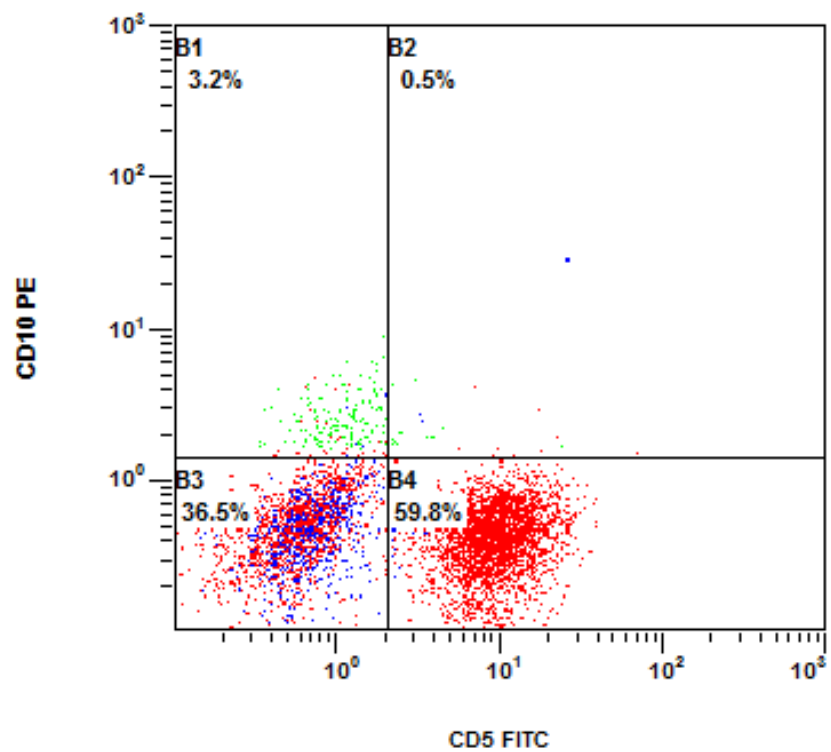




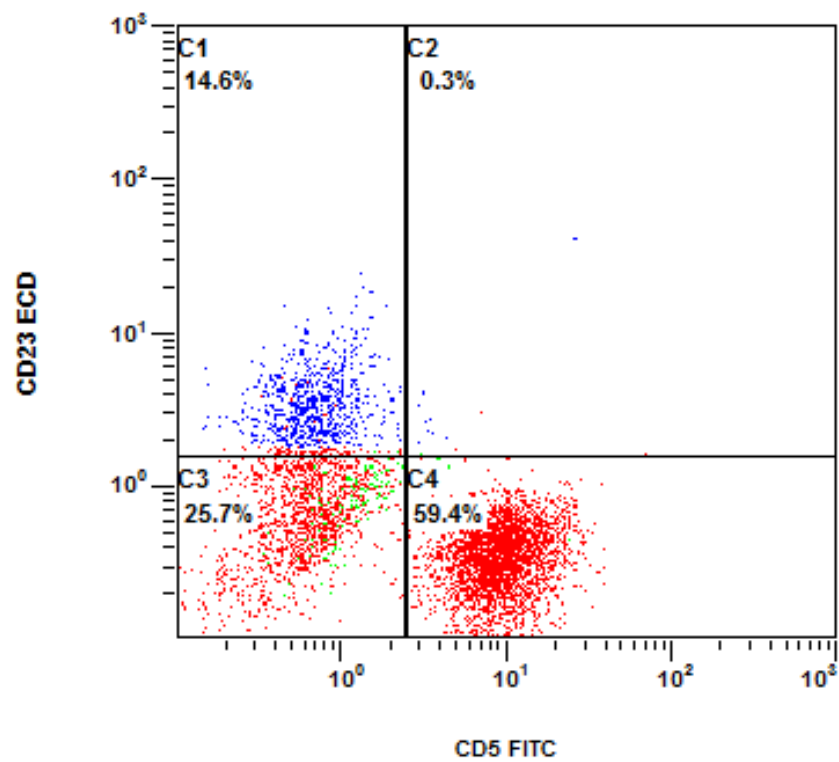
[Cells AND CD45+ Lymph] FL3 INT LOG/FL8 INT LOG

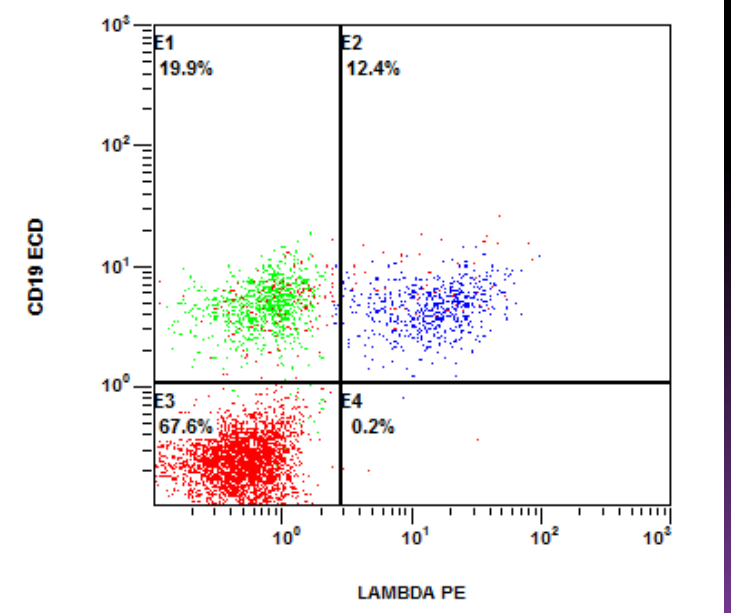
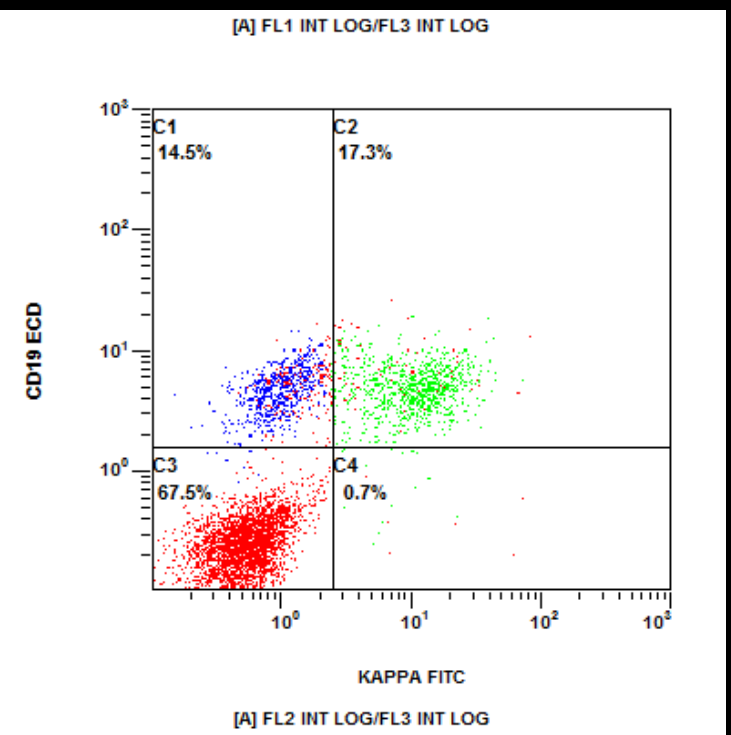
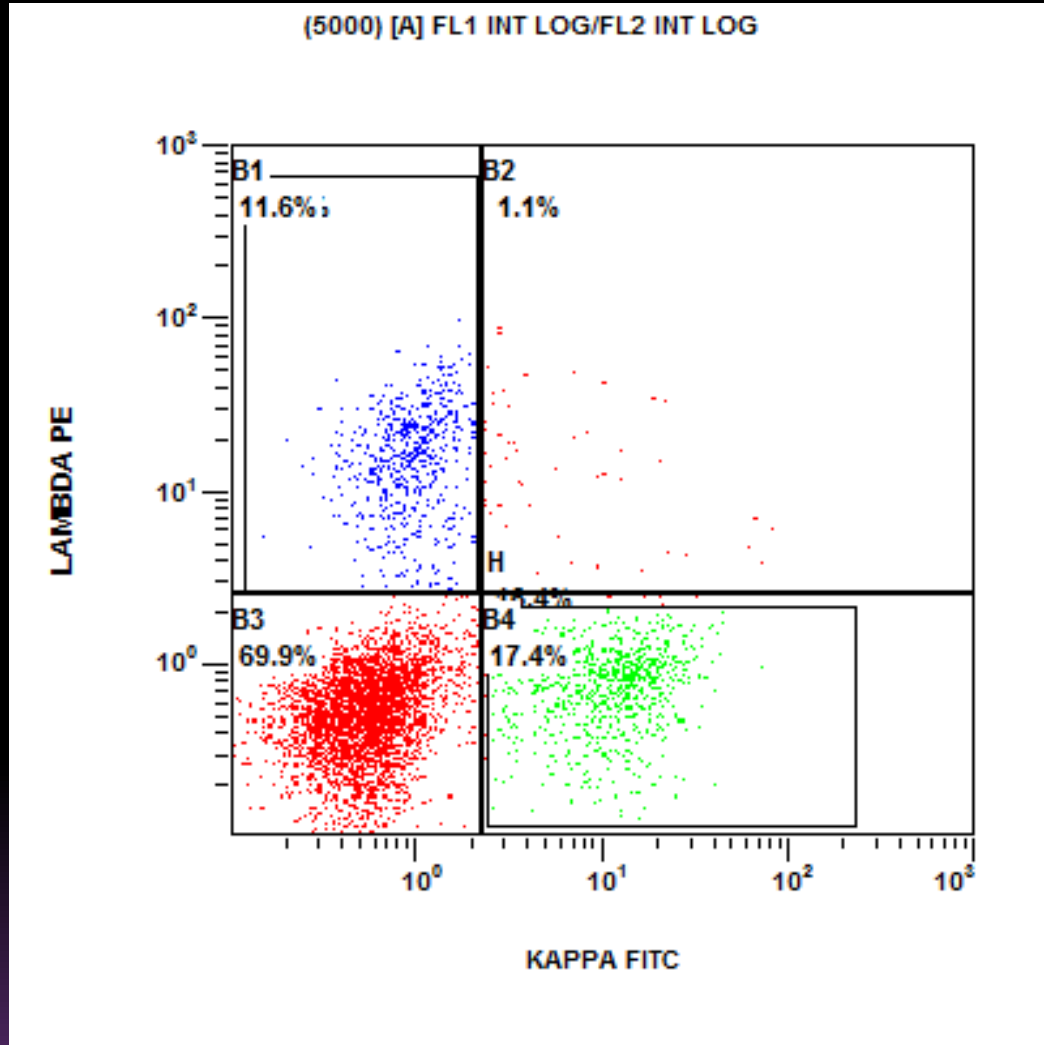


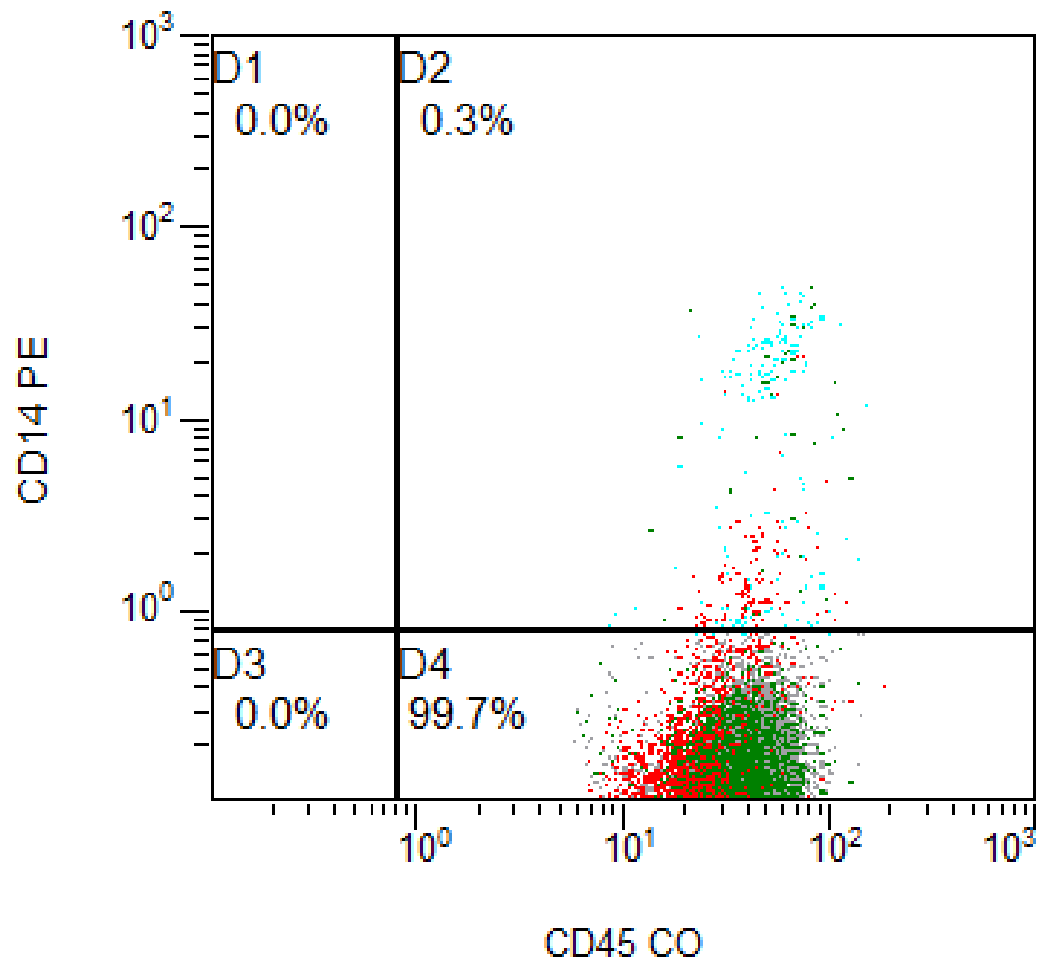
(5000) [A] FL1 INT LOG/FL2 INT LOG



[A] FL1 INT LOG/FL3 INT LOG







Patient Details

Hosp No: DoB:		Patients name:	
Lab No:		Clinical details: Persistent right inguinal? LN. 1.5-2cm non tender. Skin lesions on prescription drugs (GP, nature not known.	
Requesting Dr:		Date tested:	
Panels to be set up:			
Previous results:			
FNA	Site	Right Groin	

Technical comments



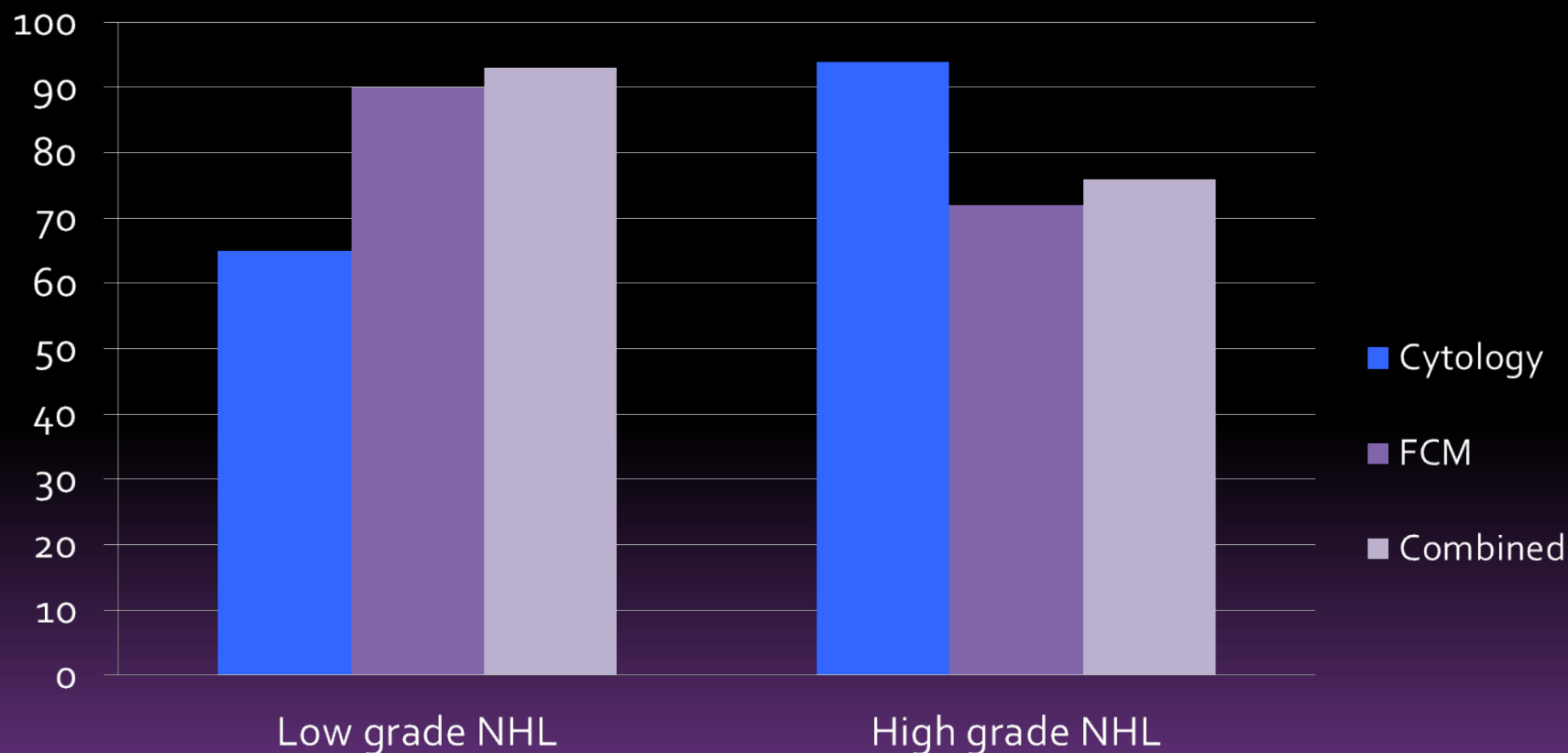
Immunophenotyping results

CD	% POS	CD	% POS
CD3	61	KAPPA	18
CD43	65	LAMBDA	13
CD4	50		
CD5	60		
CD7	57		
CD8	16		
CD10	4		
CD14	0		
CD19	32		
CD20	33		
CD20/5	1		
CD23	14		
CD45	97		

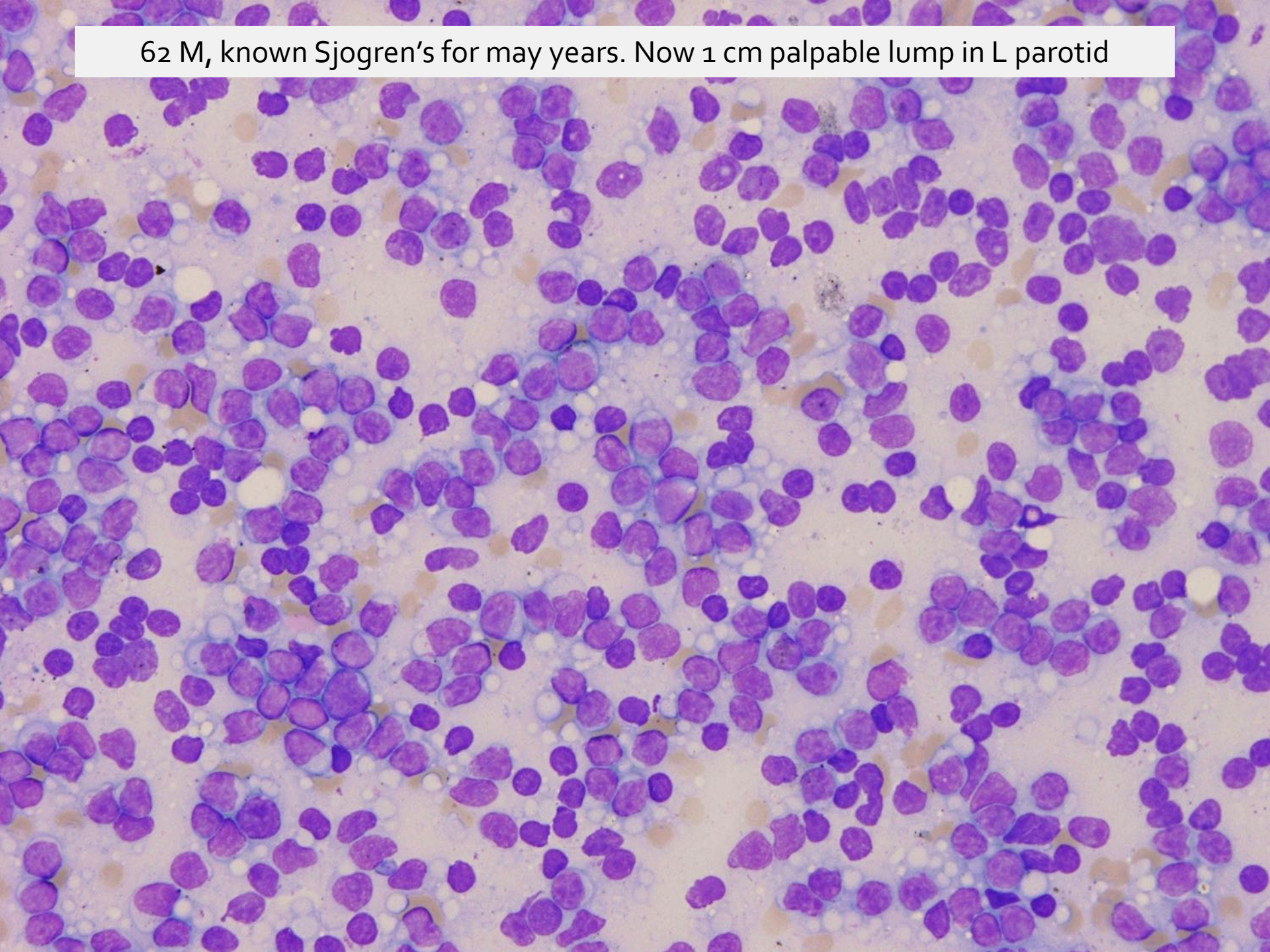
Clinical comment

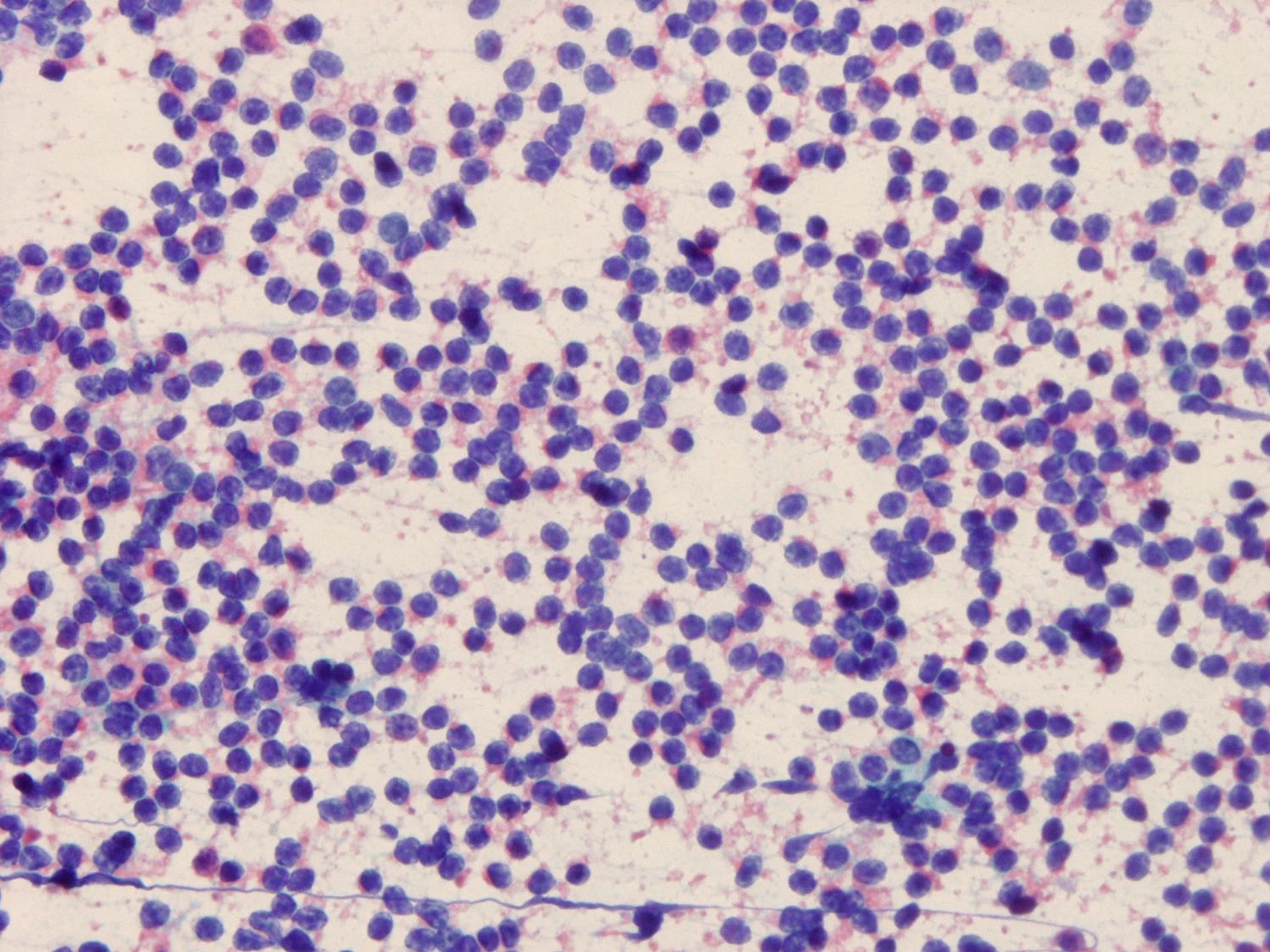
Reactive immunophenotype
No e/o B-LPD

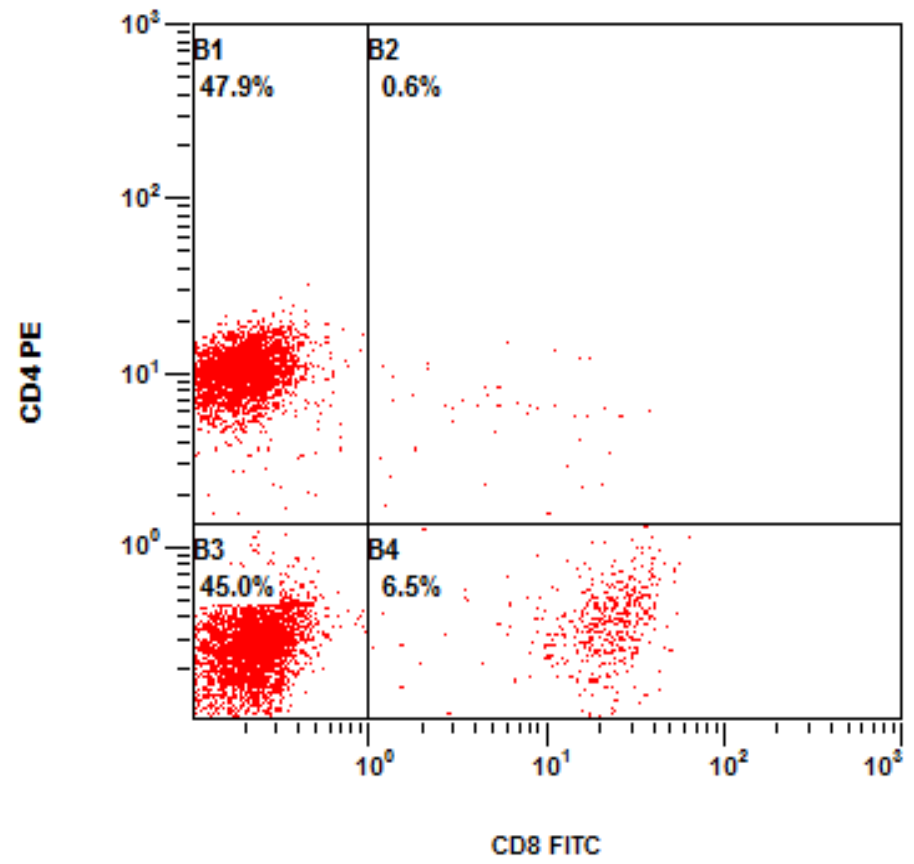
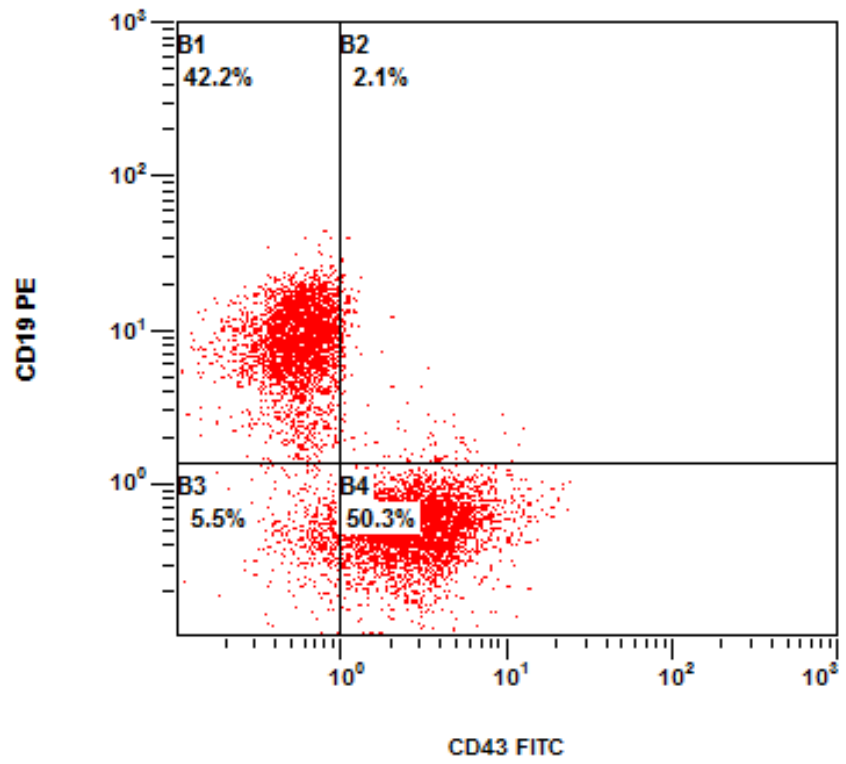
What is the actual role of flow cytometry in FNA lymphoma diagnosis?

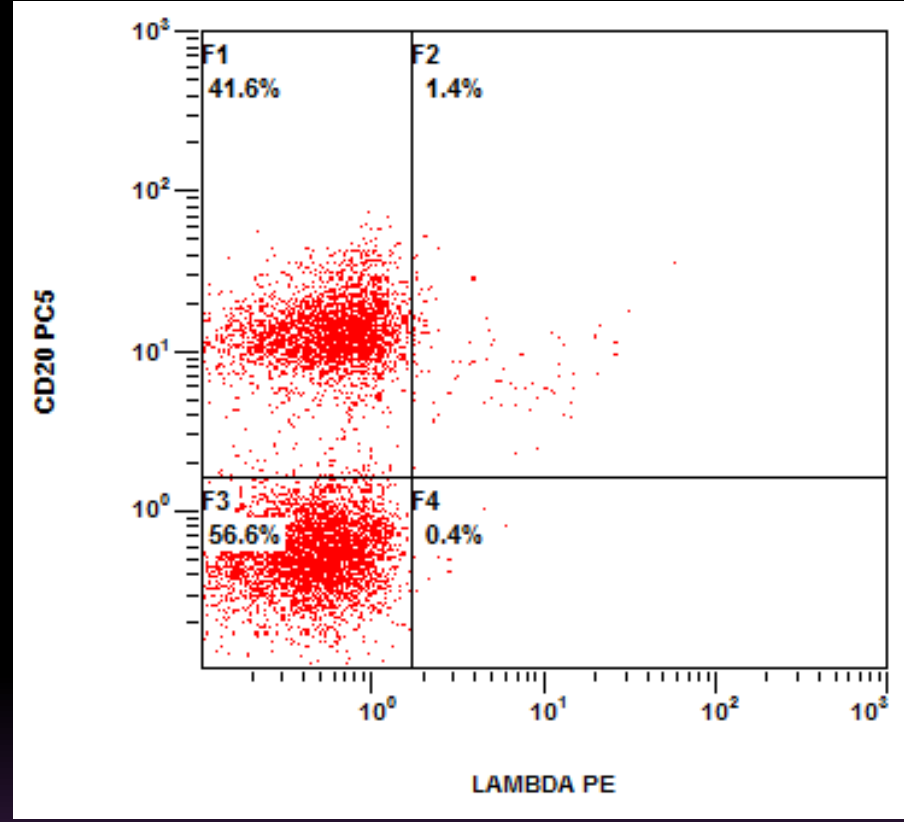
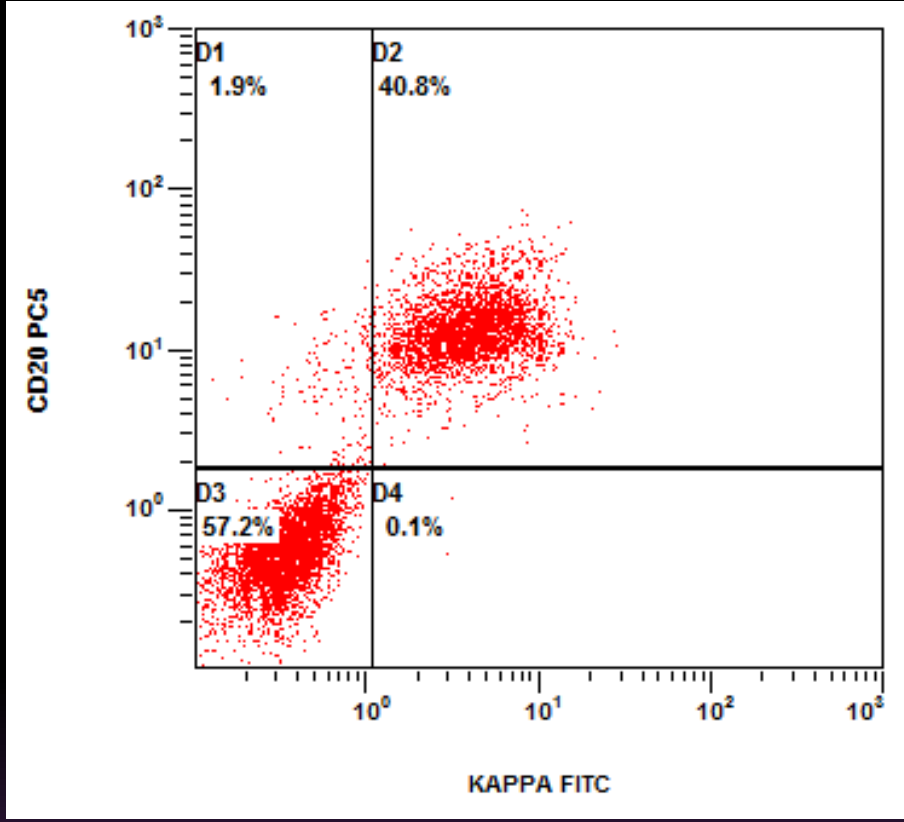


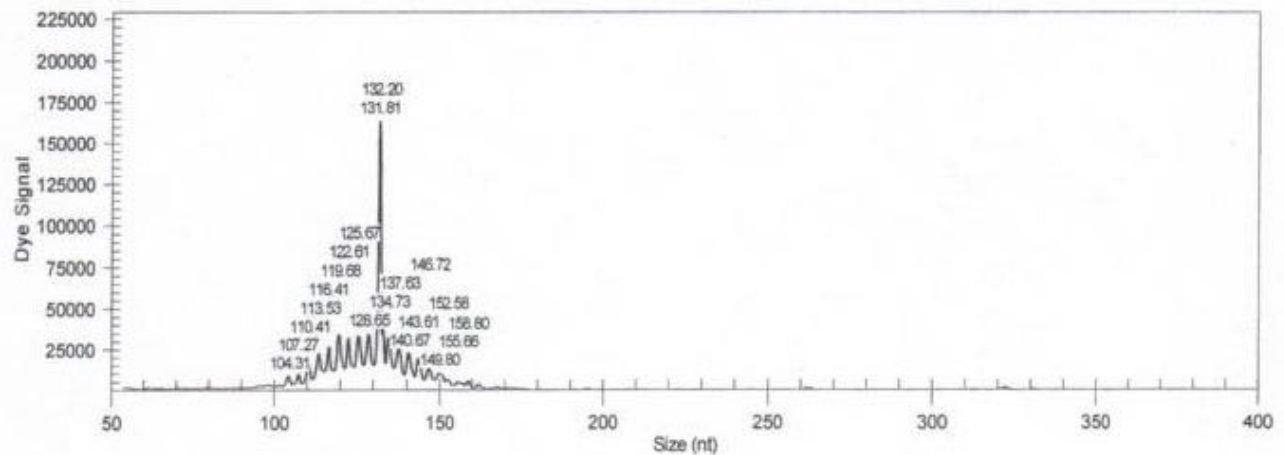
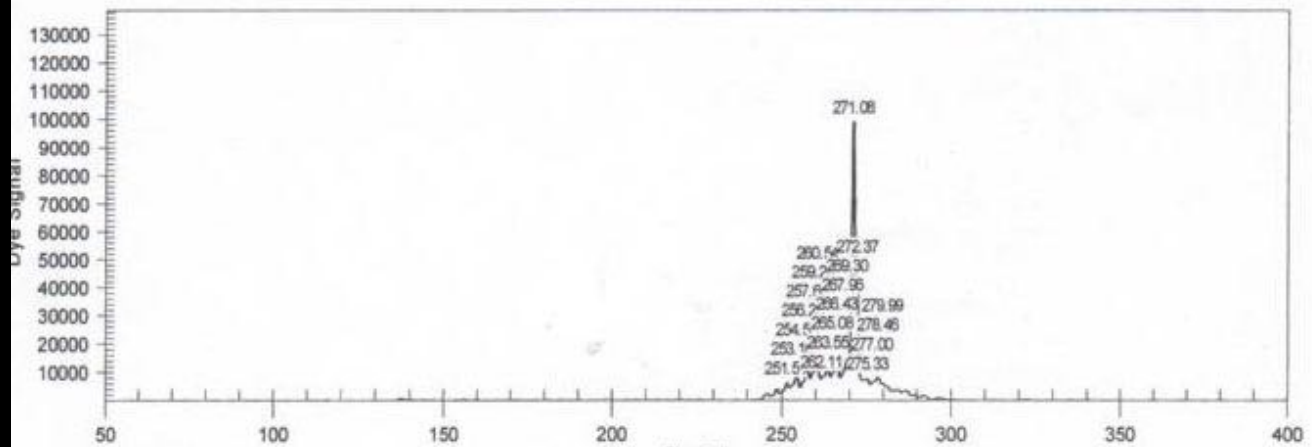
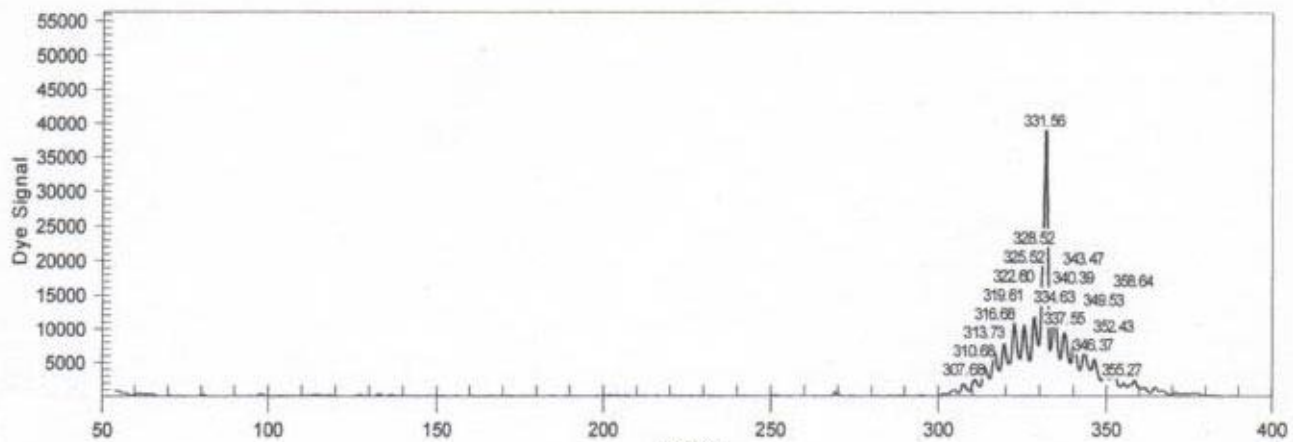
62 M, known Sjogren's for many years. Now 1 cm palpable lump in L parotid











Parotid:

Low grade B-cell Non-Hodgkin lymphoma consistent with
Marginal zone lymphoma (MALToma)

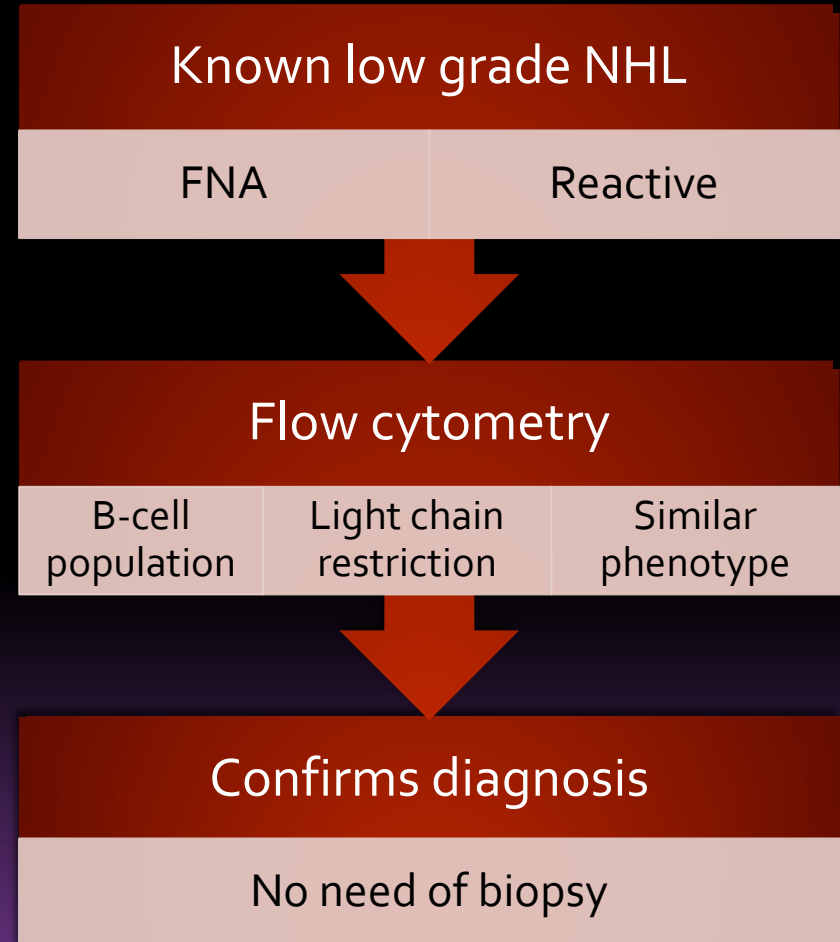
CD20(+), Kappa(+)

CD10(-), CD5(-), CD23(-)

IGH gene rearrangement studies: Clonal

Utility of FNA in low grade NHL

- Primary diagnosis: limited utility.
- Follow up / relapse / residual disease: very useful.
- If adequate material obtained – *obviates need for biopsy*.



Take home message

- Structured approach necessary
- Needle washings very useful
- Ancillary investigations often convert a consistent/suspicious report into a definitive diagnosis.
- FNA with flow cytometry is very useful in follow-up of low grade lymphoma's.

Thank you

