Fight the Flight
Simplifying Body Fluid Analysis

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Disclosure

- I am receiving an honorarium from Sysmex for today's presentation

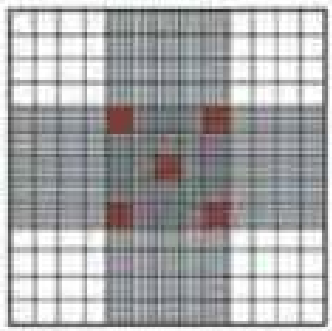
I'm just going to head to lunch now…

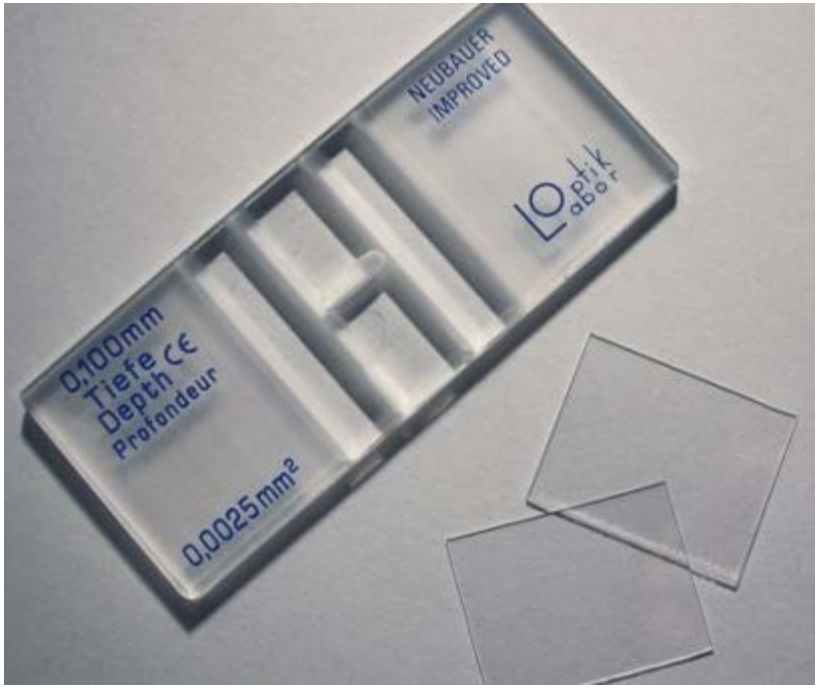
Objectives

- Discuss manual and automated counting techniques
- Recognize normal cells found in body fluids
- Recognize characteristics of malignant cells
- Review patient case studies

Manual Counting

- Time consuming
- Error prone
  - Dilutions
  - Area correction
  - More RBC than Nucleated Cells (NC) or vice versa
  - Crenated RBC vs NC
  - Tech Inexperience





HEMOCYTOMETER FLUID COUNTS

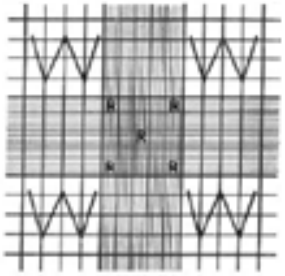
Patient Name	
Accession #	
Fluid Type	

NOTE: If the whole side is counted, multiply the results by 1.1. If you count any number of large squares other than those listed; use the following guide for the appropriate area correction multiplication factor:

# Large Squares Counted	Multiplication Factor
1	10
2	5
4	2.5
6	1.7

large square

If you count the 5 small center squares (marked R) multiply the result by 50.



Count:
Tech 1 _____ (initials)

TNC _____ x _____ = _____ RBC _____ x _____ = _____
(dilution x area correction) (dilution x area correction)

Tech 2 _____ (initials)

TNC _____ x _____ = _____ RBC _____ x _____ = _____
(dilution x area correction) (dilution x area correction)

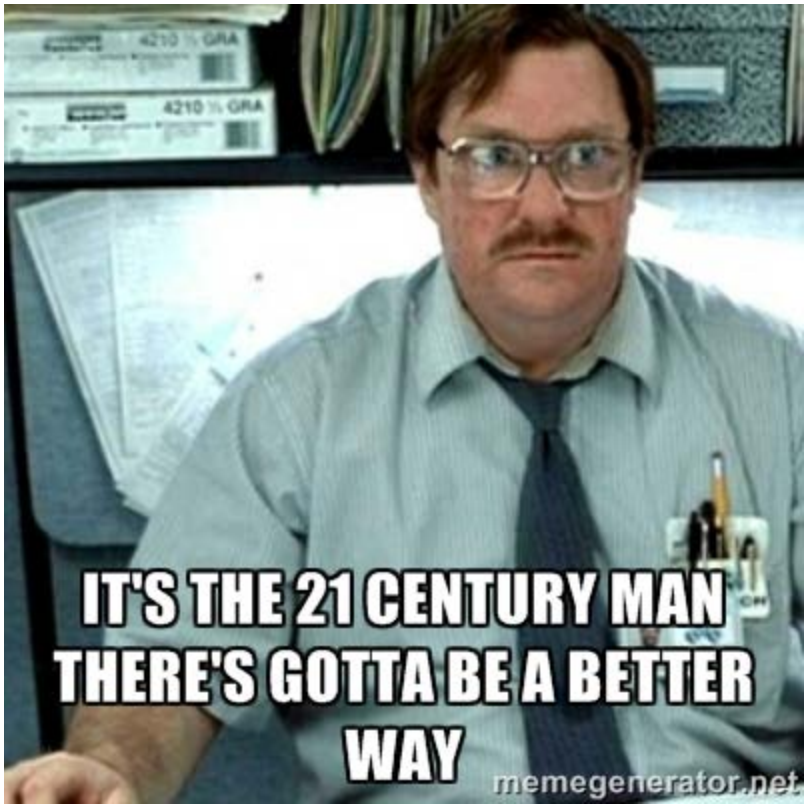
Average: The results of the two sides should be within 20% of each other - if not, flood another counting chamber and repeat the count.

Average TNC _____ Average RBC _____

Differential

Tech 1:		Tech 2:		Average	
	% Granulocytes		% Granulocytes		% Granulocytes
	% Lymphocytes		% Lymphocytes		% Lymphocytes
	% Macro/Mesos		% Macro/Mesos		% Macro/Mesos
	% Other		% Other		% Other

Describe any other morphology:


IT'S THE 21 CENTURY MAN
THERE'S GOTTA BE A BETTER
WAY
memegenerator.net

Automation!!!

Faster, Increased Precision, Fewer Dilutions

Preparing Sample for Analysis

- DON'T MAKE YOUR ANALYZER ANGRY
  - Treat synovial fluids with hyaluronidase
    - An aliquot, NOT the original
  - Remove clots and snots
    - Report count as > or Approximate

TNC? AMR? CRR?

Let's break it down…

- TNC - Total Nucleated Cell count
  - Includes lining cells (such as mesothelial) as well as WBC's
- AMR - Analytical Measurement Range
  - Range of analyte values that a method can directly measure on a specimen without any dilution, concentration, or other pretreatment not part of the usual assay process
- CRR - Clinical Reportable Range
  - How low and high YOU can report results

Establishing AMR and CRR

- XN AMR
  - 2,000-5,000,000 RBC/uL
  - 3-10,000 TNC/uL
- UF-1000 AMR
  - 0-5,000 RBC/uL
  - 0-5,000 TNC/uL
- St. Luke's CRR
  - 0-5,000,000 RBC/uL
  - 0-100,000 TNC/uL
- Why are they different?
  - If we run a body fluid on XN and RBC is 0, we know it is really <2,000; could report as <2000
  - If we run a body fluid on UF-1000 and TNC is >5,000, could report as >5,000

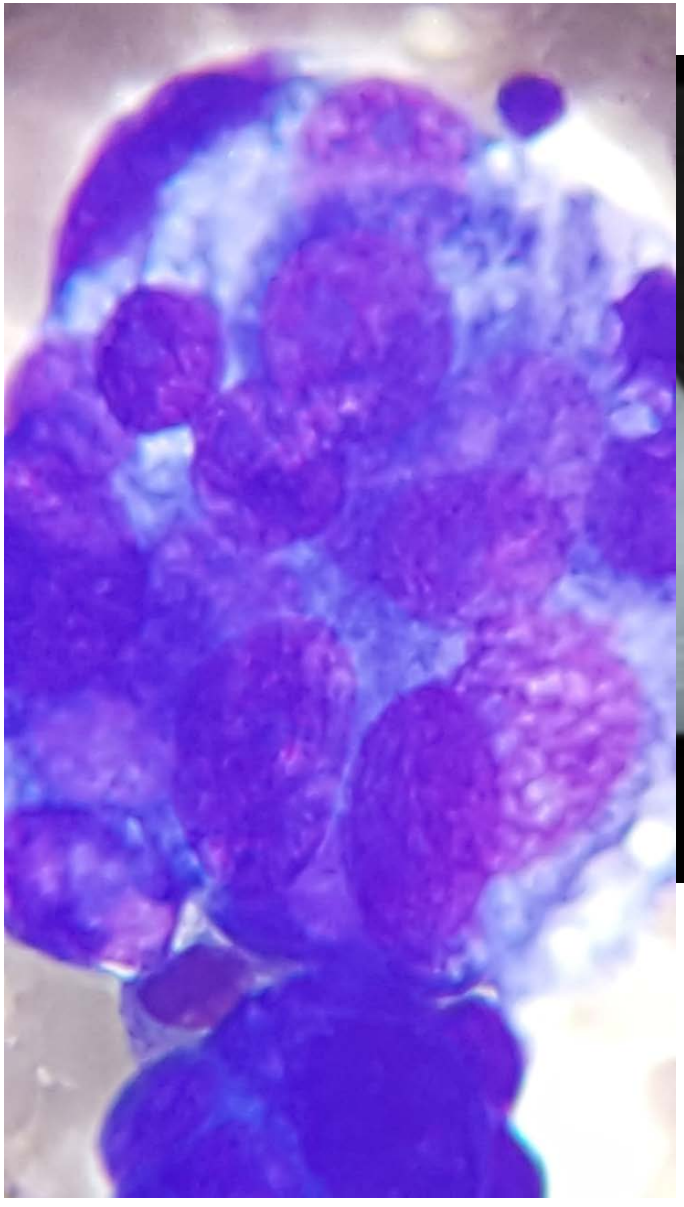
I have automation, why would I ever do a manual count?

- Low sample volumes
  - Depends on sample requirement of your testing system
    - UF-1000 sample volume is 800 u/L
    - XN sample volume is 88 u/L
- Sample not approved/correlated for automated analysis
  - Bronchial Lavages
  - Low RBC count w/ small sample volume
- Flags on Automated counts
  - Or if cytospin doesn't match automated counts
- When you want to be mean
  - Students
  - New Techs

What do these counts even mean?

- RBC counts are really only important for CSF
  - Hemorrhage (SAH) vs traumatic tap
    - RBC > in tube 1 than tube 4
- ***Type of cell present is more important than the count***
  - Can still do a diff on clotted sample
  - Can still possibly do a diff on QNS sample

If you thought cell counts were scary…



A few things to help simplify

- Know how to make an adequate cytospin
  - How to recognize when it's not good
- Be familiar with what cells are present in each fluid type
- Be familiar with characteristics of malignant cells
- Always scan on 10x first, perform diff on 50x
- Have 2 techs perform differential
- Have a good resource available for reference
  - Books
  - Pathology

Cytospin

- 20 fold concentration of cells
  - Cell count in fluids lower than peripheral blood
- Preserves cellular morphology
- Monolayer of cells

Nucleated cell count	# of Drops of Fluid	Add Saline
1 – 500	5 drops	To bottom line if QNS fluid
501 – 1,000	3 drops	To bottom line
1,001 – 2,500	1 drop	To bottom line
2,501 – 10,000	1 drop	To top line
10,001 – 25,000	1 drop of 1:5 diluent (1 drop fl. + 4 drops NaCl)	To top line
25,001 – 50,000	1 drop of 1:10 dilutent (1 drop fl. + 9 drops NaCl)	To top line
>50,000	1 drop of 1:15 diluent (1 drop fl. + 14 drops NaCl)	To top line

#WBC/ cumm	#Drops 30% Albumin	Amount of CSF	Add Saline
< 200	1	Fill to top line if enough CSF	To bottom line if QNS CSF
200 – 499	2	15 drops	To bottom line if QNS CSF
500 – 999	2	10 drops	To bottom line
1000 – 2499	3	4-5 drops	To bottom line
2500 – 4999	3	2 drops	To bottom line
5000 – 9999	4	1 drop	To bottom line
10000 – 24999	4	1 drop 1:5 dil (1CSF + 5 NaCl)	To bottom line
25000 – 49999	5	1 drop 1:10 dil (1CSF + 9 NaCl)	To bottom line
≥ 50000	6	1 drop 1:15 dil (1 CSF + 14 NaCl)	To bottom line

Albumin or not?

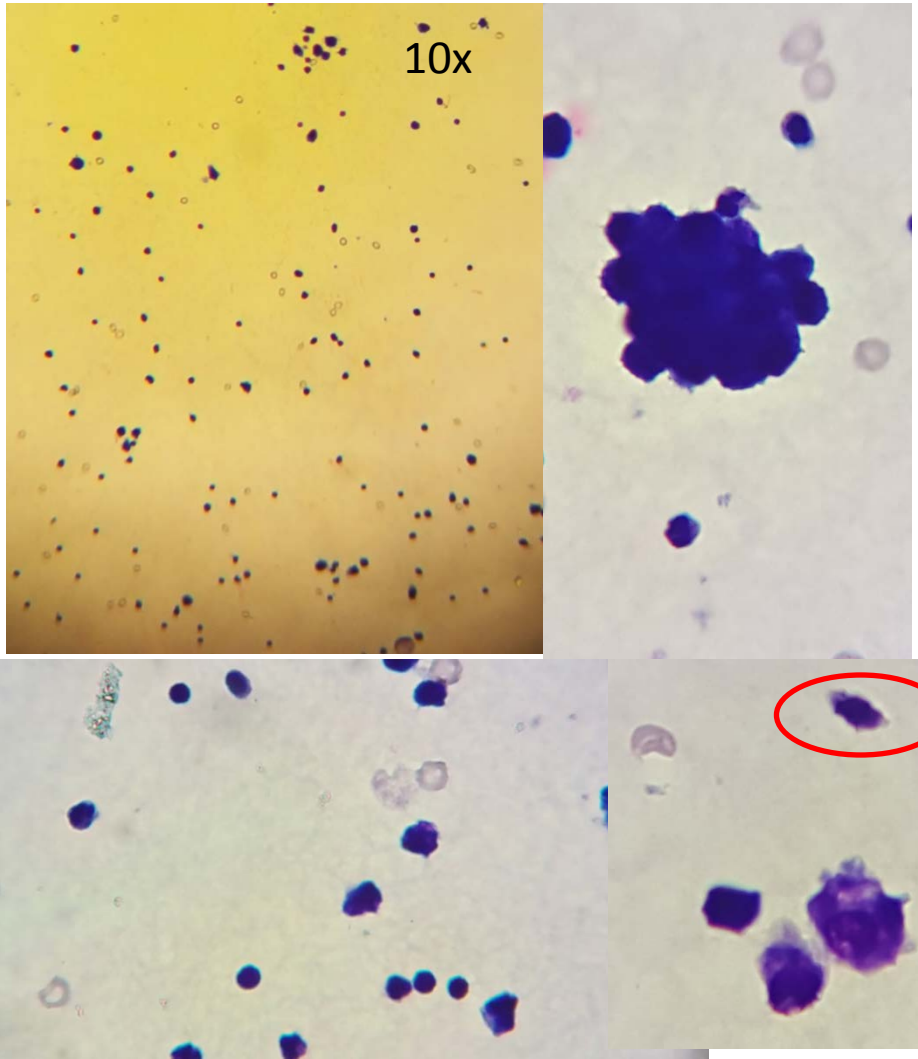
- Textbook[1] says to add albumin to CSF and serous fluids
  - If protein is too high cells shrink, making them difficult to identify
    - Remake slide without albumin
    - If no albumin used, use a little saline when preparing the cytospin
  - Lots of smudge cells = add albumin

[1]McKenzie, Shirlyn B. (1996). *Textbook of Hematology.* Baltimore, MD: Williams & Wilkins.

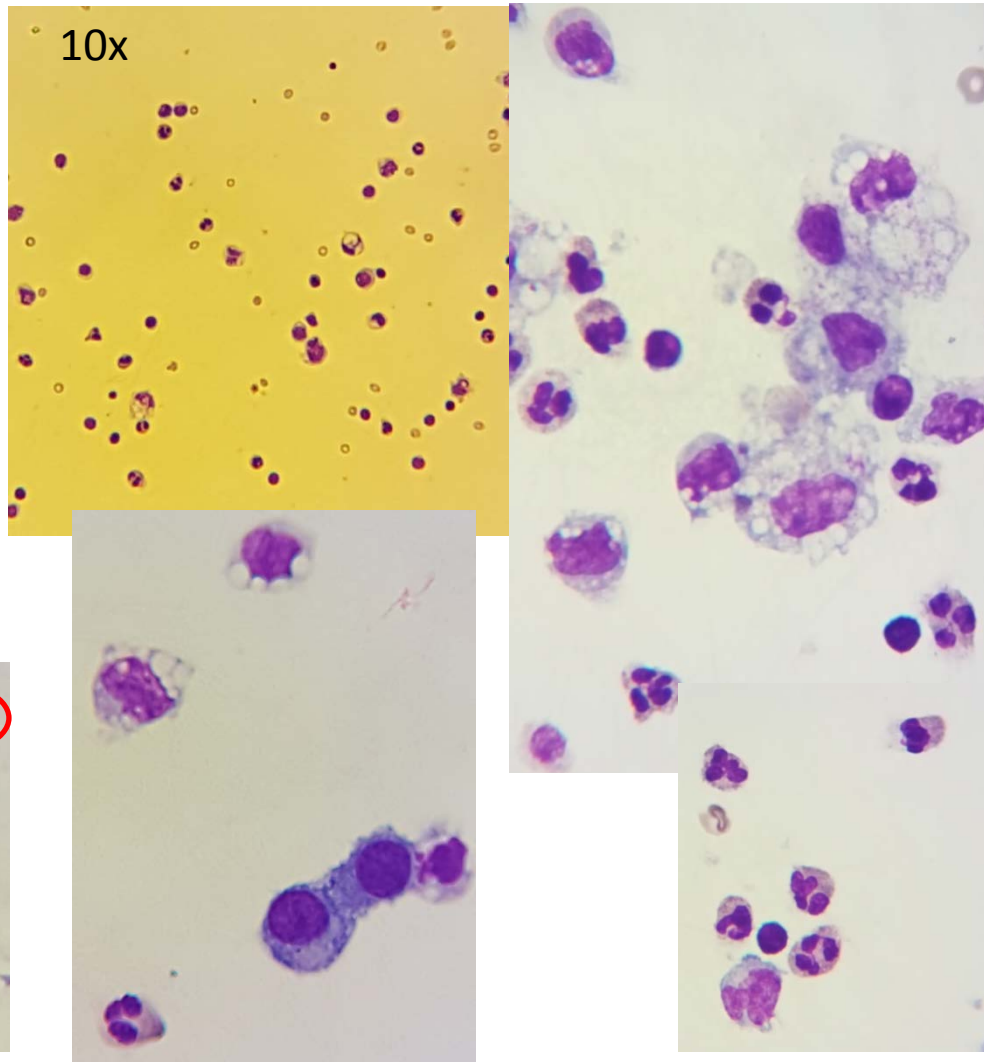
Albumin or not?

Pleural Fluid

Albumin



No Albumin



Cell Types Present in Body Fluids

- All body fluids can have
  - WBC's
    - Become comfortable with the characteristics of cell types in peripheral blood where they look less scary
      - Neutrophils can look hypersegmented or degenerated
      - Lymphs often appear more reactive with artifactually prominent nucleoli and cytoplasmic projections
      - Monocytes may have more abundant or vacuolated cytoplasm, or have phagocytosed material
  - RBC's
  - "phages"
  - Malignant cells
    - Found most often in serous fluids

Cell Types Present in Body Fluids

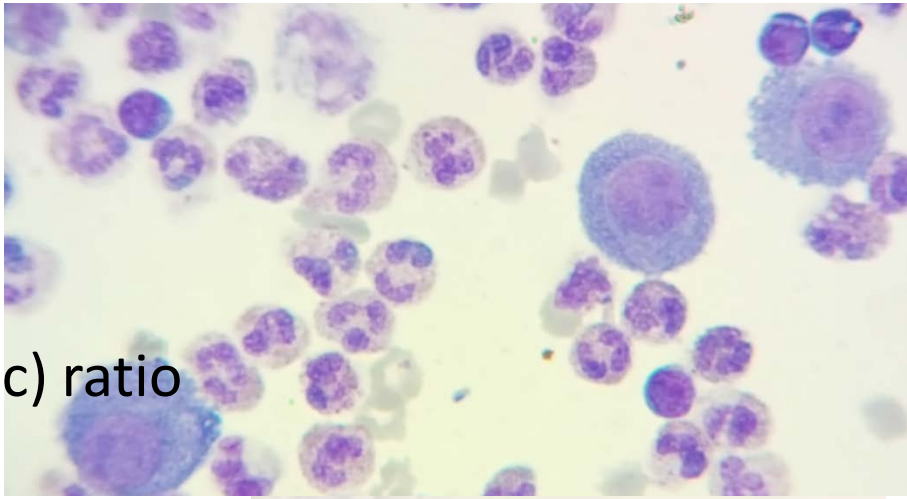
- Cerebrospinal fluid (CSF)
  - Lining Cells-often present in clumps, more common in neonates
    - Ependymal
    - Choroid Plexus
    - Arachnoid

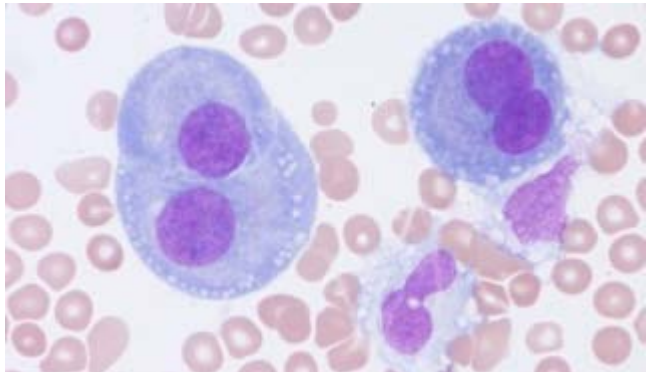
Choroid Plexus

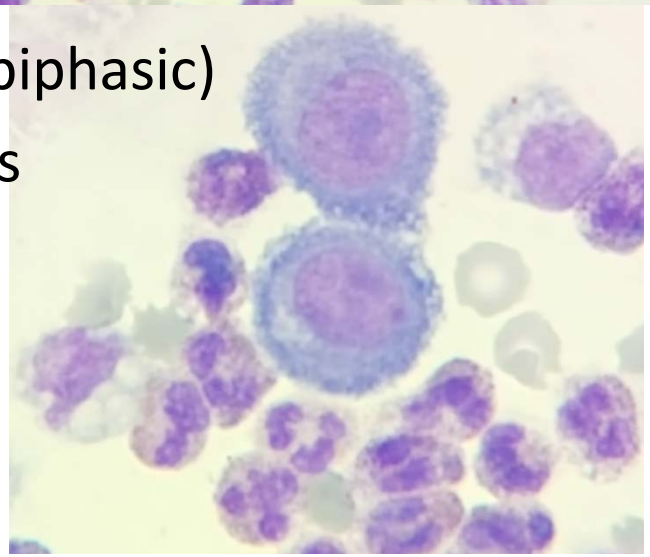
  - Mesothelial cells are NOT present in CSF
    - Presence of large tissue cells are suspicious for malignancy

Cell Types Present in Body Fluids

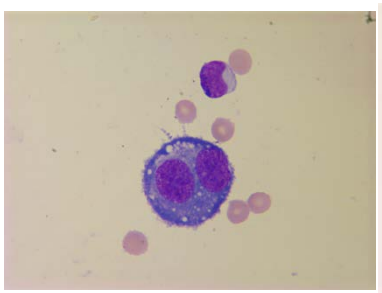
- Serous - Pleural, Pericardial, Peritoneal
  - Mesothelial-lining cell
    - Individual or seen in clumps
      - No cytoplasmic molding
      - Flat clusters
    - Low NC (nuclear-cytoplasmic) ratio
    - Multinucleate
    - Cytoplasm can be light or dark blue (biphasic)
    - Galagan book-illustrations and images

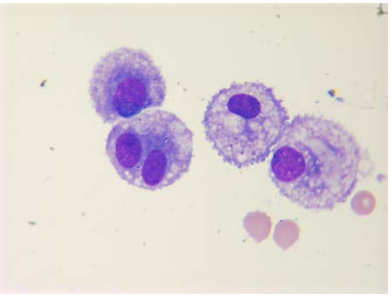




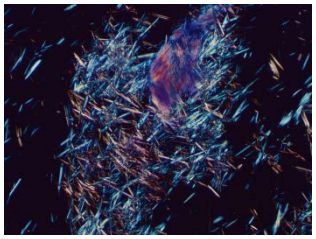


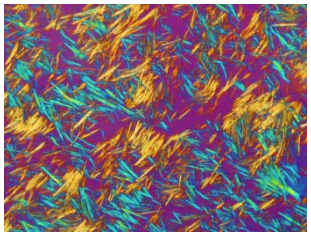
Cells Present in Body Fluids





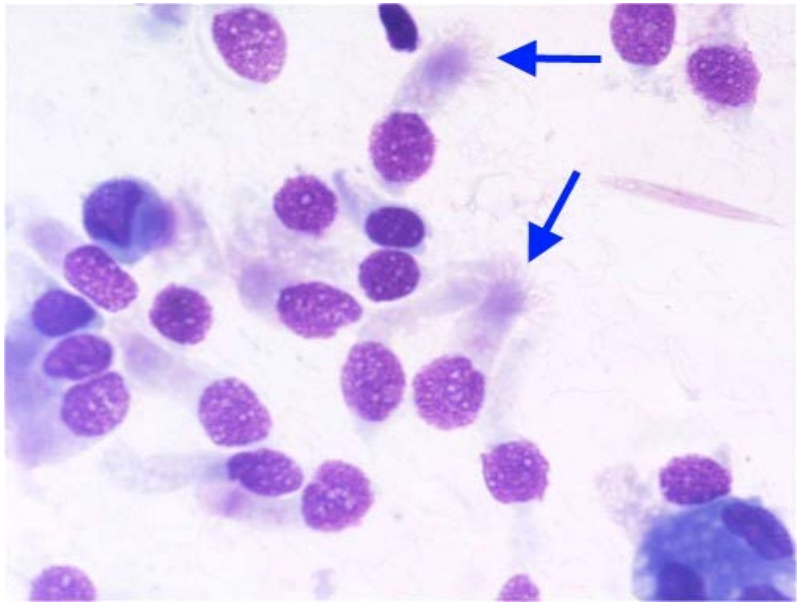
- Synovial - joint fluid
  - Synovial lining cells
    - Resemble mesothelial cells, but have denser cytoplasm
  - Crystals
    - Monosodium Urate - gout
    - Calcium Pyrophosphate - pseudogout
    - Cholesterol - chronic arthritis (RA)
  - Malignant cells are extremely rare



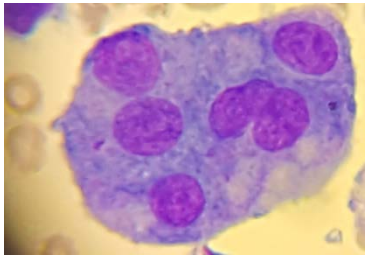


Cells Present in Body Fluids

- Bronchial Lavage/Brushing
  - Bronchial lining cells-row of cilia at one end
    - Considered “contaminant”
      - We report as # seen per 100, not including them in the differential

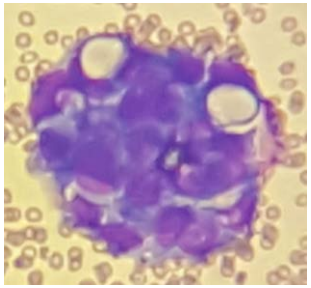


Characteristics of Malignant Cells



Benign

- Individual/flat clusters
- Separation window
- Low N:C ratio
- Uniformity
  - Size and shape of nucleus
  - Loose, homogeneous nuclear chromatin
  - Small/regular nucleoli



Malignant

- Ball like clusters
- Nuclear molding
- High N:C ratio (varies)
- Non Uniformity
  - Nuclear shape/size variation
    - Irregular/jagged/folded
  - Unevenly distributed chromatin
  - Nucleoli
    - Prominent, frequently multiple, irregular membrane

Characteristics of Malignant Cells

- None of the features can be used alone
- What not to use to differentiate benign and malignant cells
  - Mitotic activity-reactive mesos also undergo mitosis
  - Cytoplasmic vacuoles-can also represent early degradation

Characteristics of Malignant Cells

The most common nonhematopoietic malignancies seen in body fluids are small-cell carcinoma and adenocarcinoma

Small cell carcinoma

- High N:C ratio
- Blast like chromatin
- Absent or non-prominent nucleoli
- Frequent nuclear molding
- Paranuclear blue bodies

Adenocarcinoma

- Overall larger cell size
- Moderate to abundant cytoplasm (Low N:C ratio)
- Nuclear chromatin partially clumped and heterogeneous
- Prominent irregular nucleoli

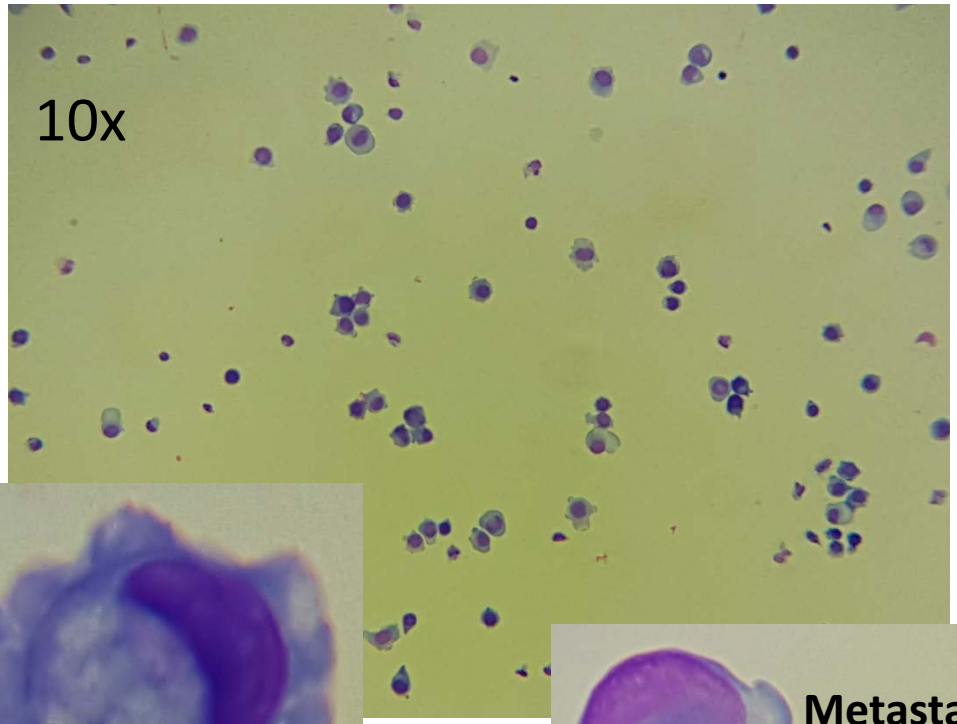
How do I know I'm not missing something

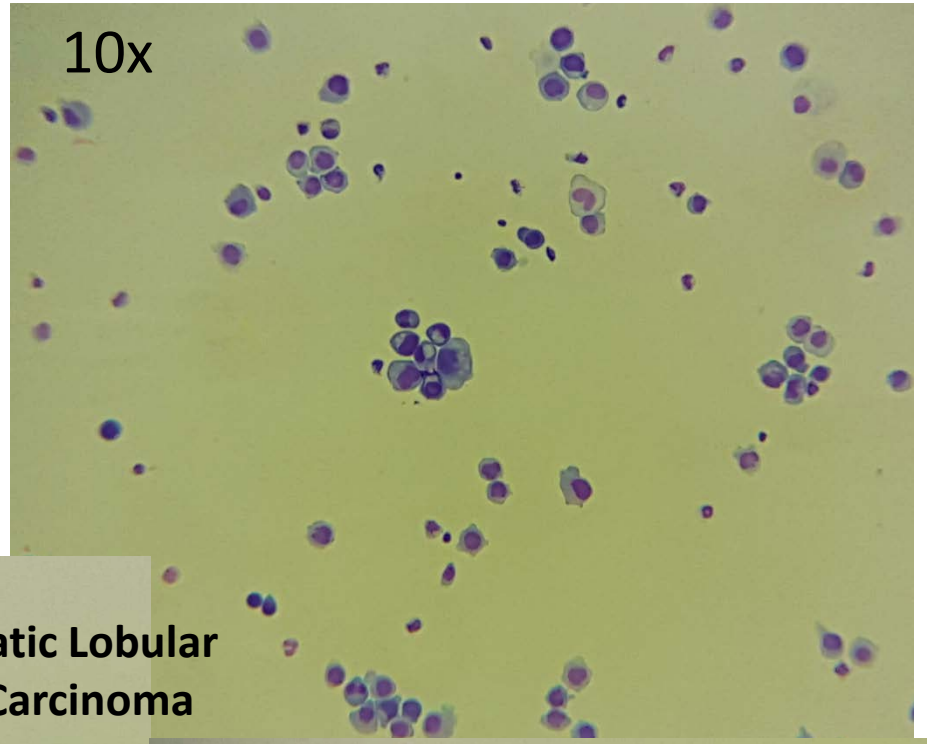
- Start out all diffs by scanning on 10x
  - Look for malignant cells
  - Find representative area to count
- Perform count on 50x
- Always have 2 techs diff body fluids
  - Most don't have abnormal cells, so it's easy to assume that they won't or techs can be in a hurry and miss these cells

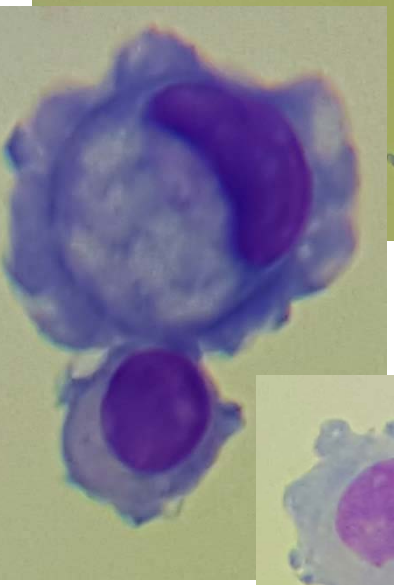
Patient 1

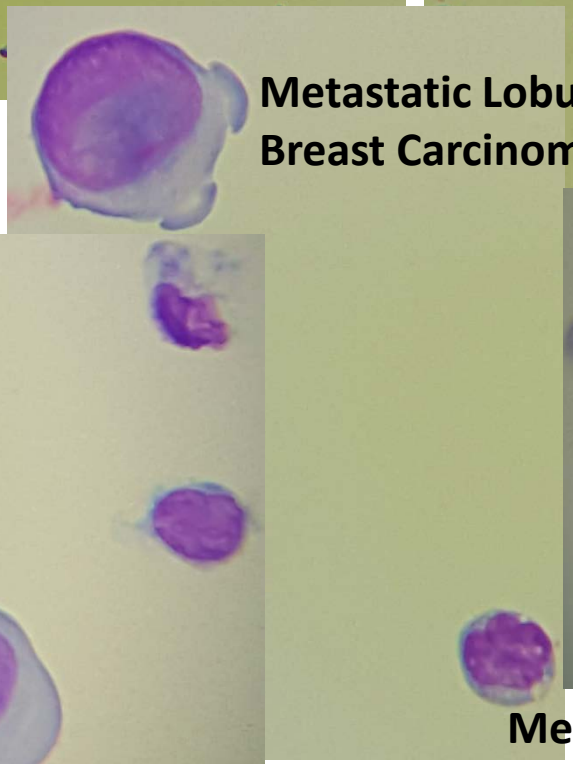
- 60yr Female
- History of lobular carcinoma of the breast
- CSF
  - TNC-31
  - RBC-1
  - Diff= 1% Neuts,6% Lymphs, 13% Macrophages, **80% other**

CSF

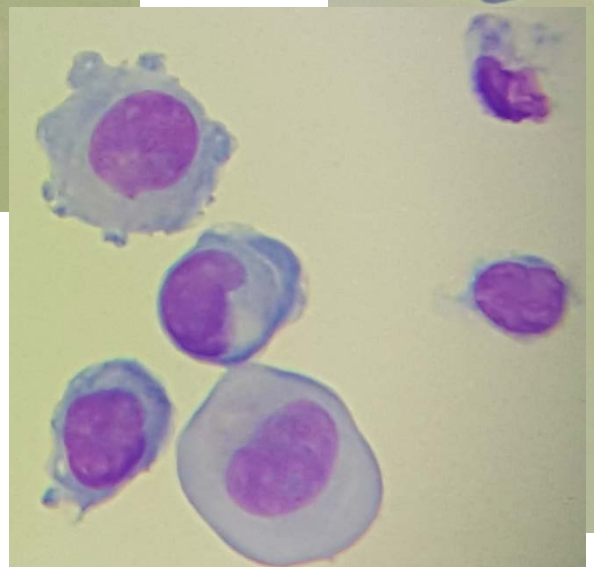


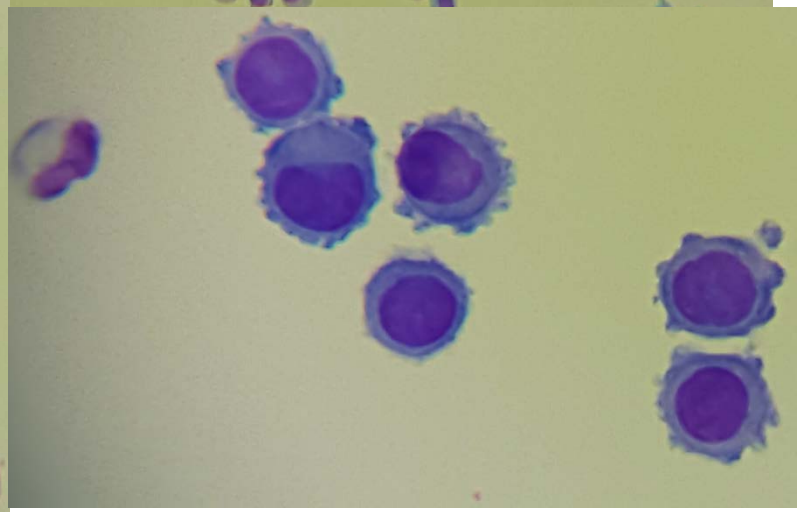






Metastatic Lobular Breast Carcinoma



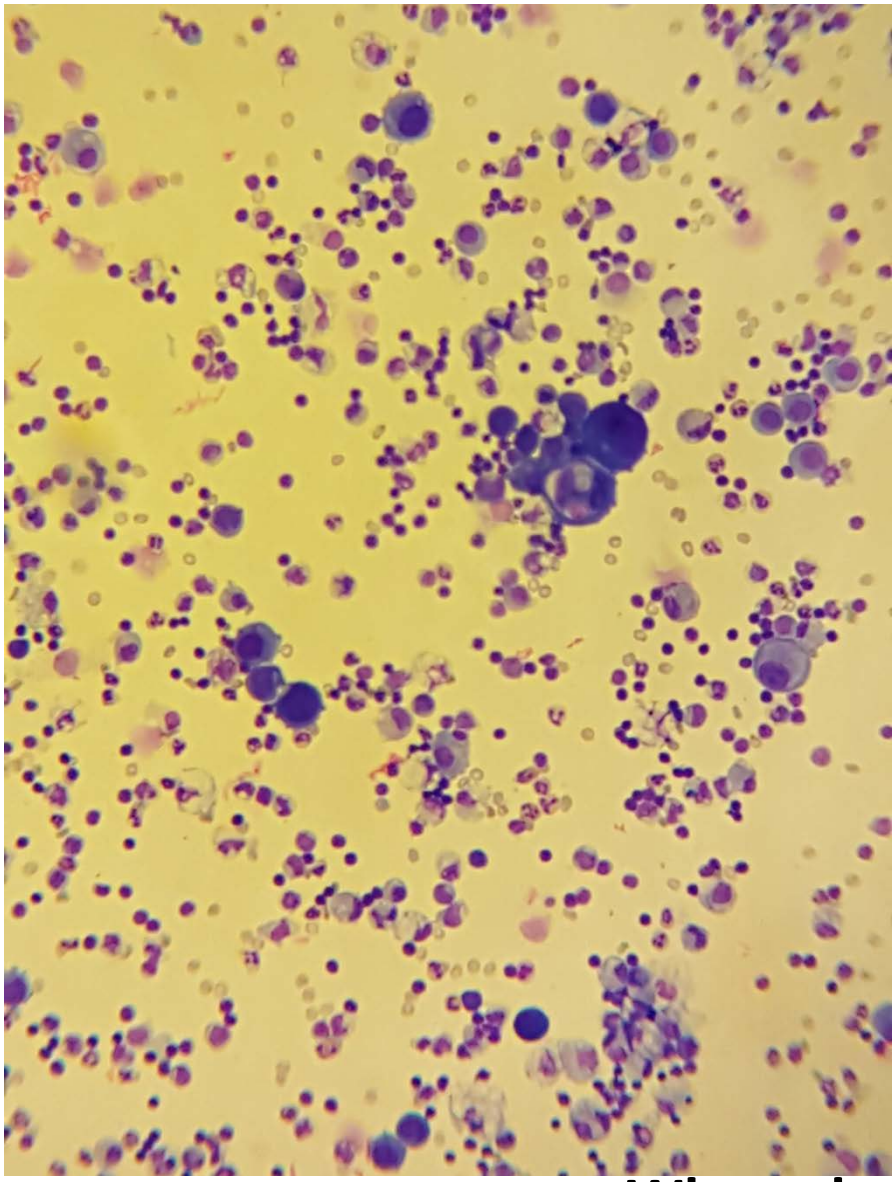


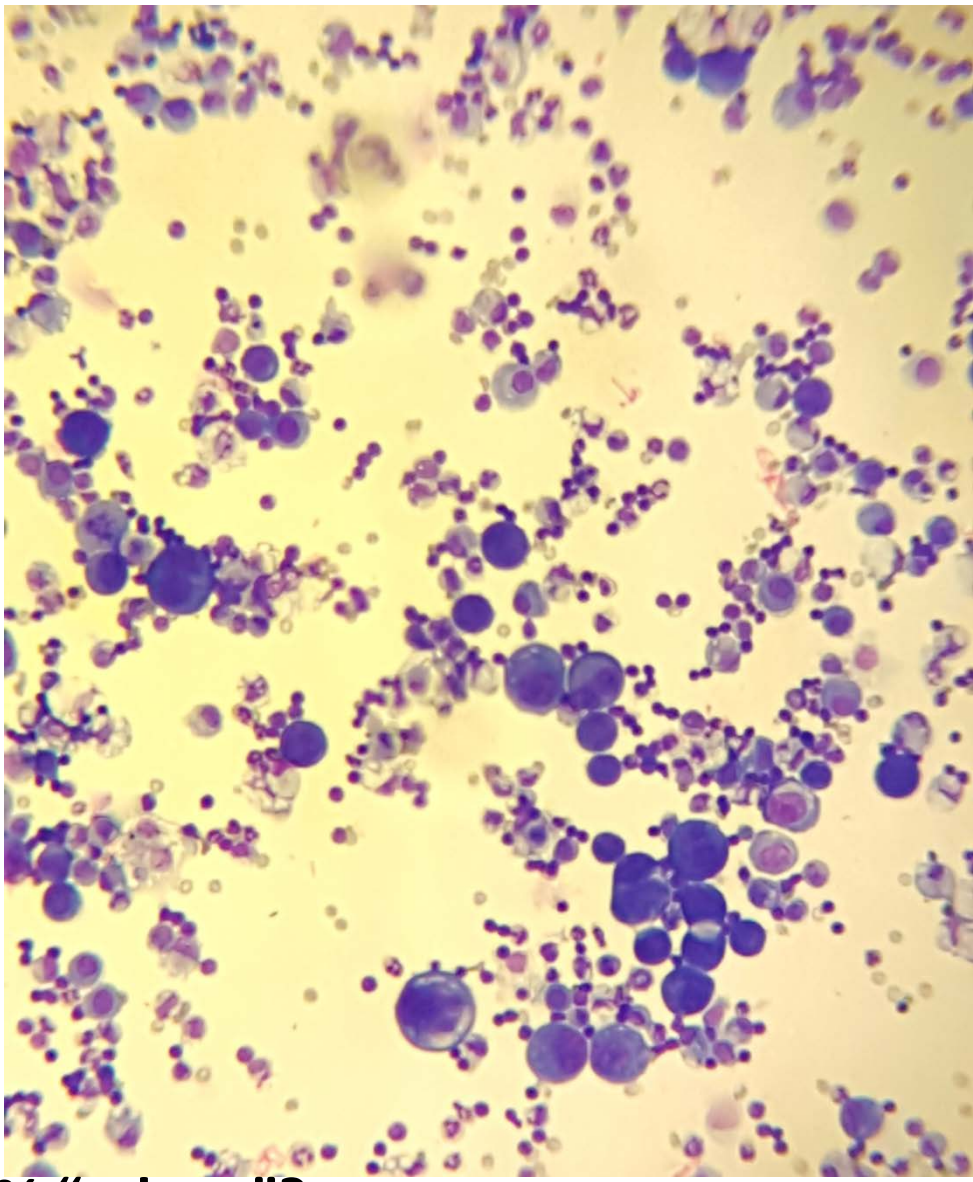
Mesothelial cells are NOT present in CSF

Patient 2

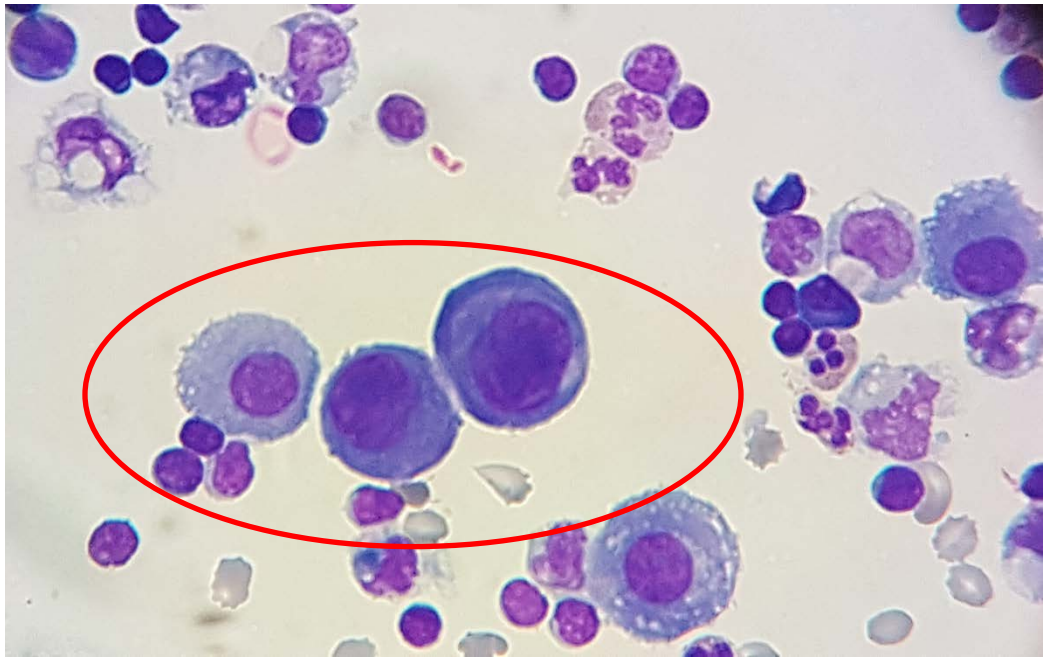
- 75 yr Female
- Presents with pleural effusion
- Pleural fluid
  - TNC-4300
  - RBC-2900
  - Diff= 18% Neut, 53% Lymph, 26% Mono/Macro/Meso, **3% Other**

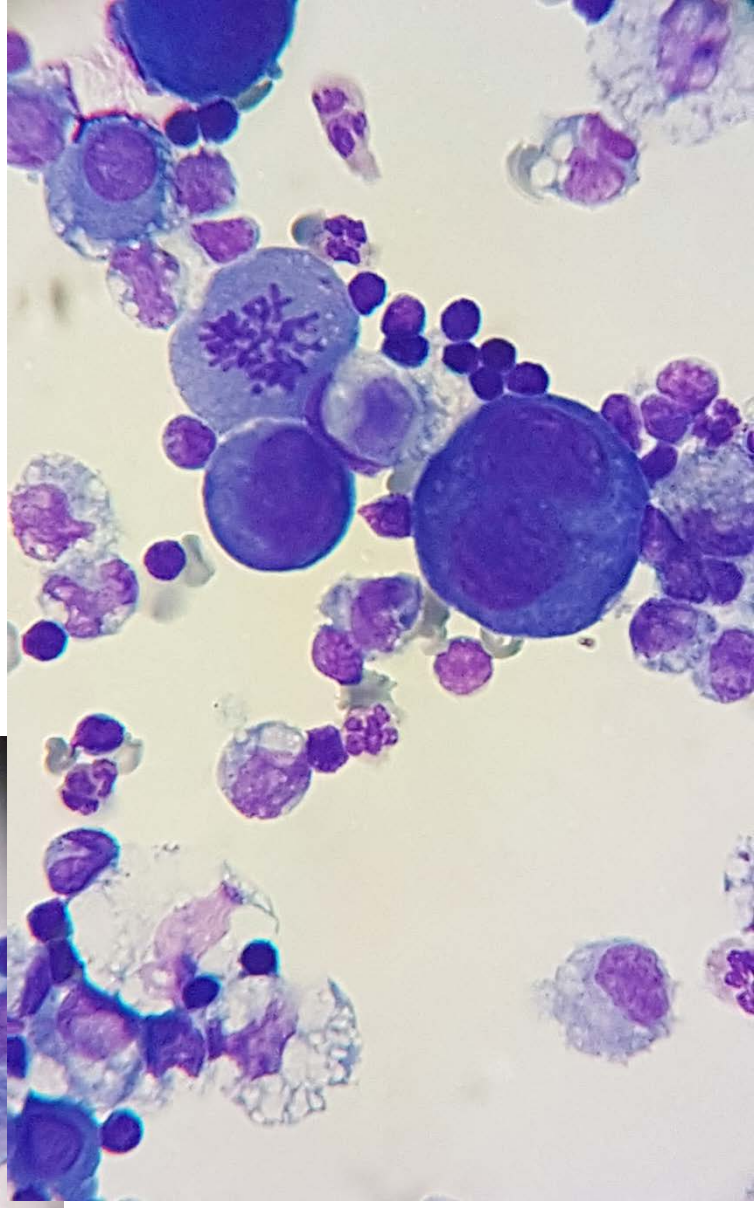
Pleural 10x

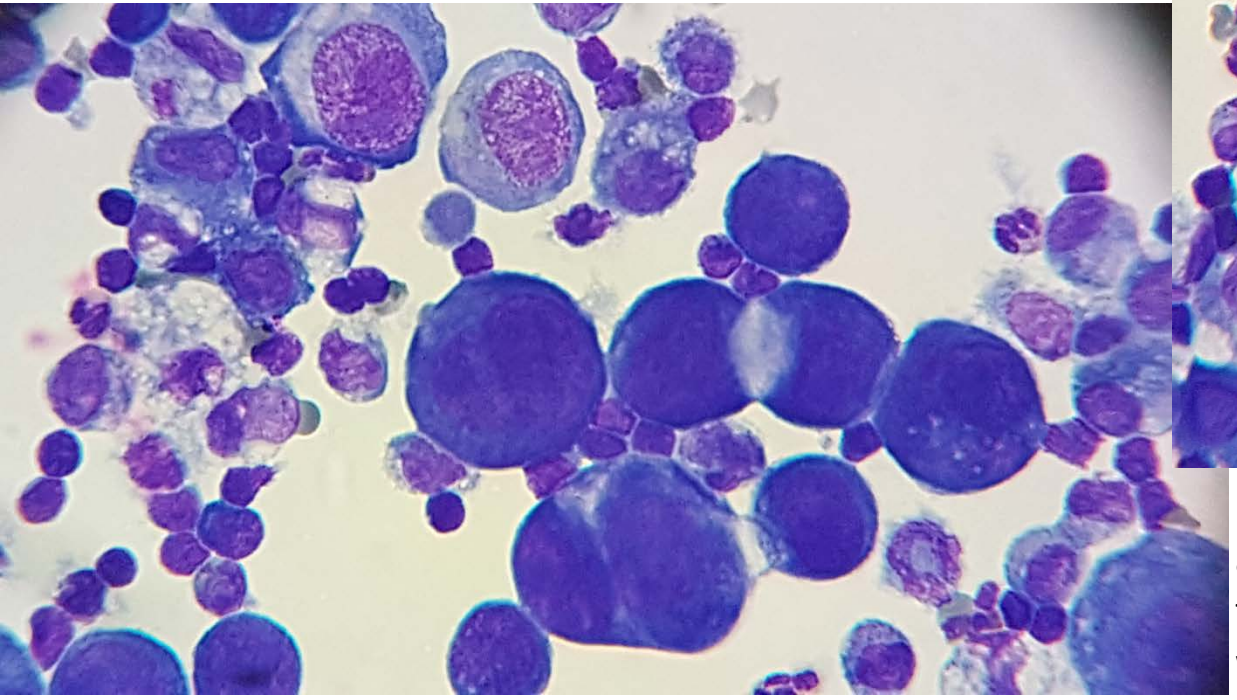




Why only 3% “others”?





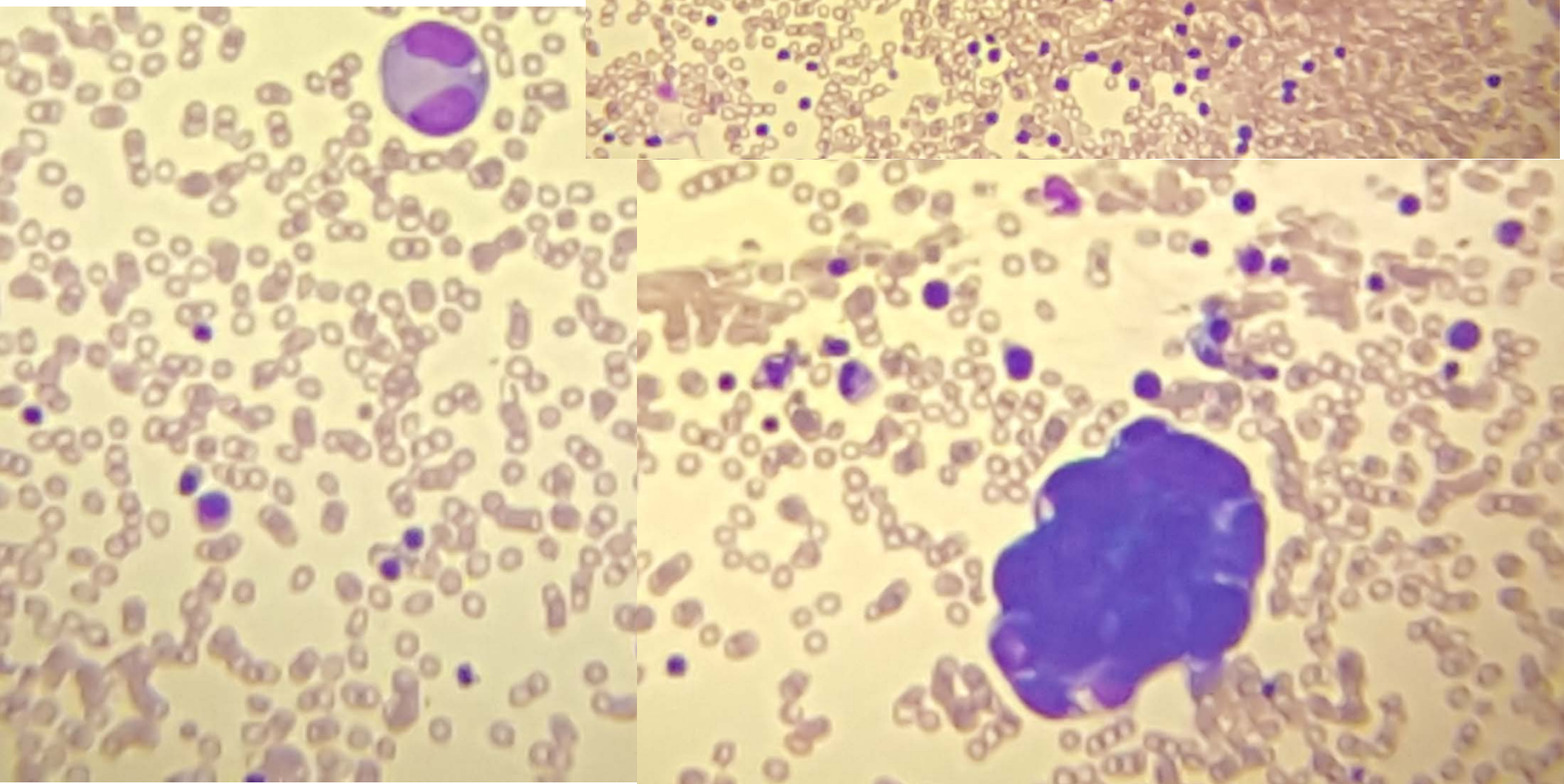


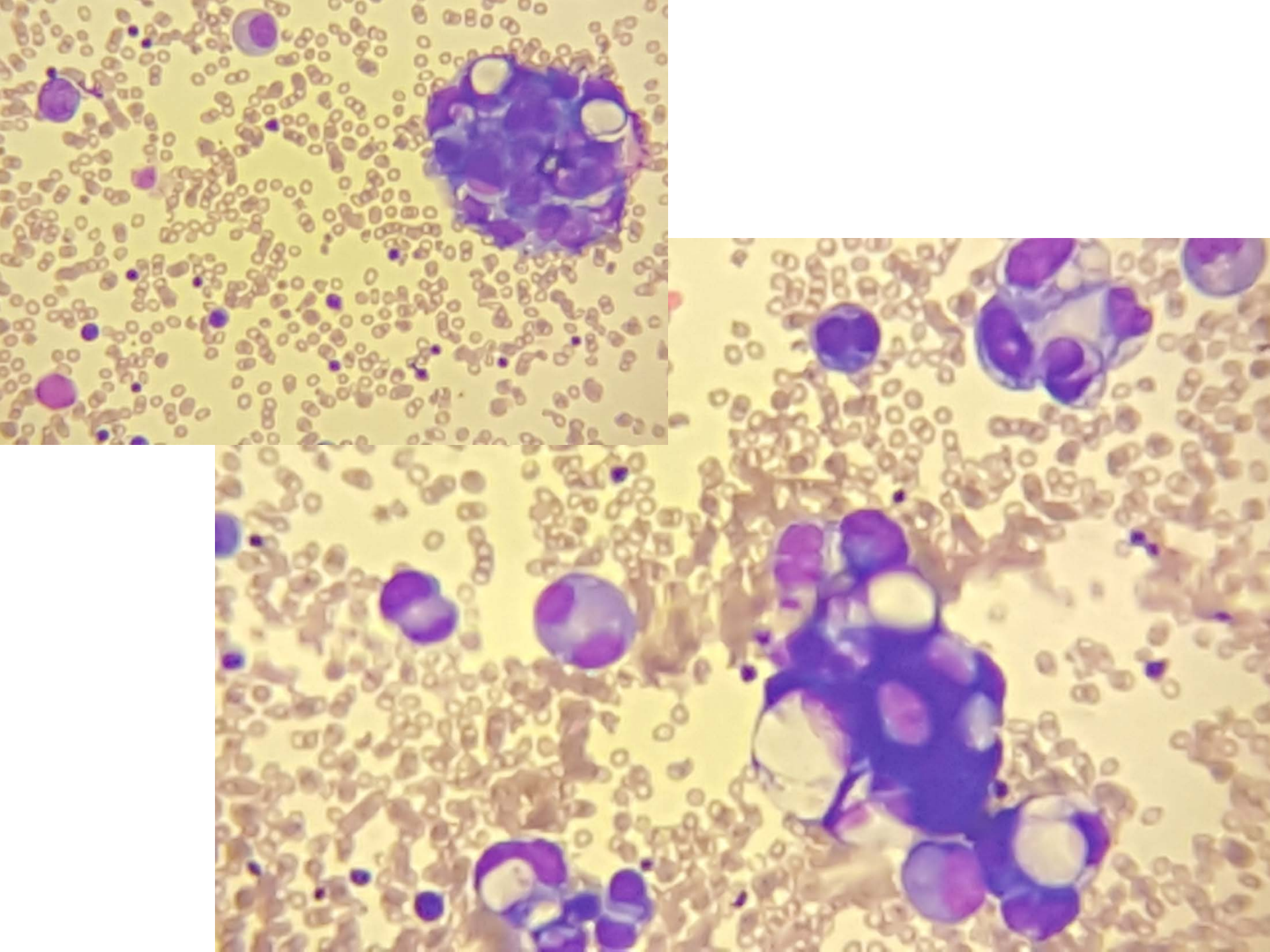
Non-small cell carcinoma- cytologic and immunohistochemical features favor adenocarcinoma (large cells with moderate cytoplasm)

Patient 3

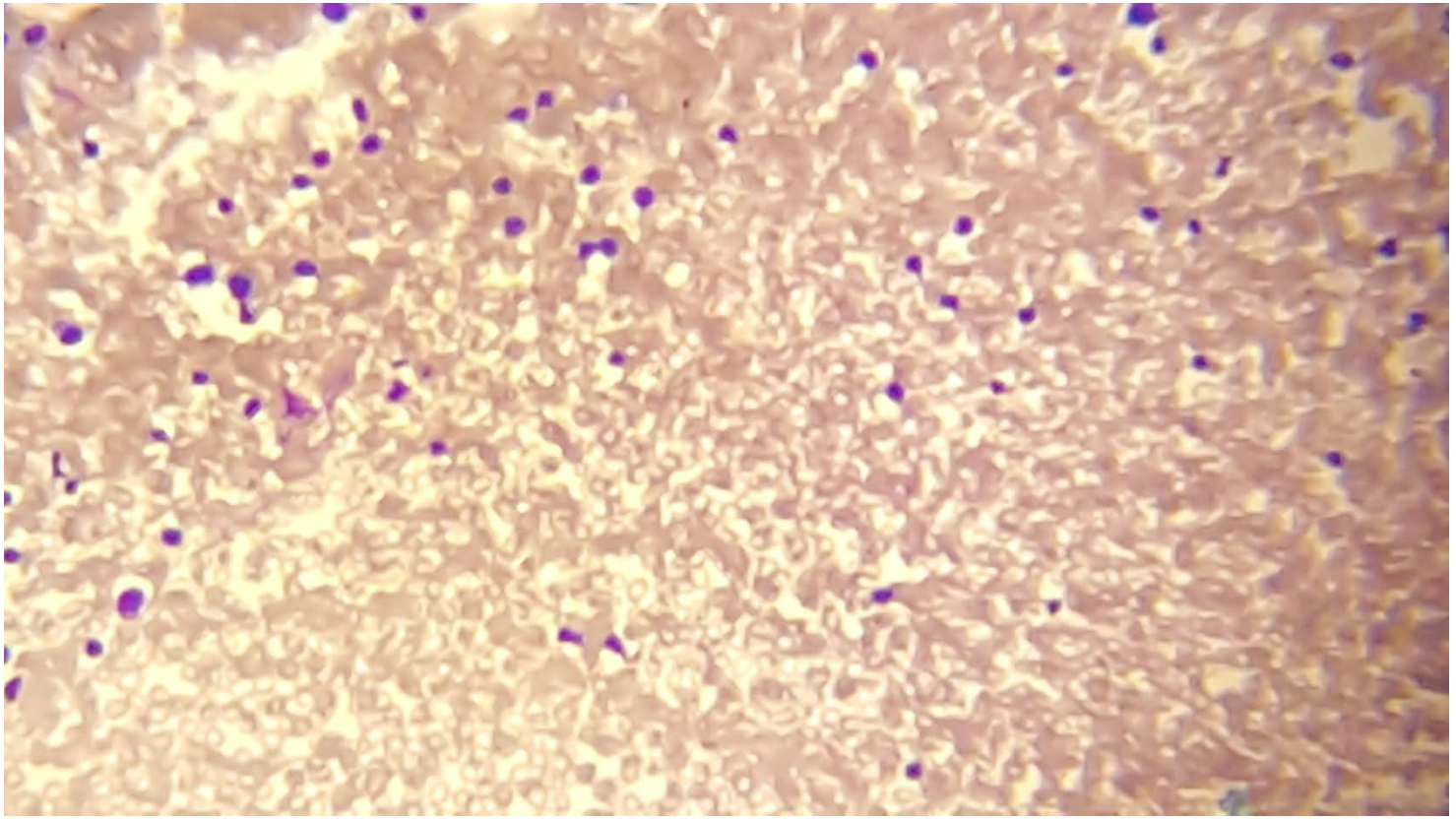
- 69 yr Female
- History of ovarian cancer
- Presents with ascites and left sided pleural effusion on evening shift
- Peritoneal fluid
  - TNC-2172
  - RBC-2848
  - Diff=31% Neuts, 30% Lymphs, 39% Macrophages
- Pleural fluid
  - TNC-476
  - RBC-25,825
  - Diff left for days

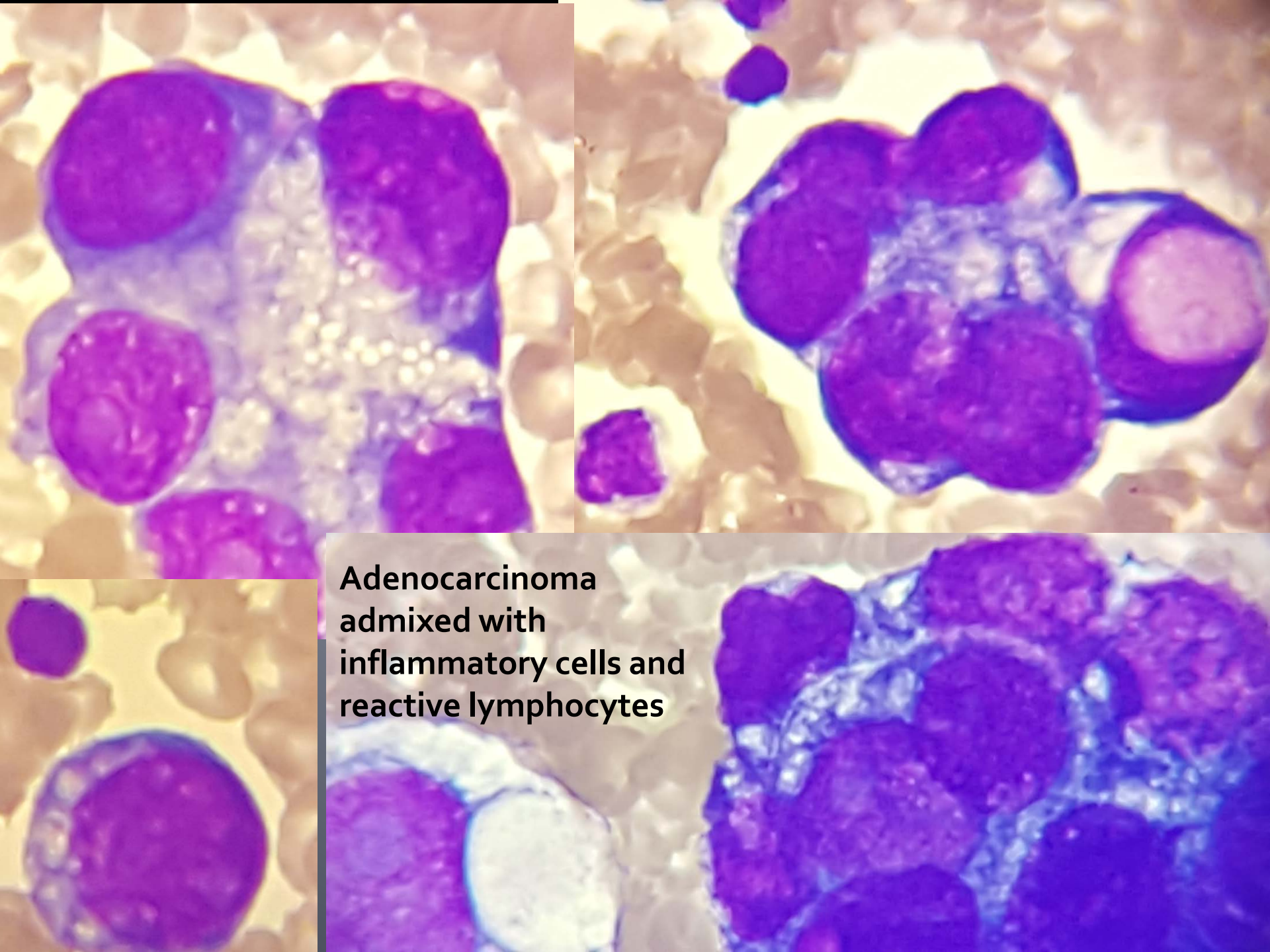
PLEURAL
10X





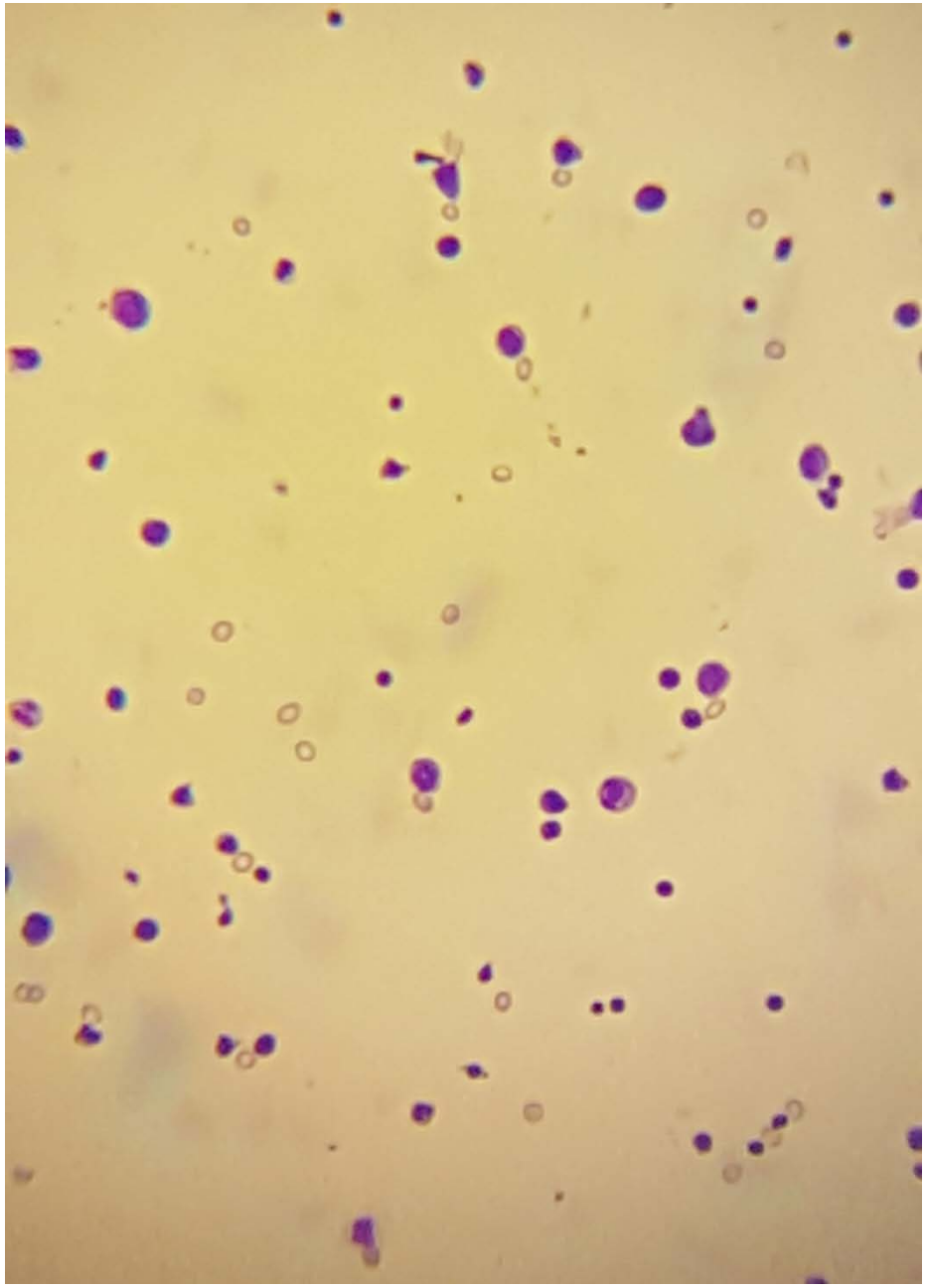
I FOUND ONE NORMAL FIELD!!

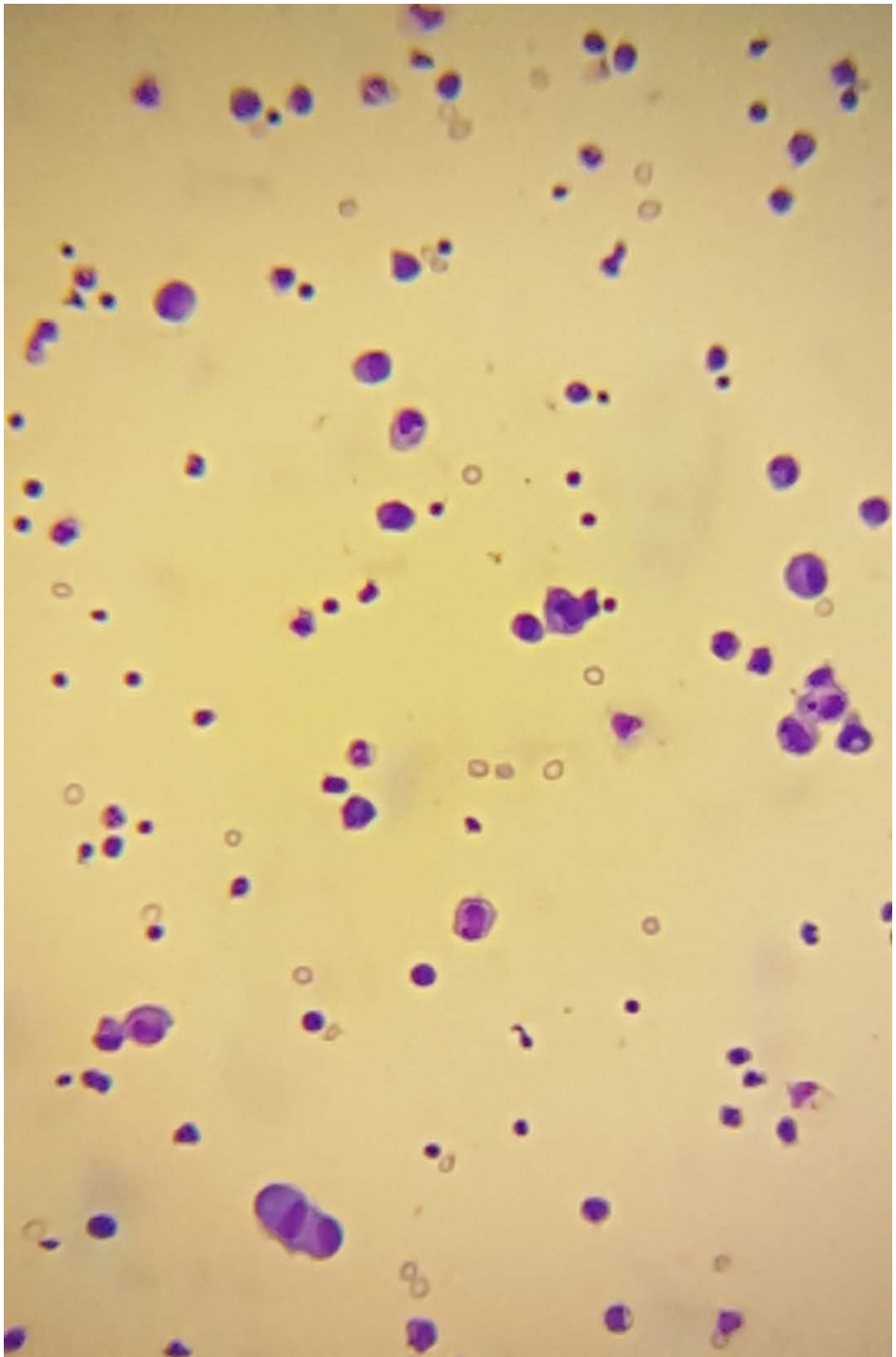


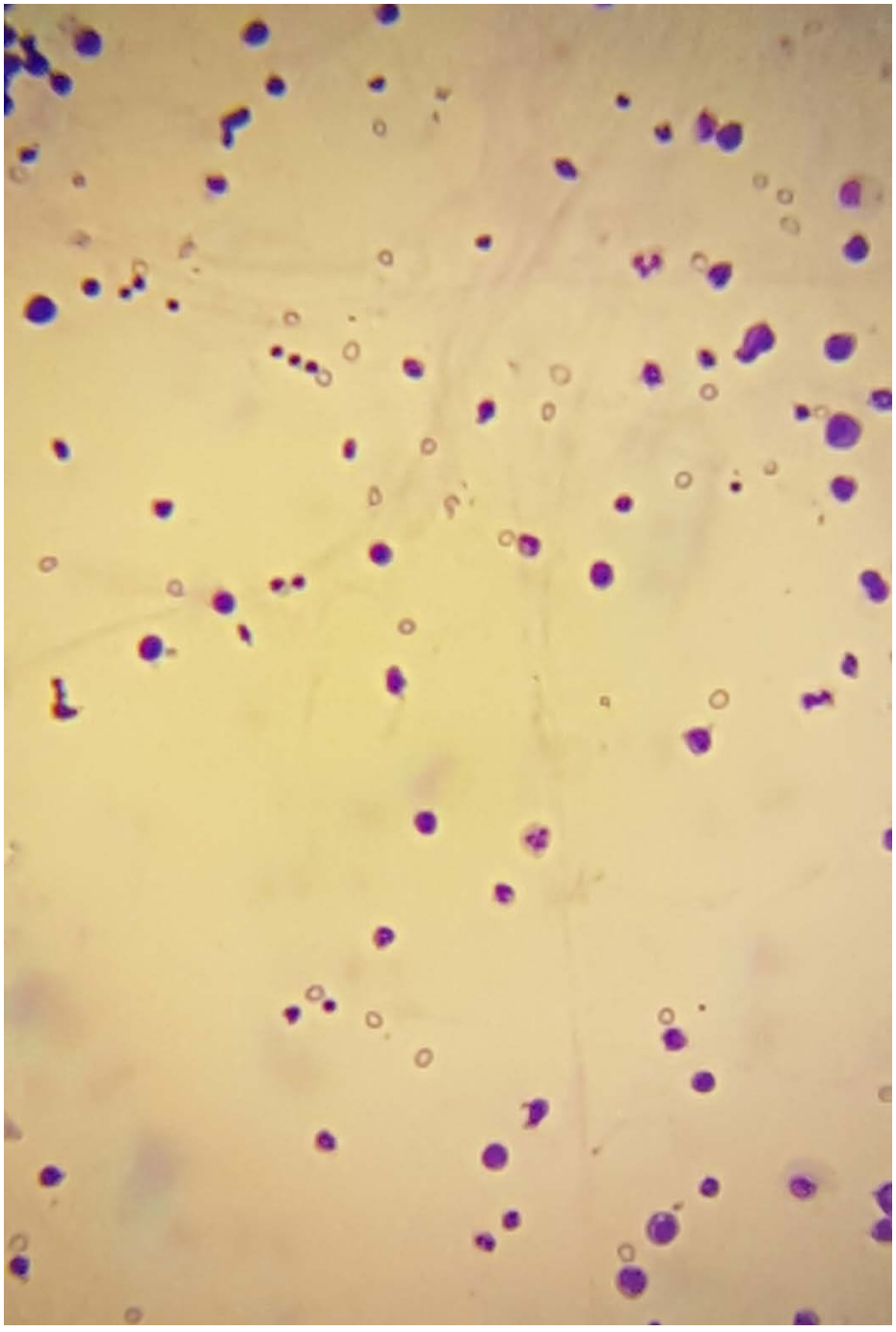

Adenocarcinoma
admixed with
inflammatory cells and
reactive lymphocytes

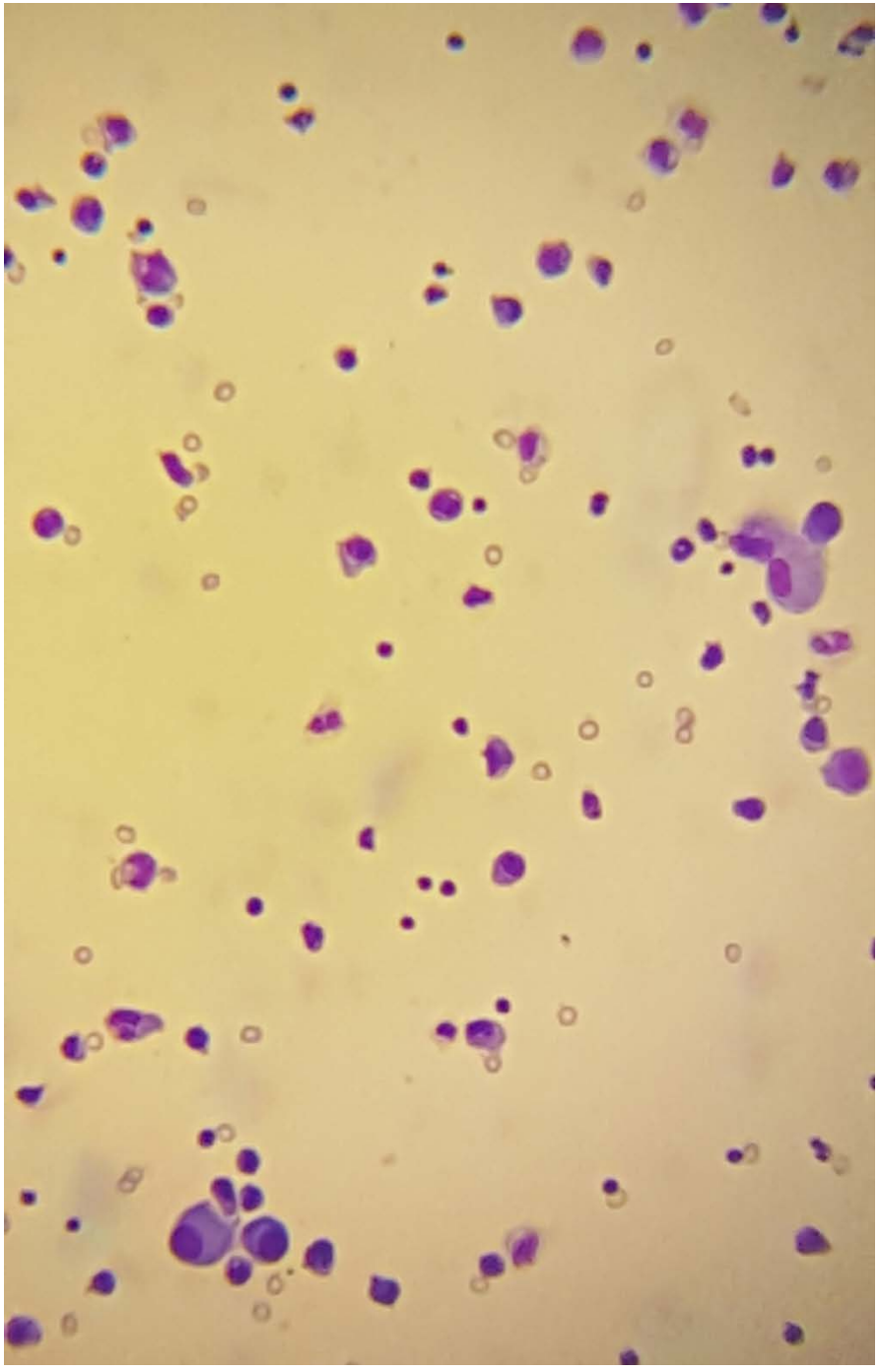
My inner nerd is showing….

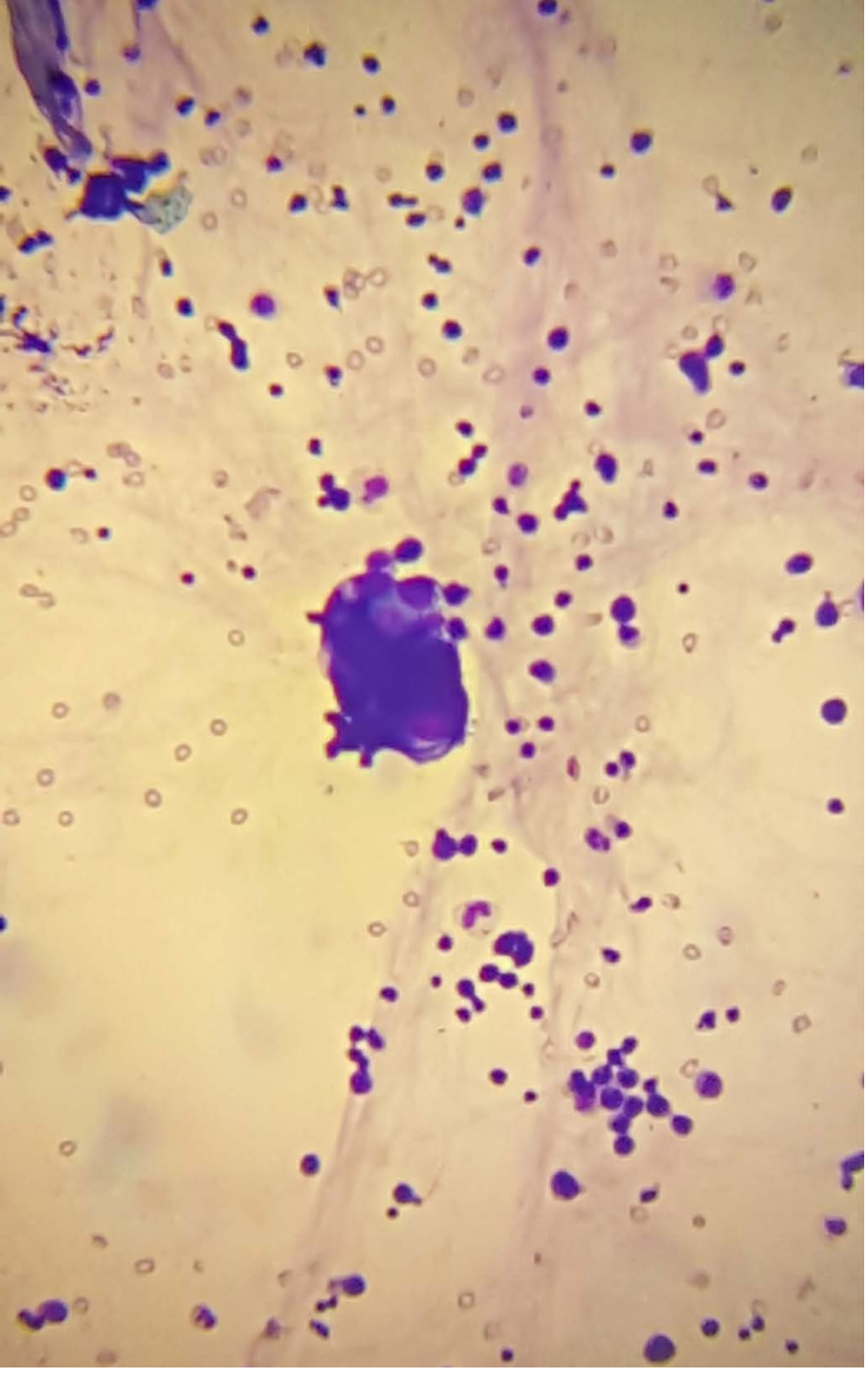
PERITONEAL FLUID 10X

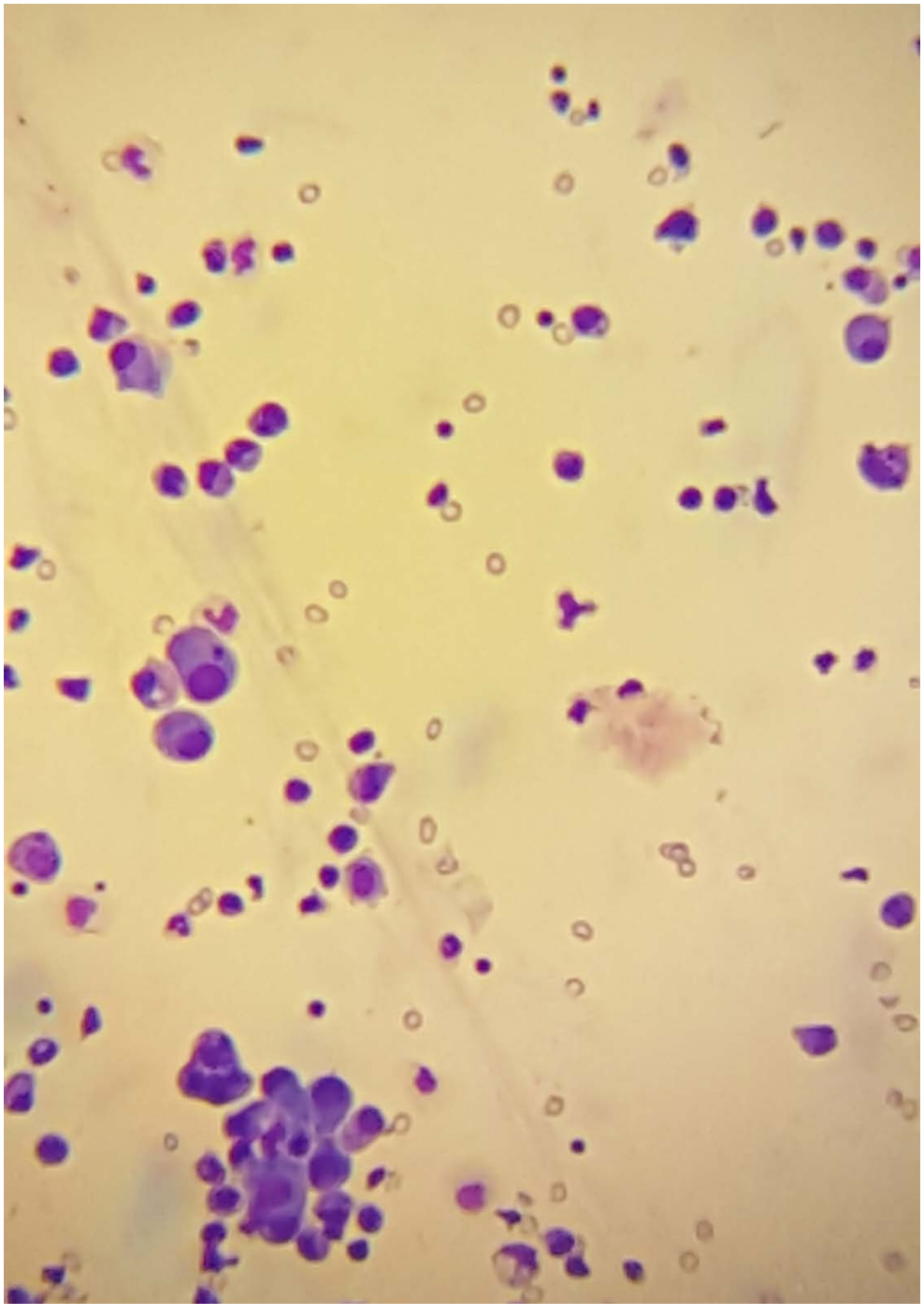


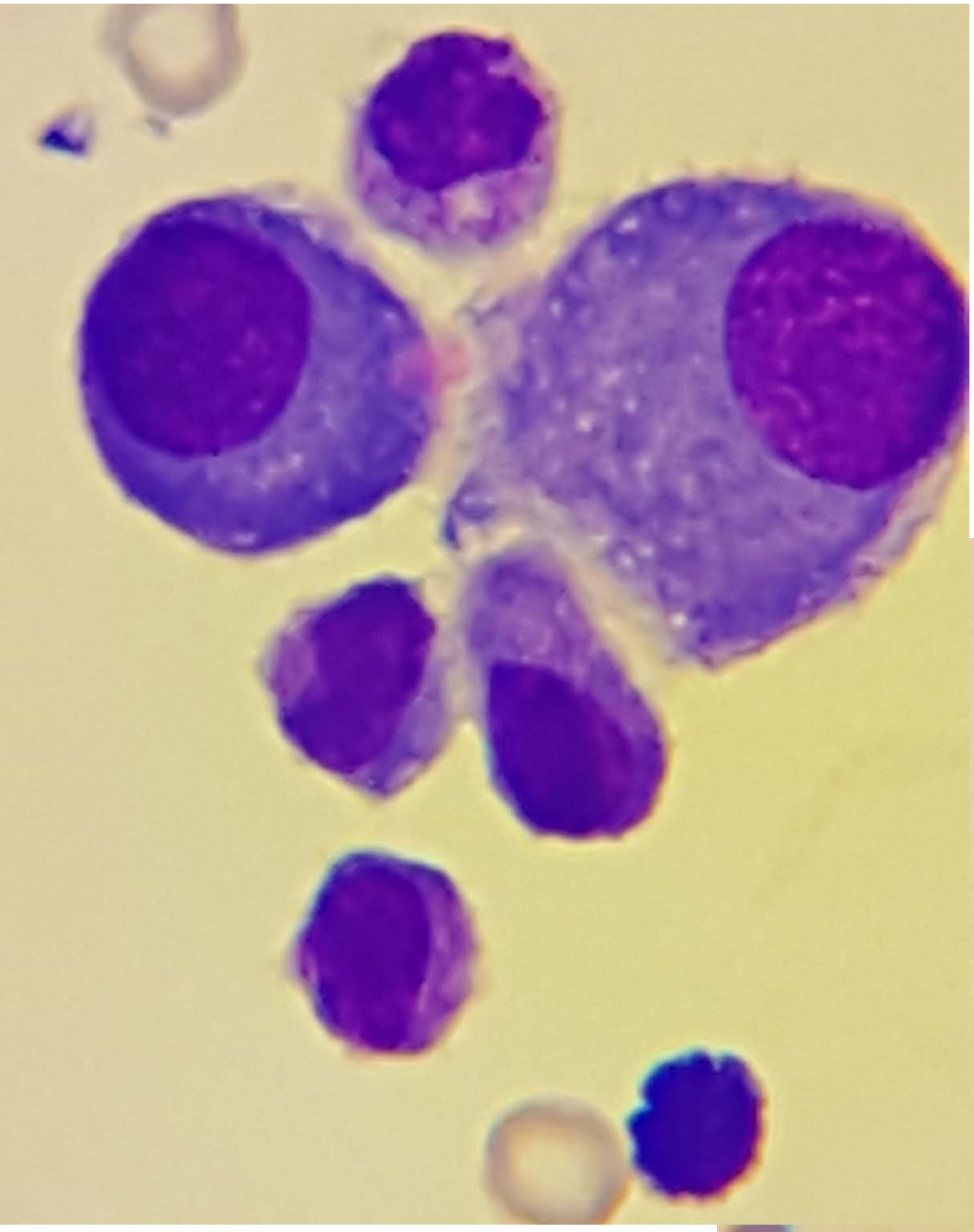


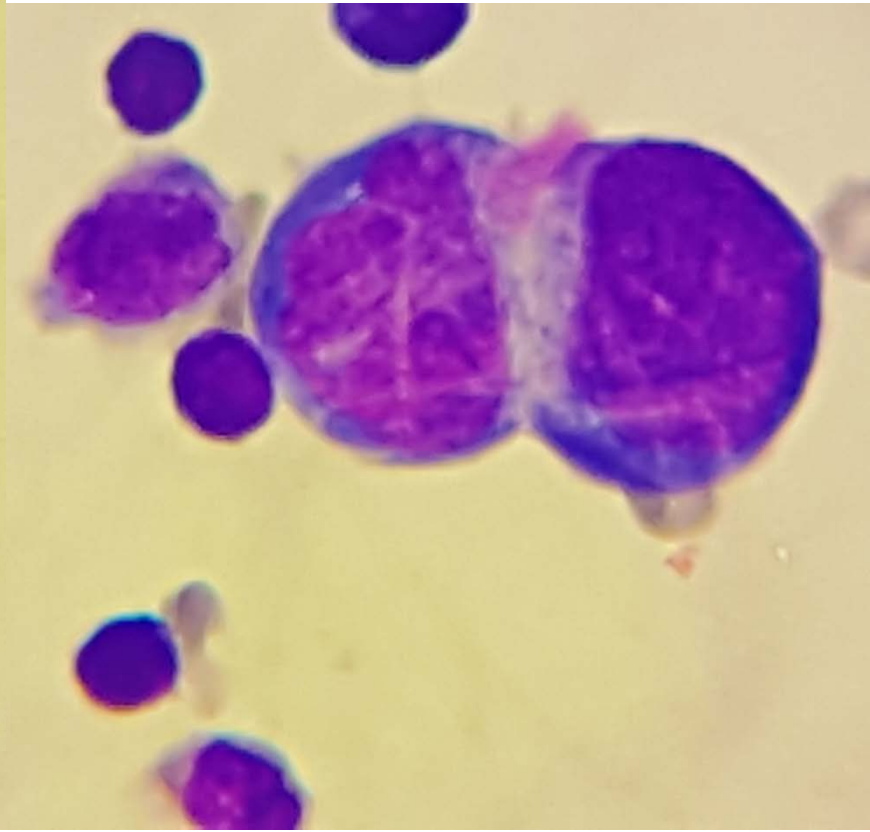


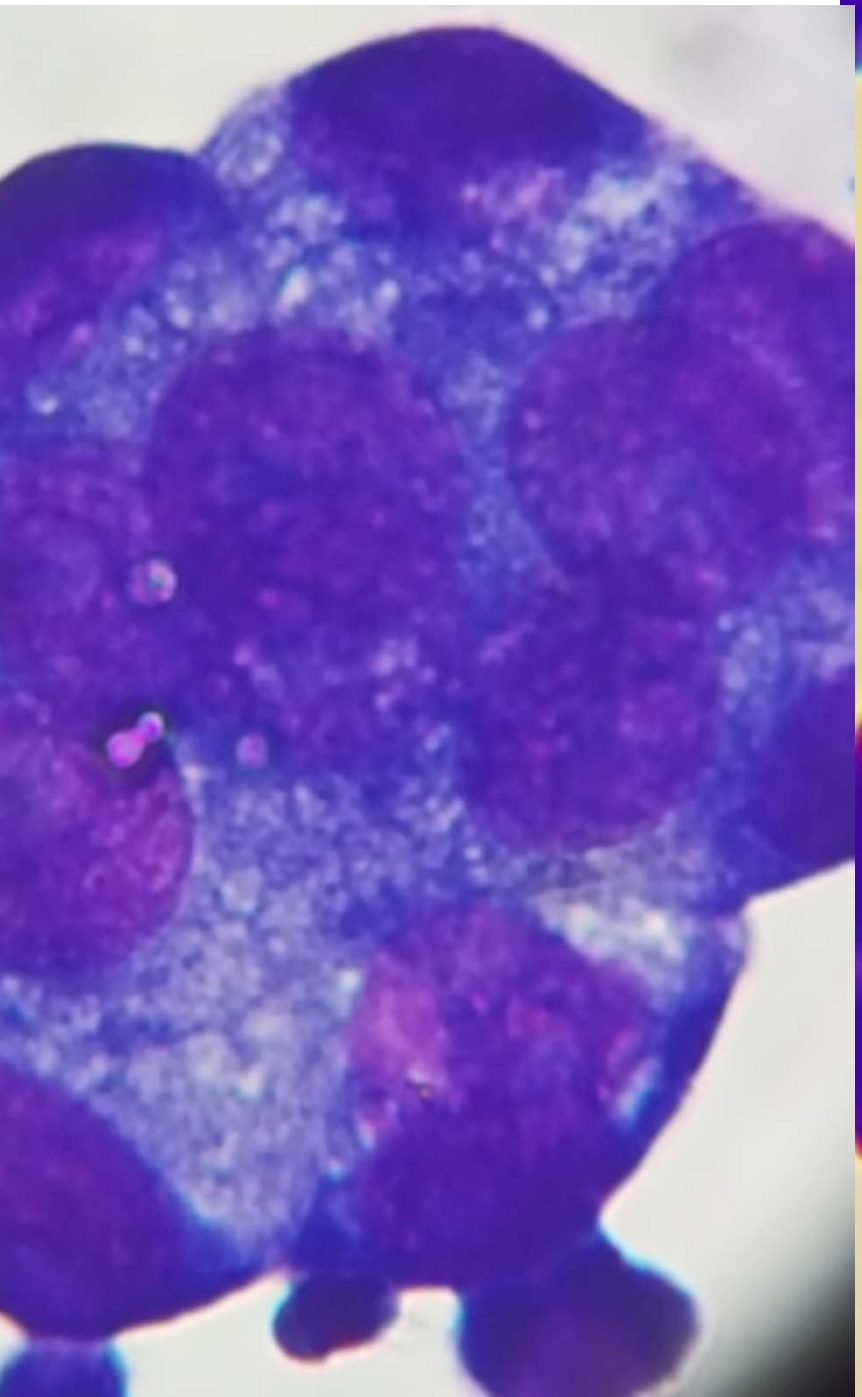


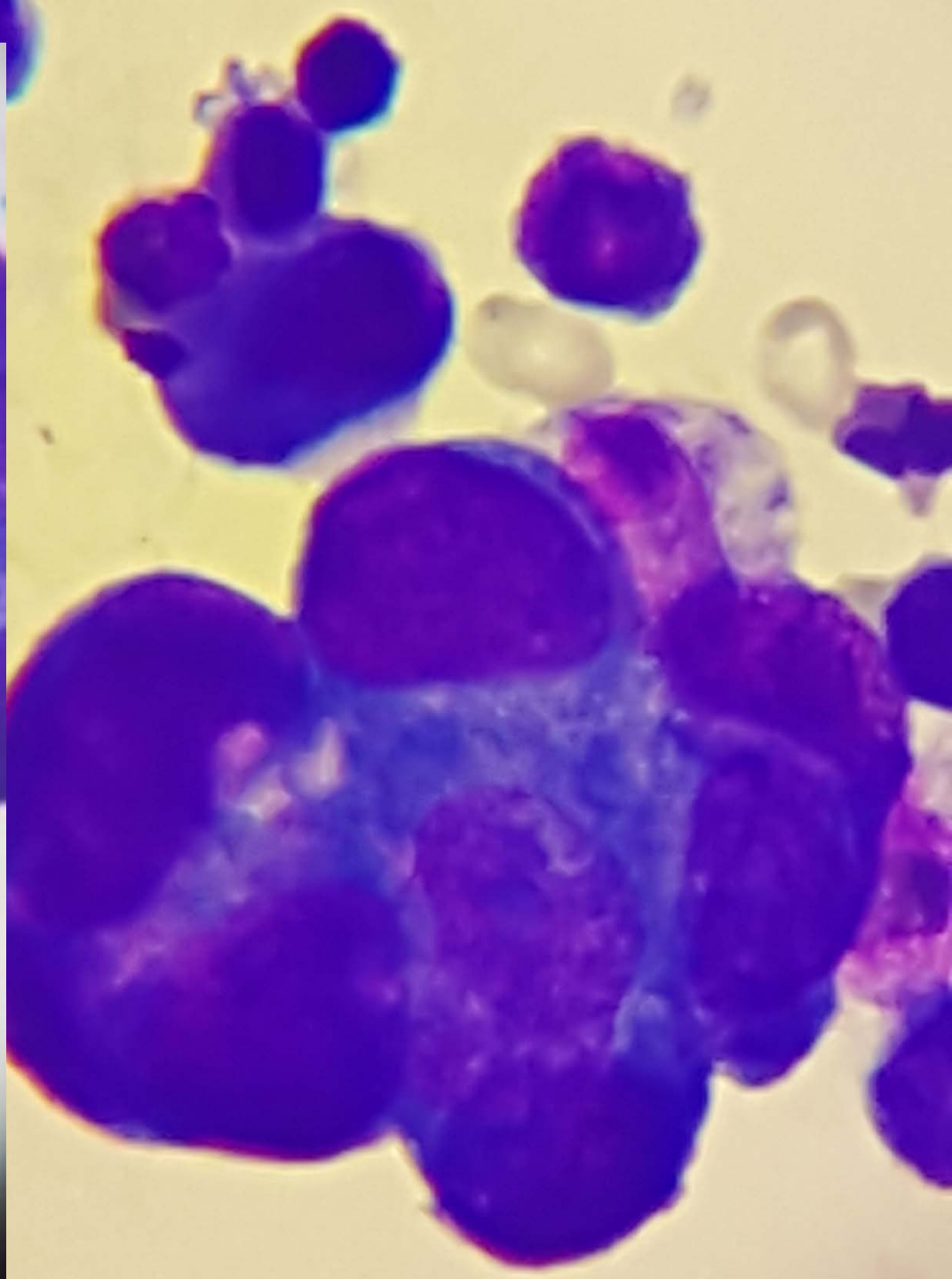


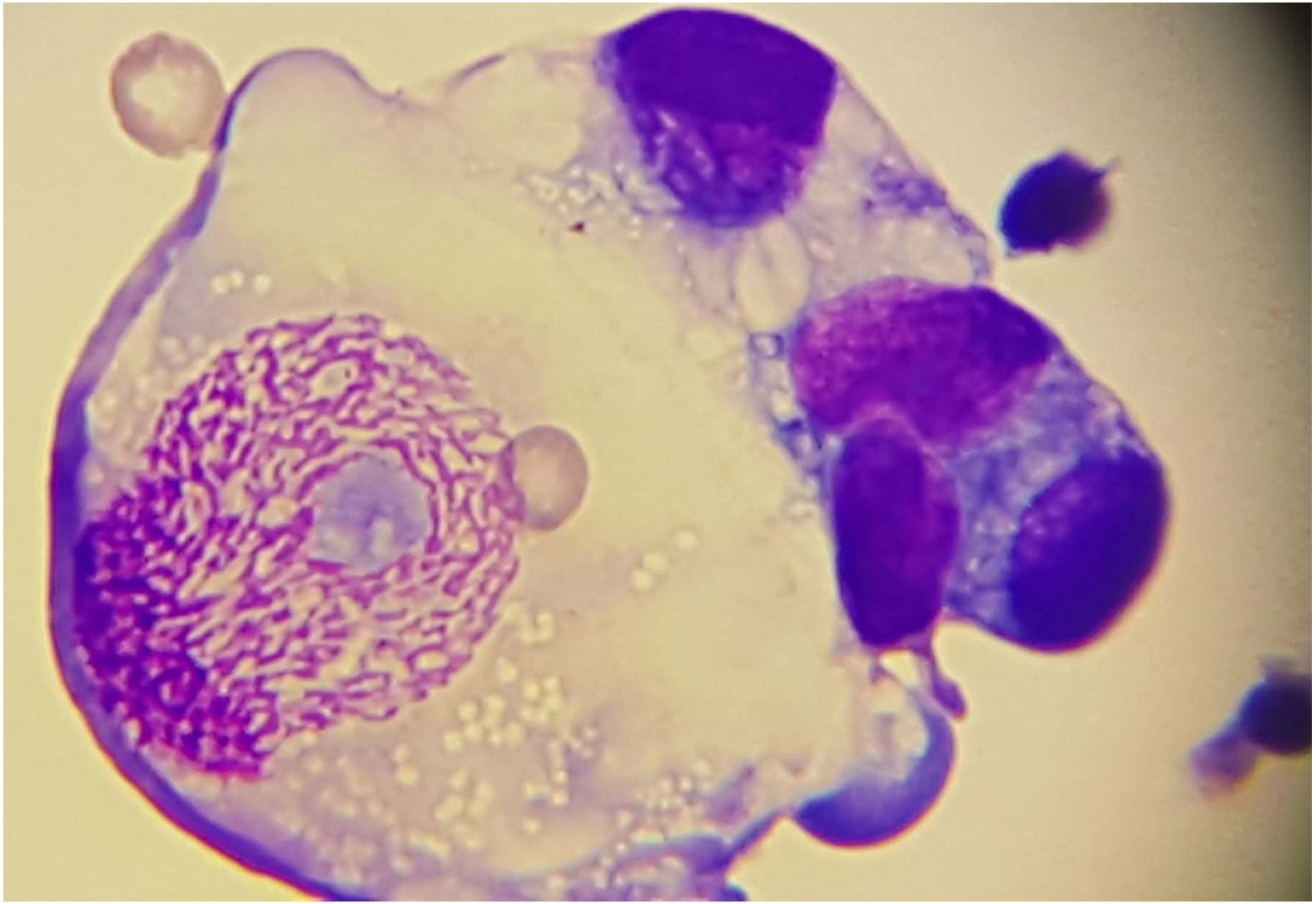


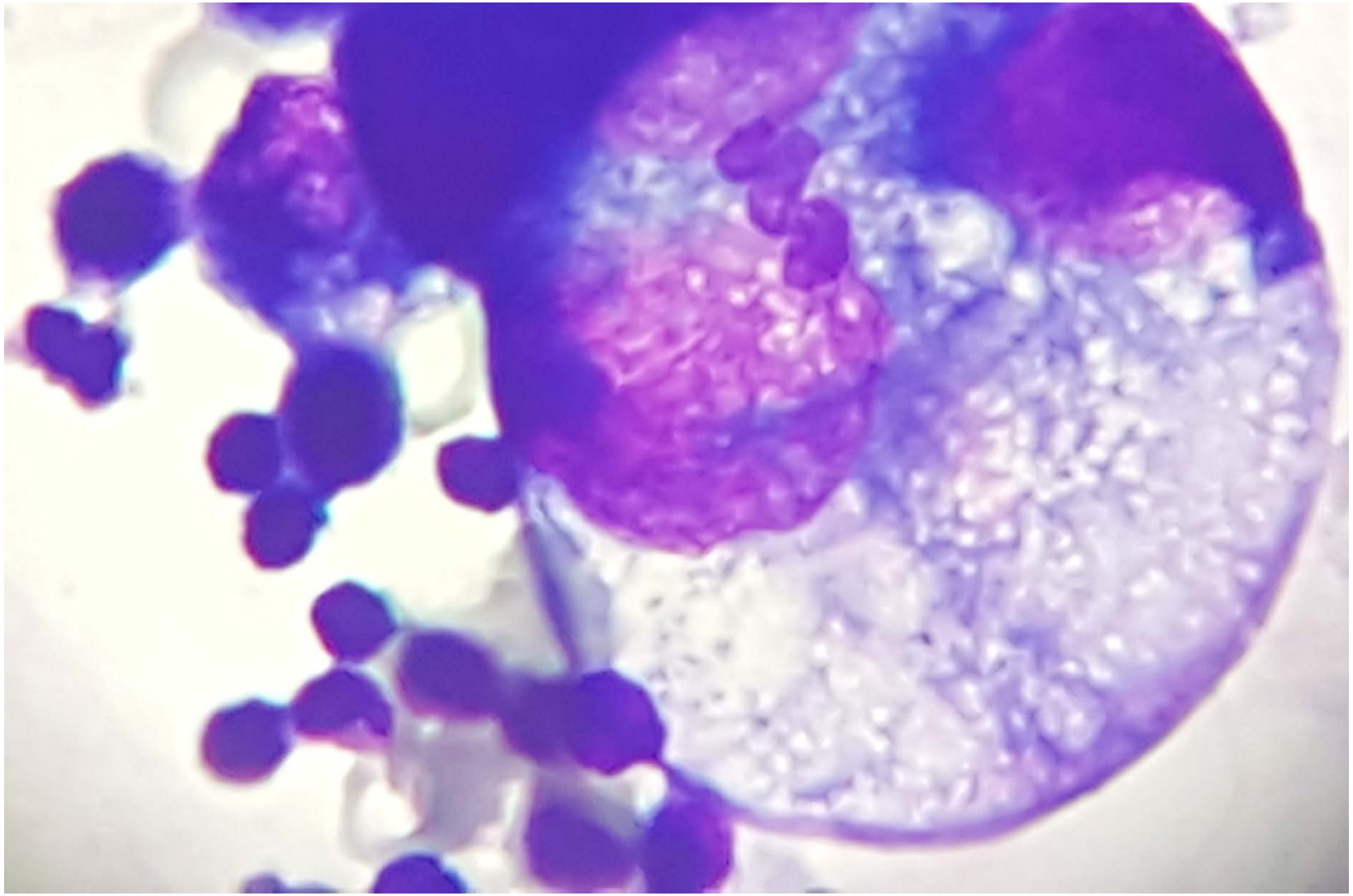


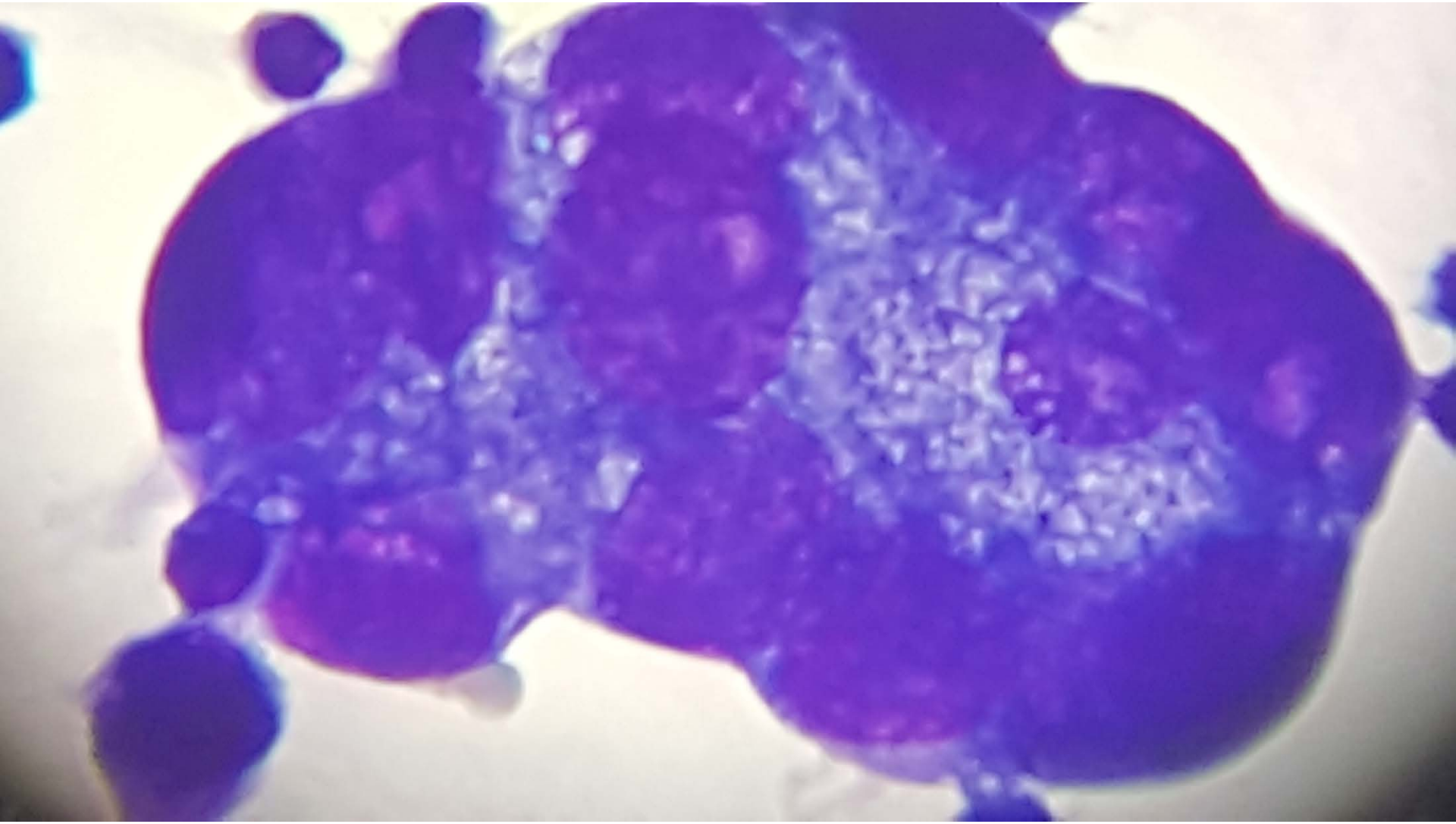












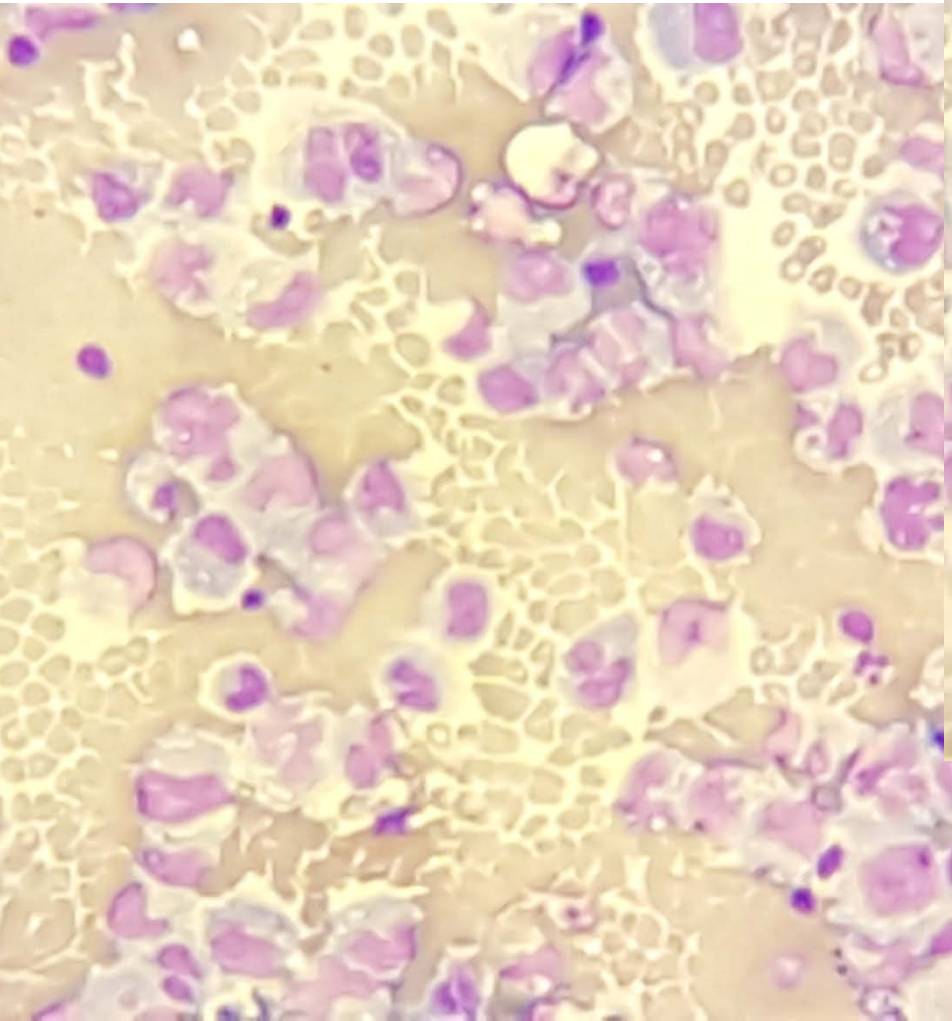
Patient 3 con’t

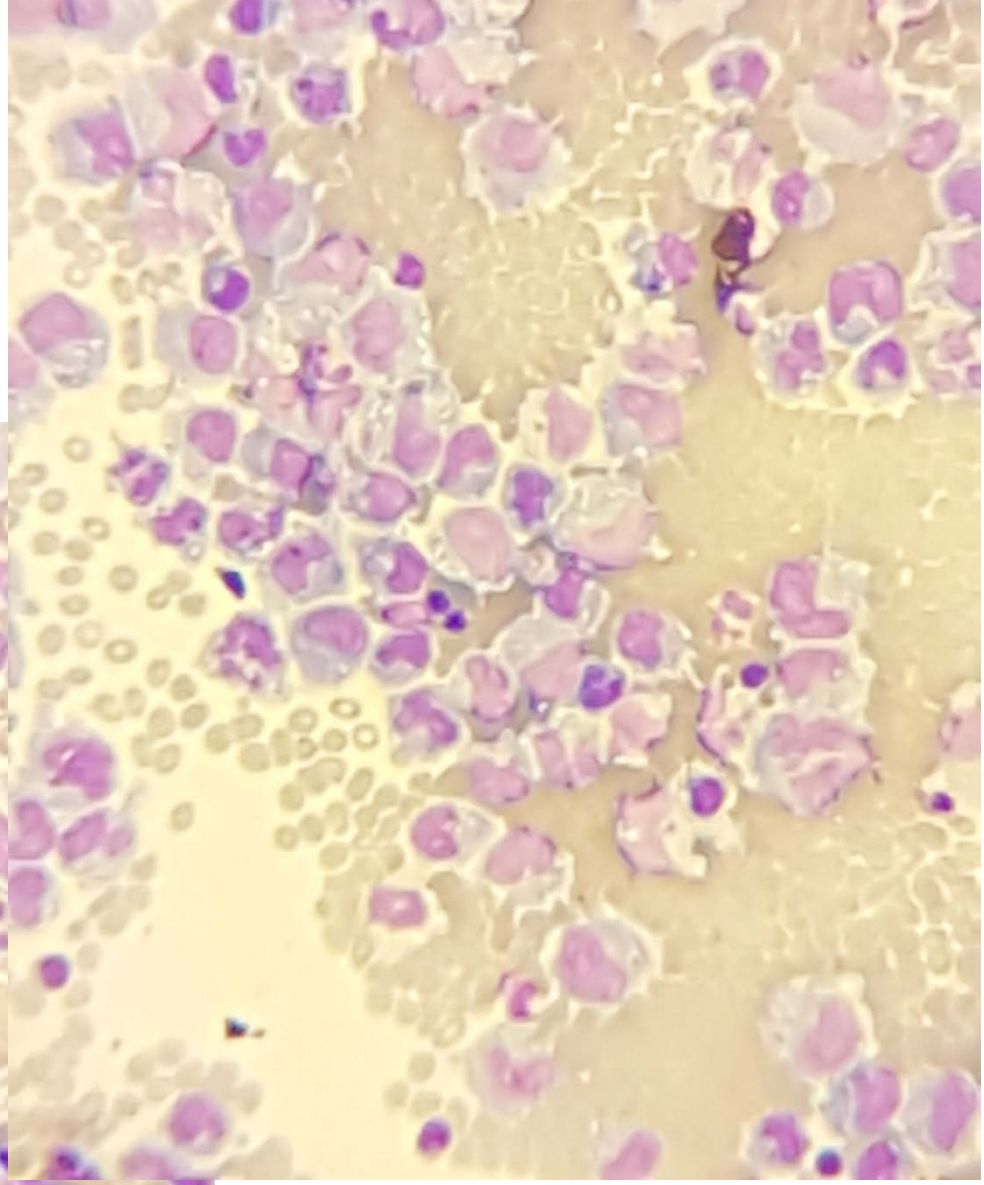
- Patient report had to be amended, had this been the only fluid sent, it would have probably been missed

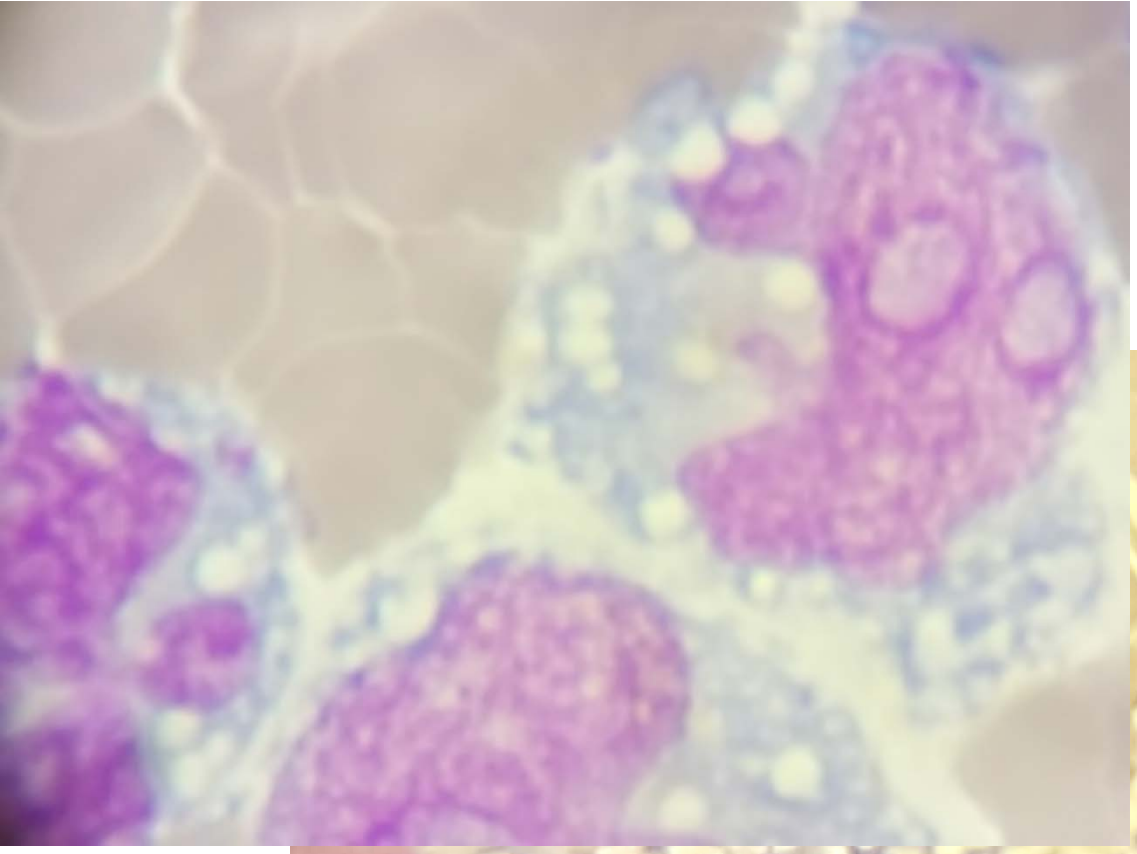
Patient 4

- 67 yr Male
- History of renal transplant
- Pleural Fluid
  - TNC-3331
  - RBC-179,000
  - First tech counted primarily macrophages

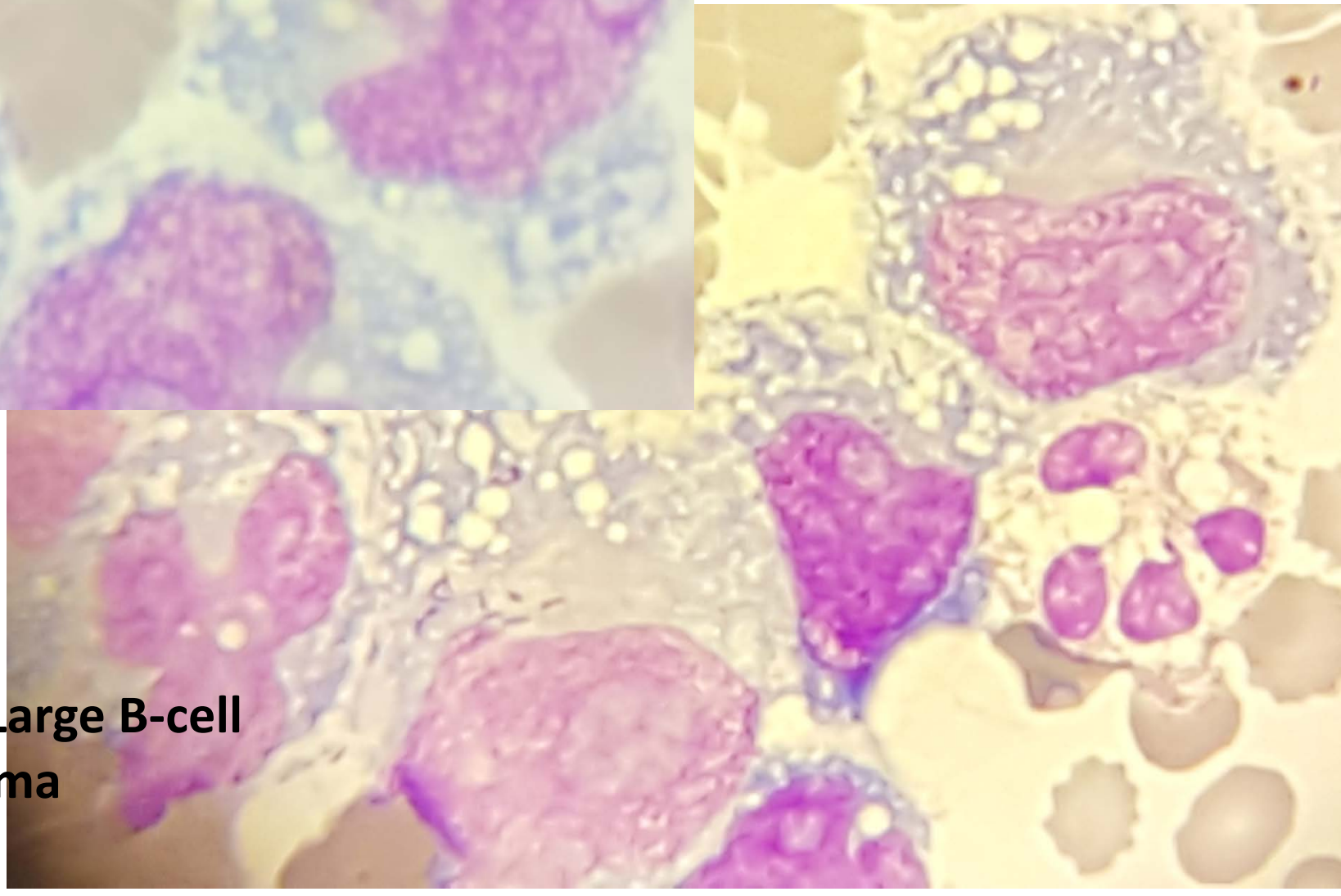
10x



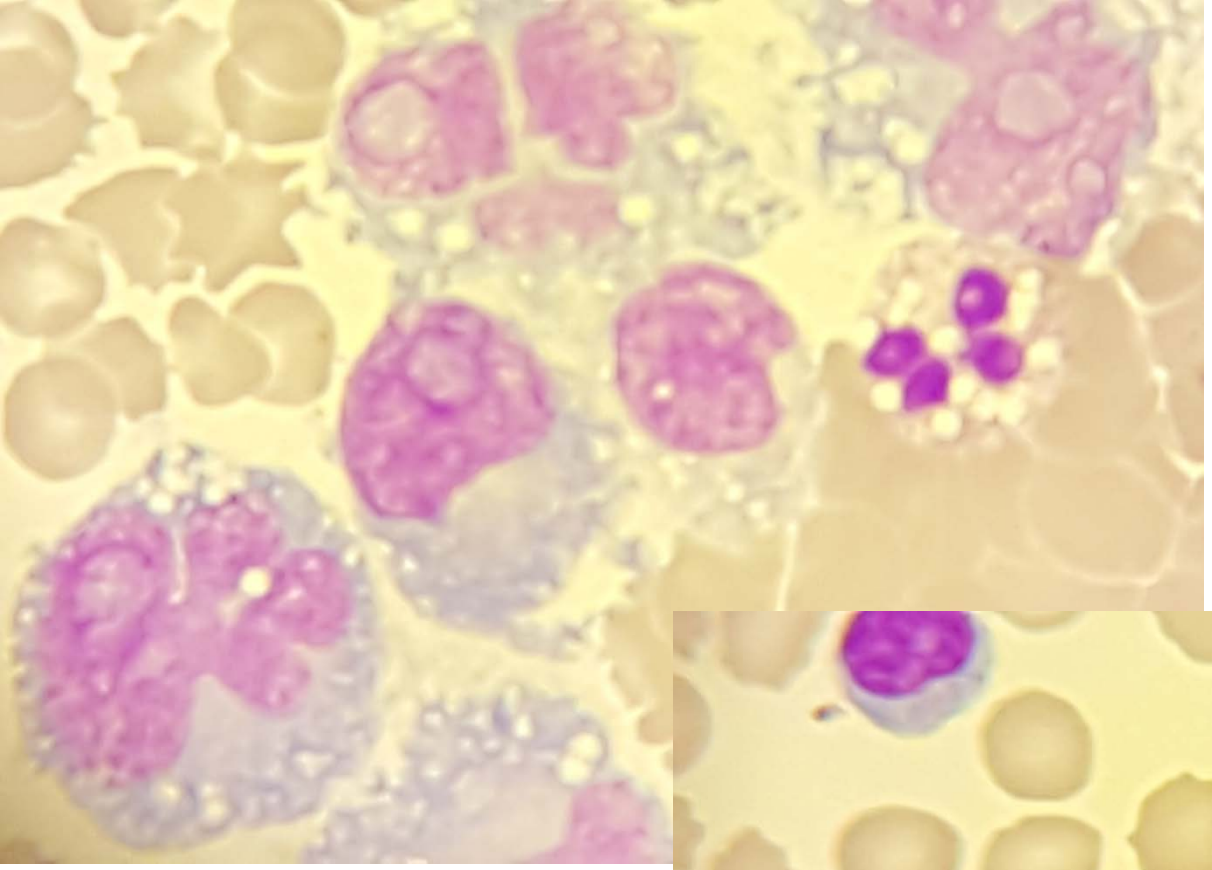


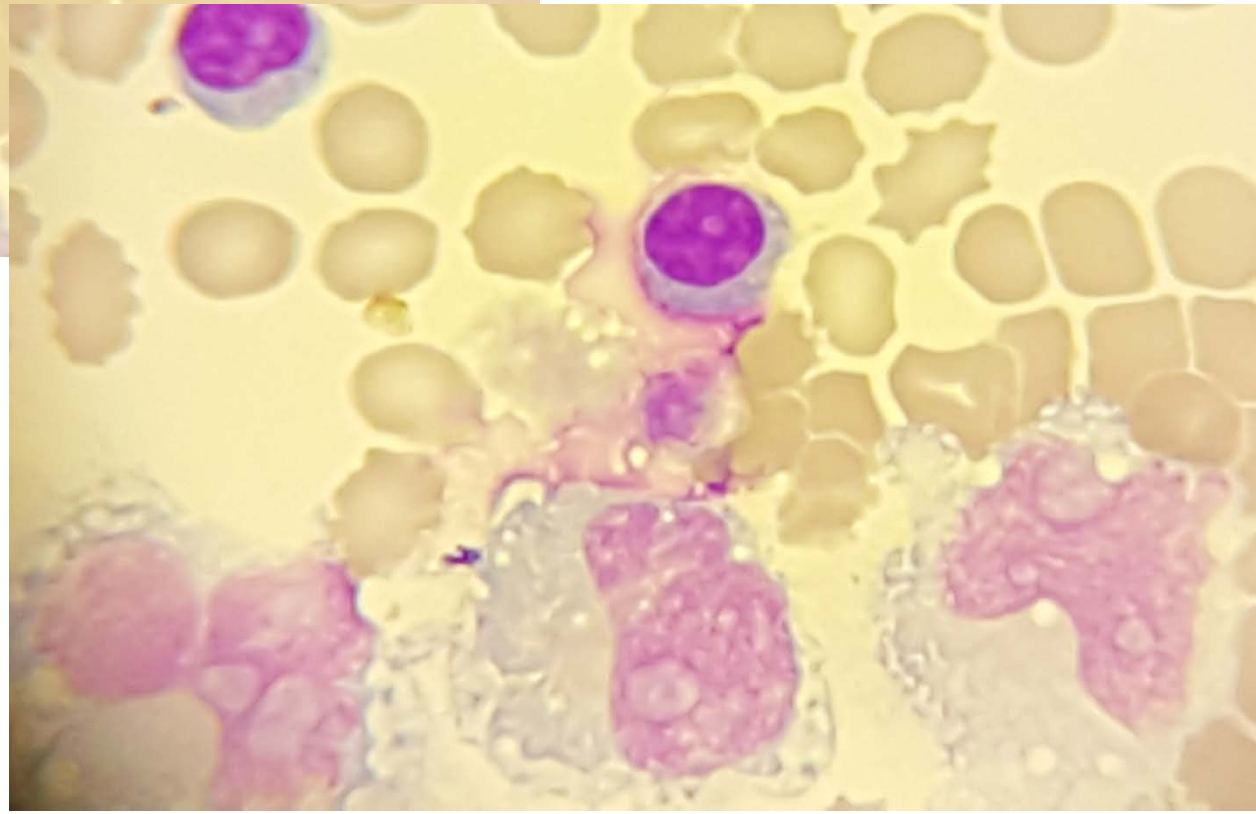


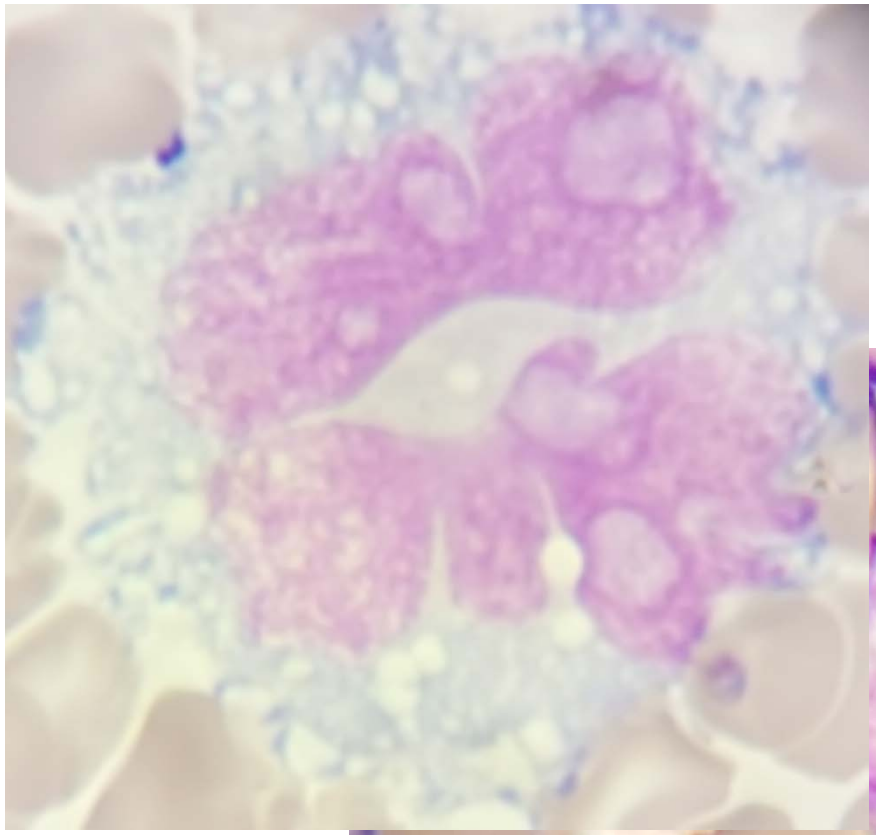
SECOND TECH COUNTED 87% "OTHER"

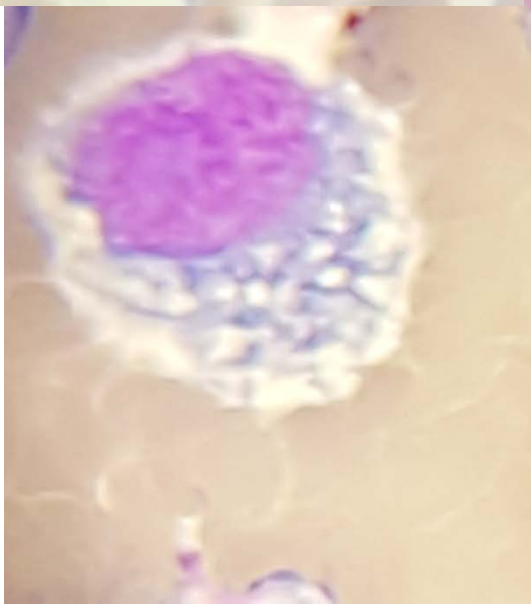


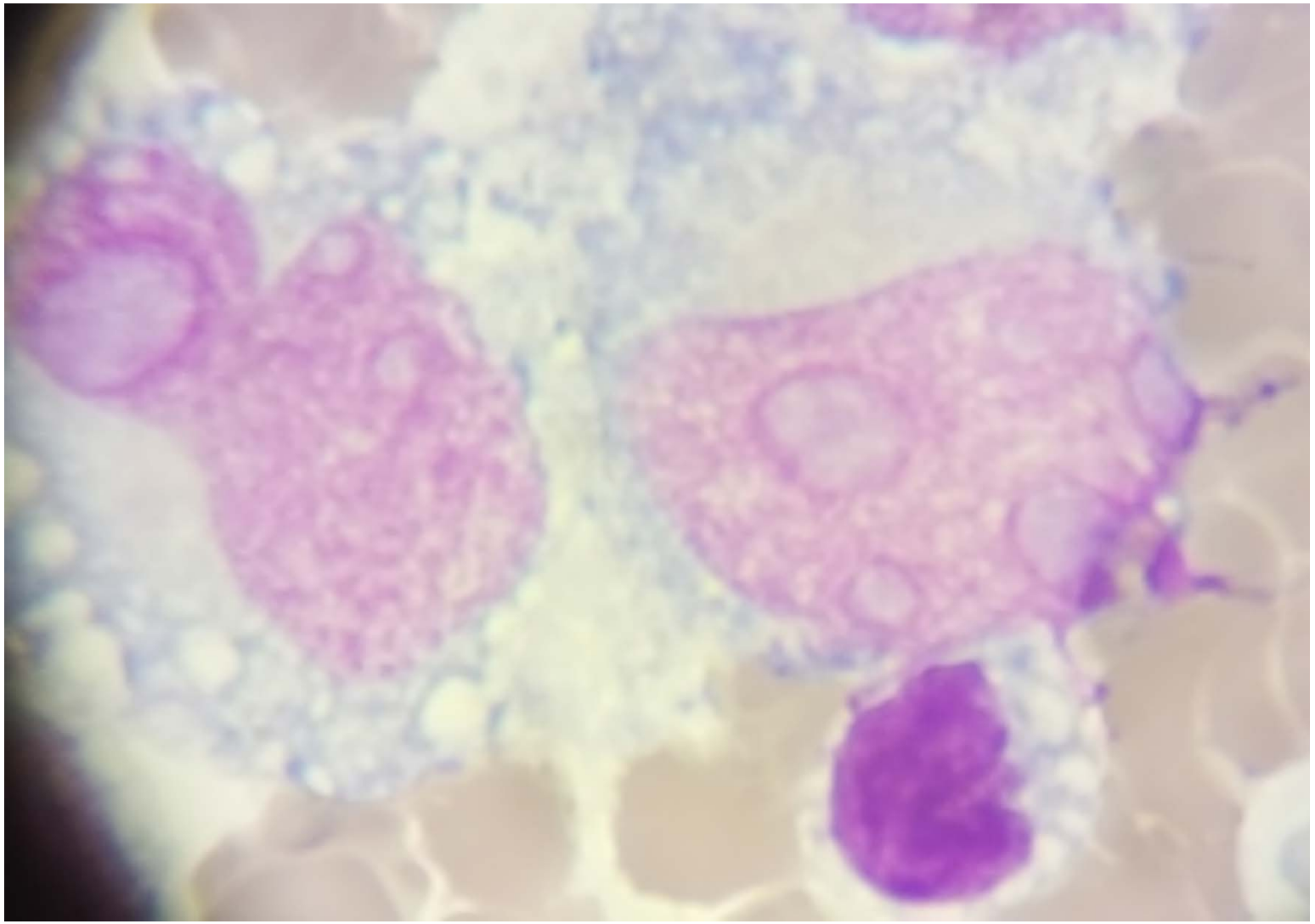
Diffuse Large B-cell Lymphoma





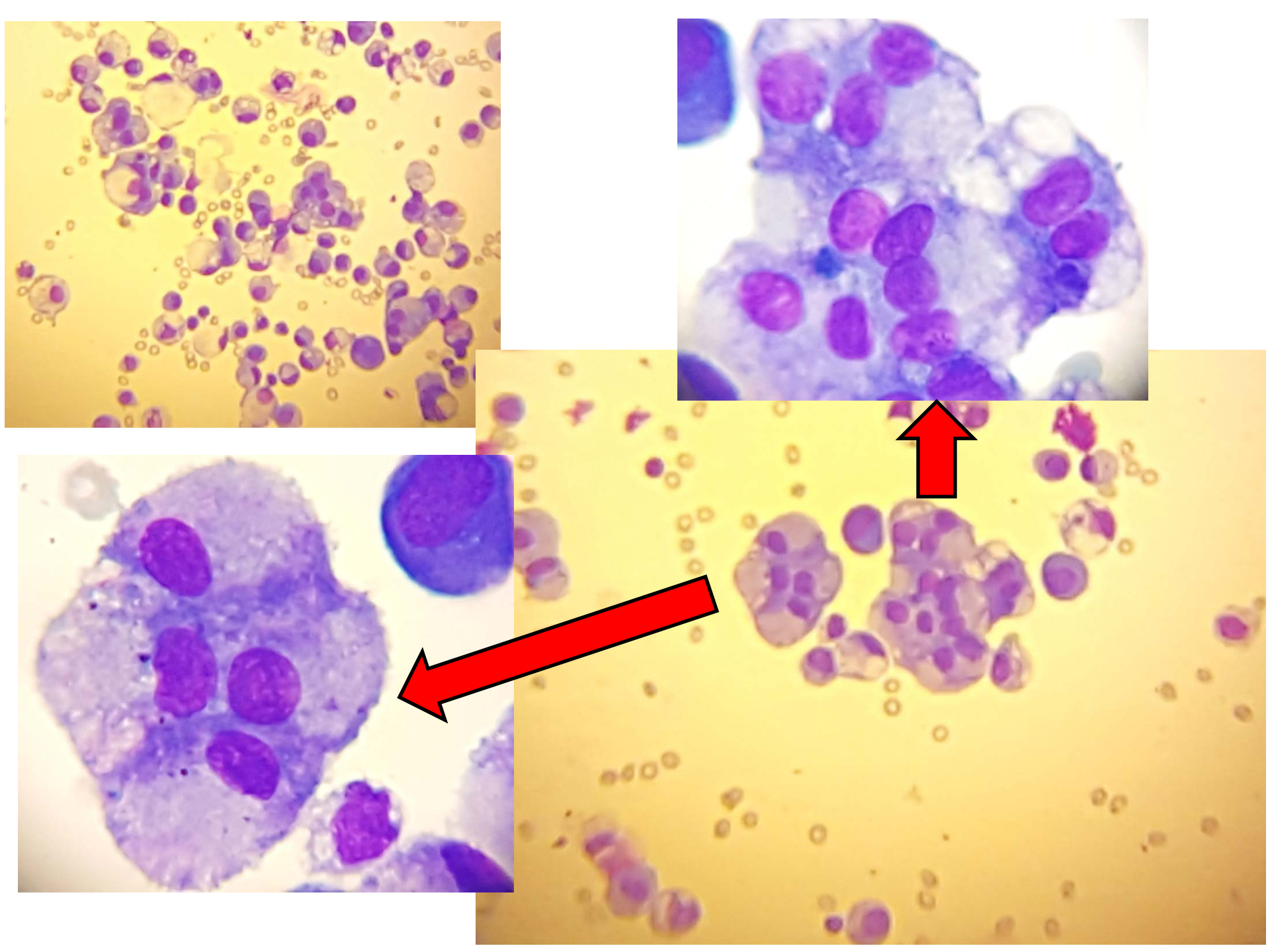


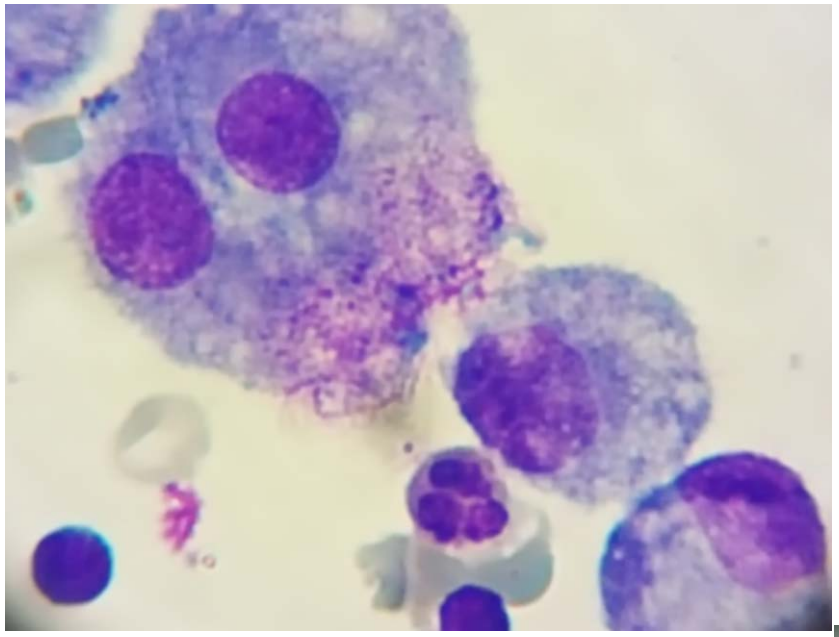


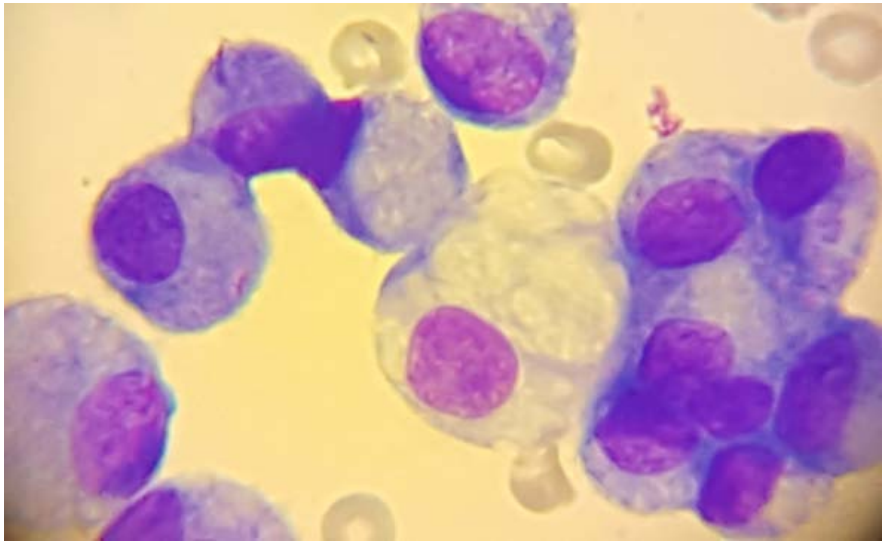


Patient 5

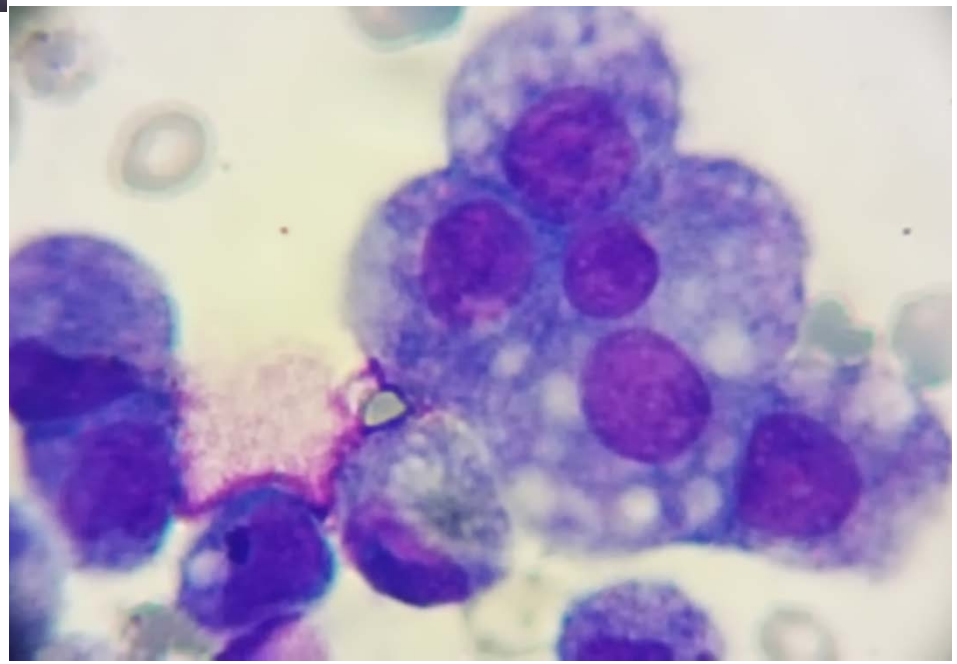
- 93yr Female
- Presents with bilateral pleural effusion
- Pleural fluid
  - TNC-531
  - RBC-2346
  - Diff= 5% Neut, 15% Lymph, 80% Macro/Meso





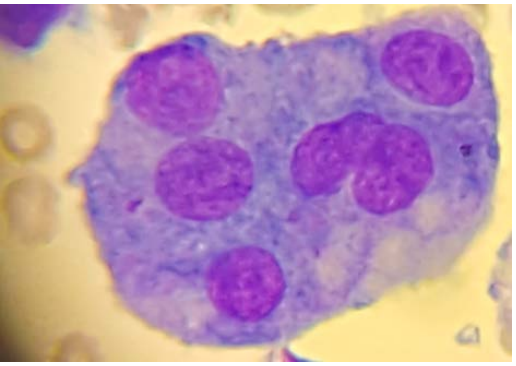


Patient had pneumonia of both lower lobes.
Mesothelial cells look reactive, but very uniform throughout the sample.

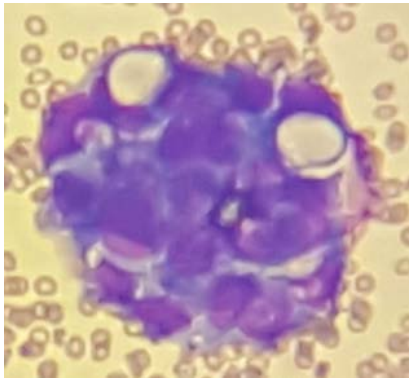


Why are these cells ok and the others weren't?

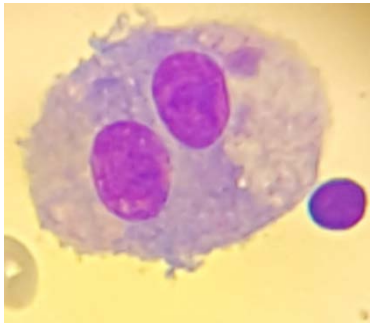
OK



Not OK



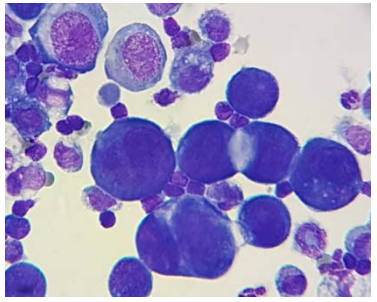
Flat vs Ball like clusters
Separation window vs Nuclear Molding

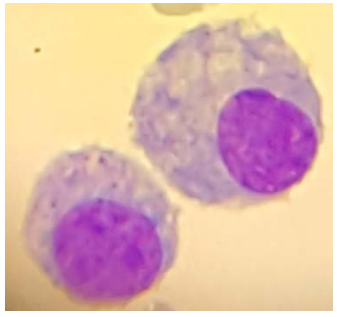


Low N:C ratio vs High N:C ratio

Loose homogenous chromatin vs Unevenly distributed chromatin

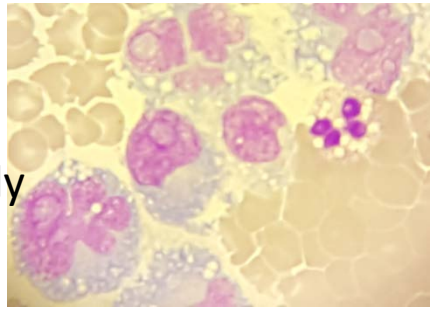
Uniform nuclear size/shape vs Varied nuclear size



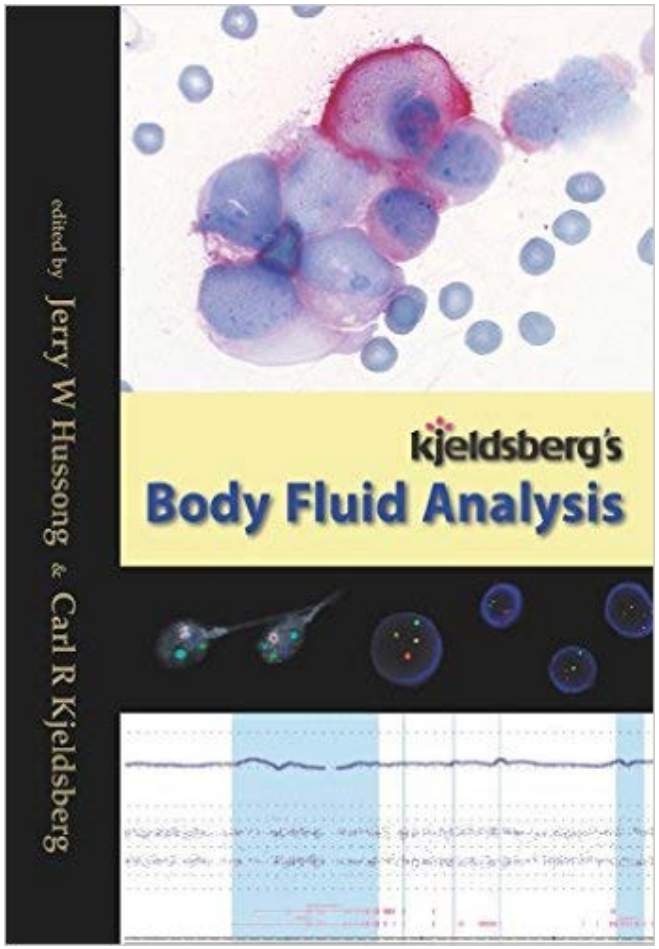


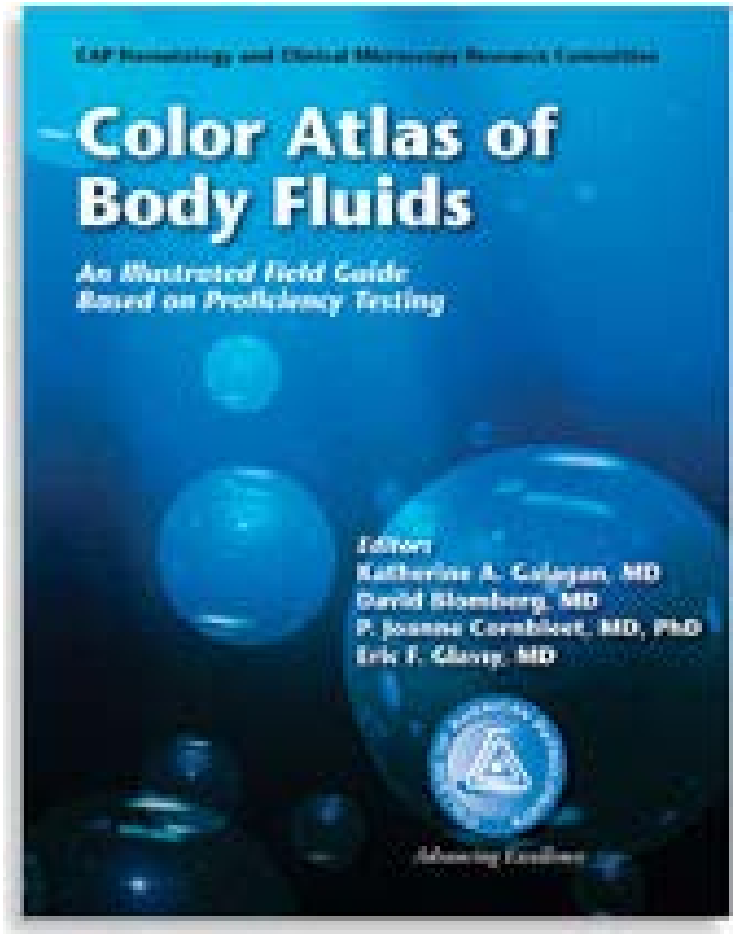
Uniform nuclear size/shape vs Varied nuclear size

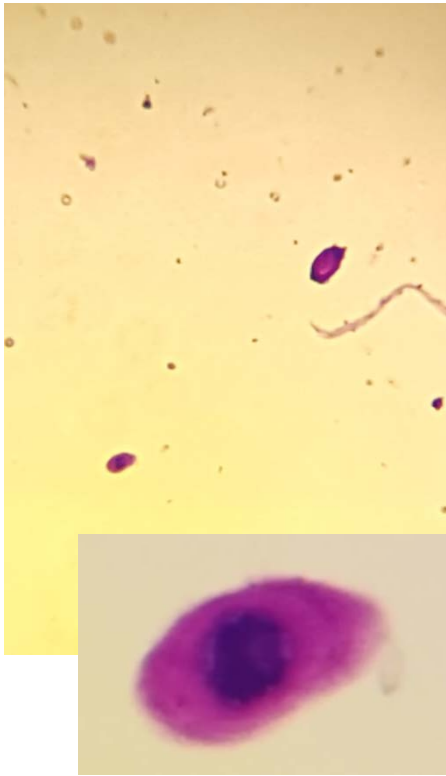
No visible nucleoli vs Mulitiple prominent, irregularly shaped nucleoli



Resources







Being unfamiliar or unsure of what a cell is doesn't make you a bad tech. Pretending it's not there does.

Summary

- Don’t make it harder than it needs to be
- Know what’s normal
- Practice what you aren’t comfortable with
- Utilize rules that prevent errors
- Use your resources

Questions?