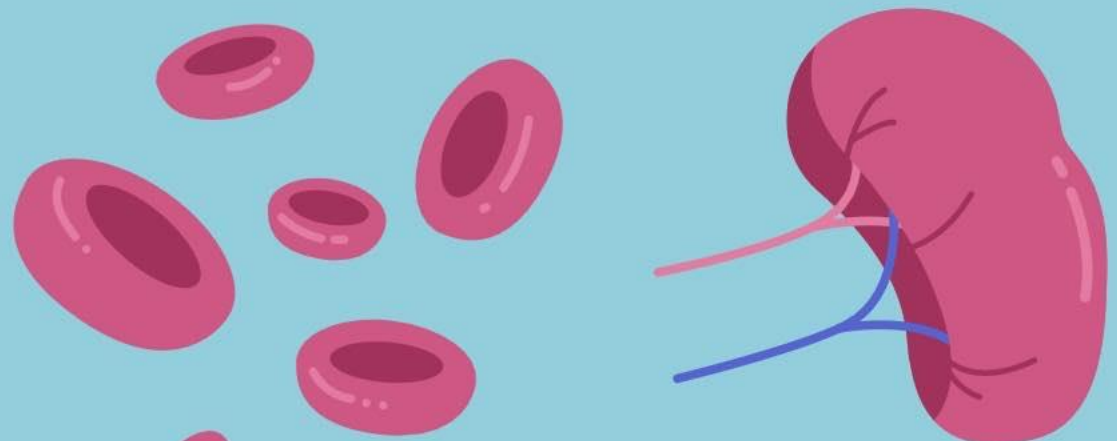
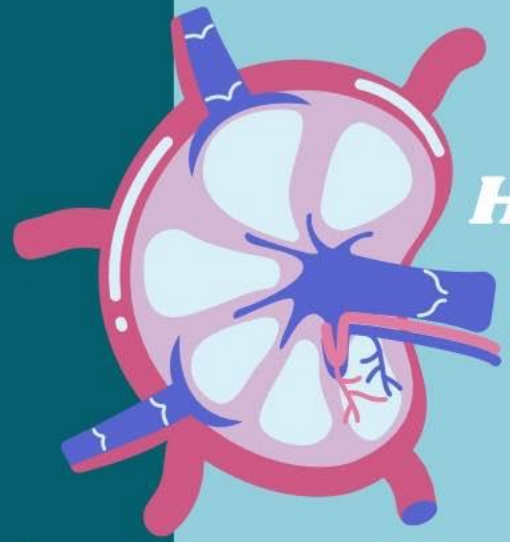
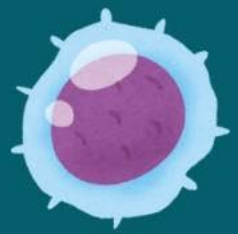
Histology Lab





Hematolymphatic System



Writer: Zahraa Al-Haddad, Mohammad Al-Saidah

Corrector: Mahmoud Al-Zoud , Mohammad Abusido

Hemato-lymphoid system

Practical Part

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The color code for these modified slides:

Black text: the professor's slides

Underlined Black text: what the professor has read from the slides

Green text: what the professor has mentioned during the lecture but isn't written in her slides

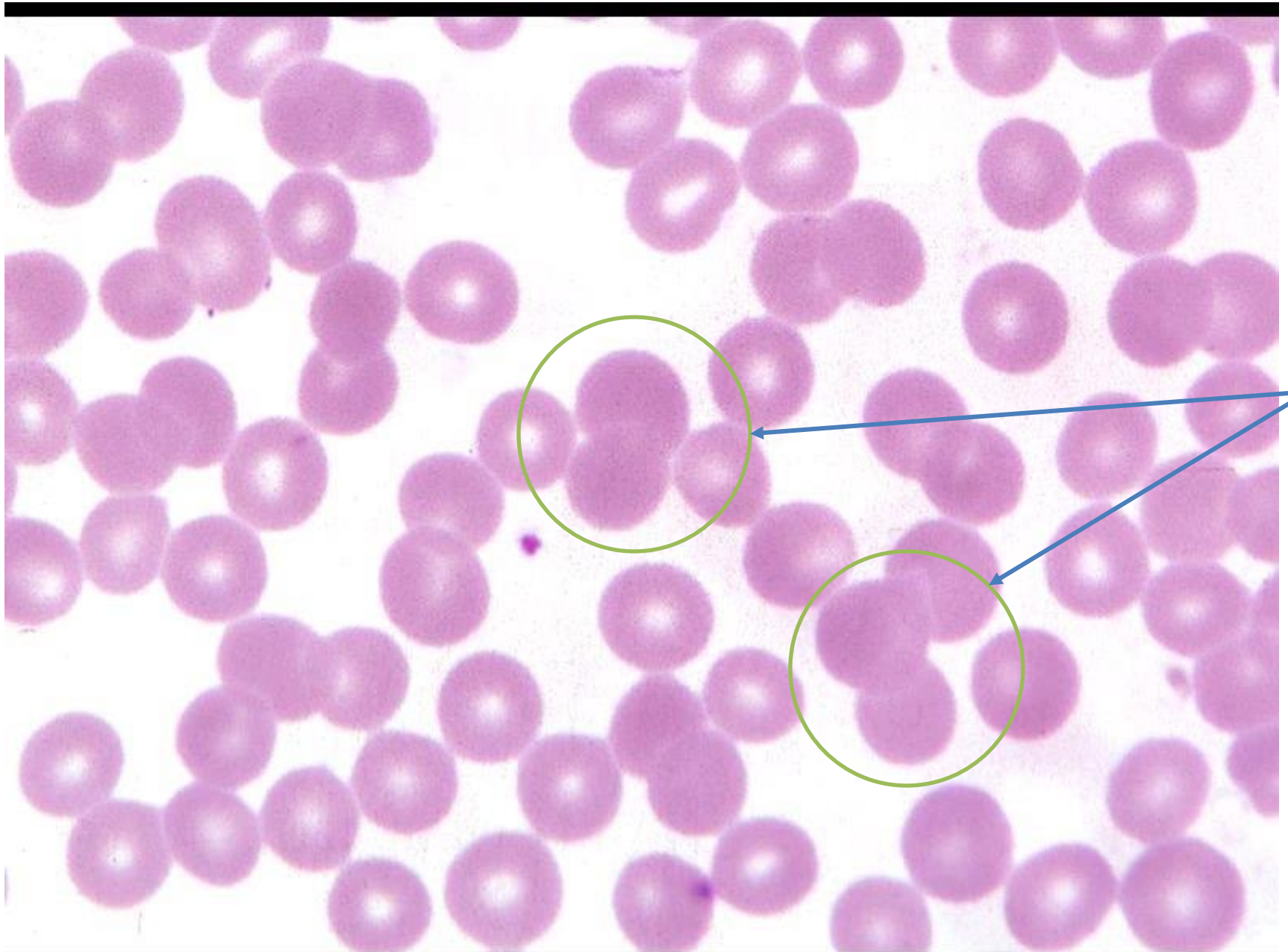
Purple text: extra information that may be useful

Highlighted text: information thought to be of greater importance

Blood

Erythrocytes

(Normal blood film)

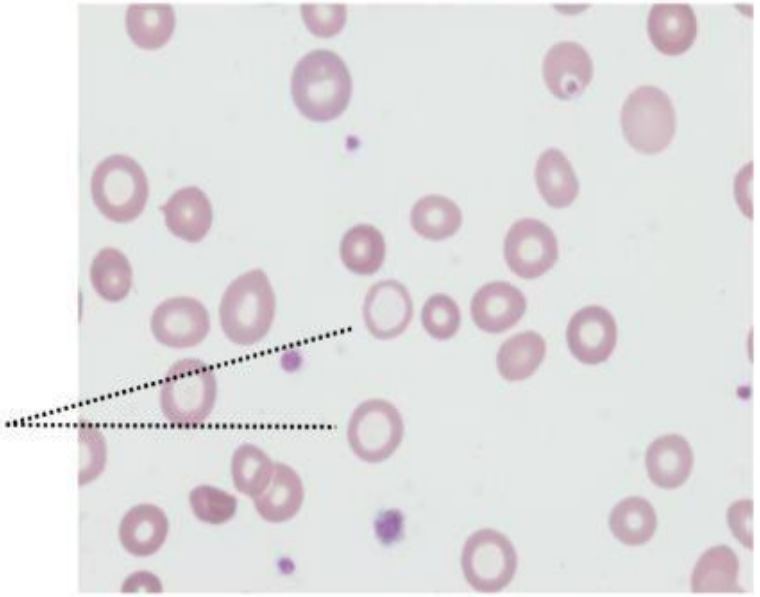


Here we can't see the central pale area due the over-lapping of RBCs (rouleaux-formation) during the histological preparation.

For example……

Hypochromic Red Blood Cells

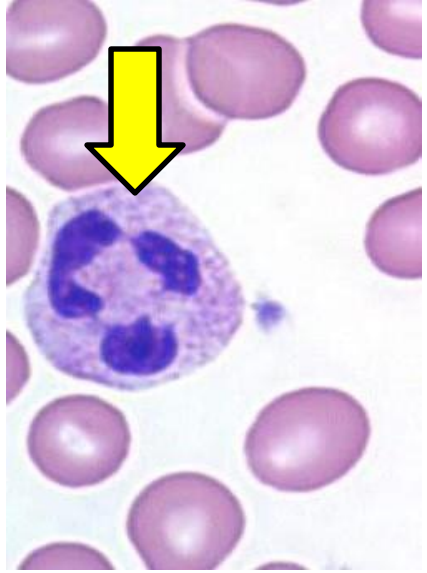
Patient with iron deficiency anemia

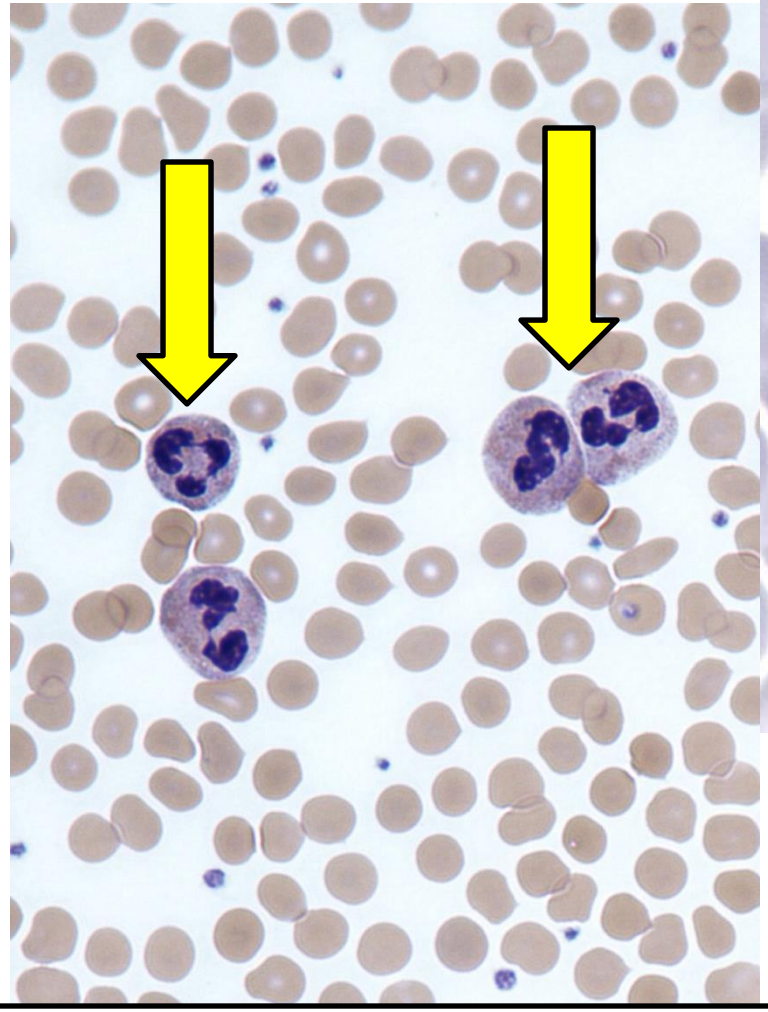


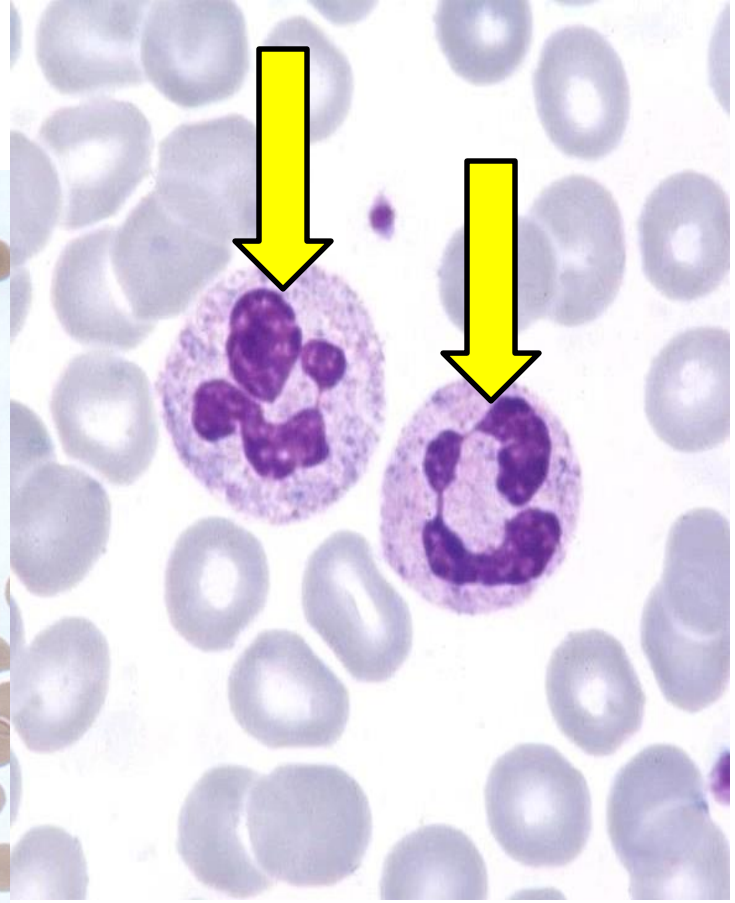
Marked increase in central pallor in iron deficient RBCs

If the pale area occupies more than a 1/3 of an RBC, then it's hypochromic. Which appears in cases like IDA.

Neutrophils



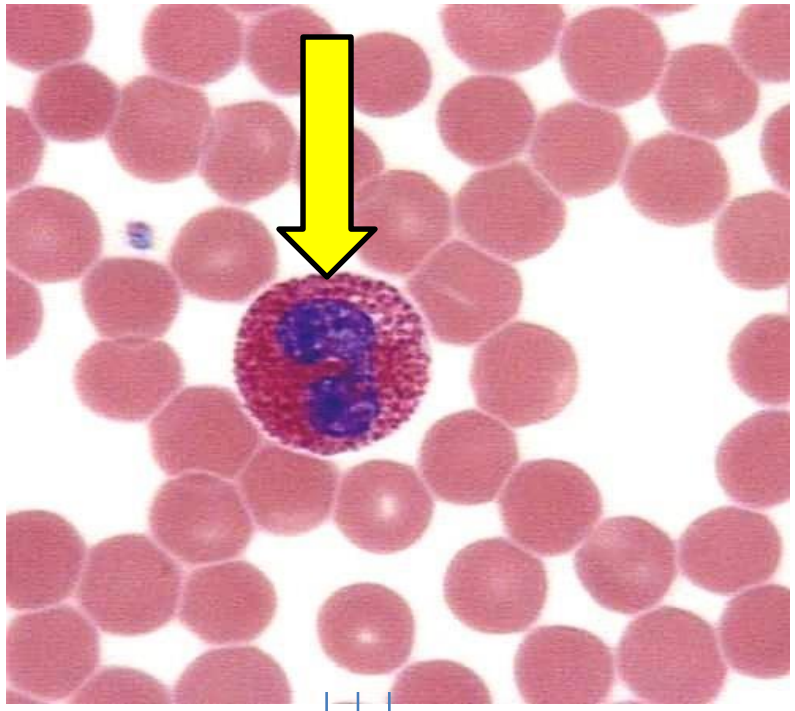




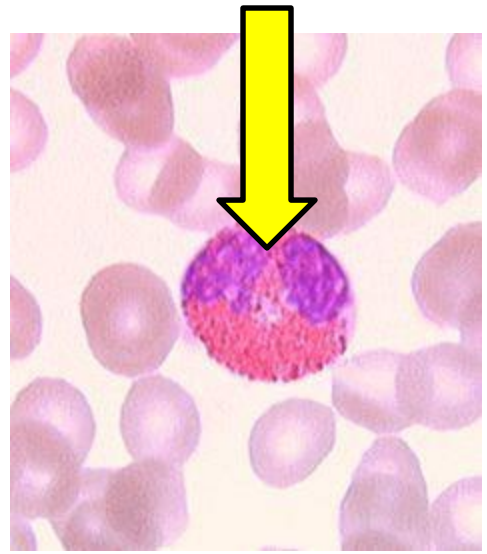
This shows a neutrophil in a blood smear. The neutrophils are 12-14 µm diameter, and so look bigger than the surrounding red blood cells. There is a single nucleus, which is multilobed (characteristic) not eye-glass shape, and can have between 2 and 5 lobes. Also, note the presence of fine granules.

In histology lab questions, regarding pictures from the mid-term, you'll not be tested upon your knowledge of the theoretical material. So, options like "cells that are involved in pus-formation" won't be there. Rather, you'll be asked to directly identify the cells in the pictures.

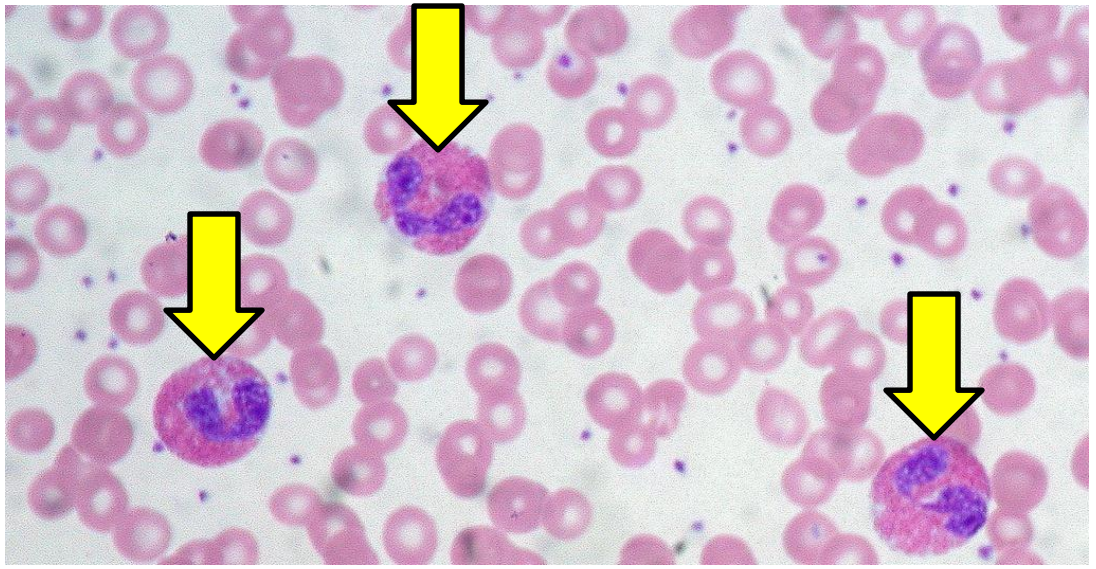
Eosinophils



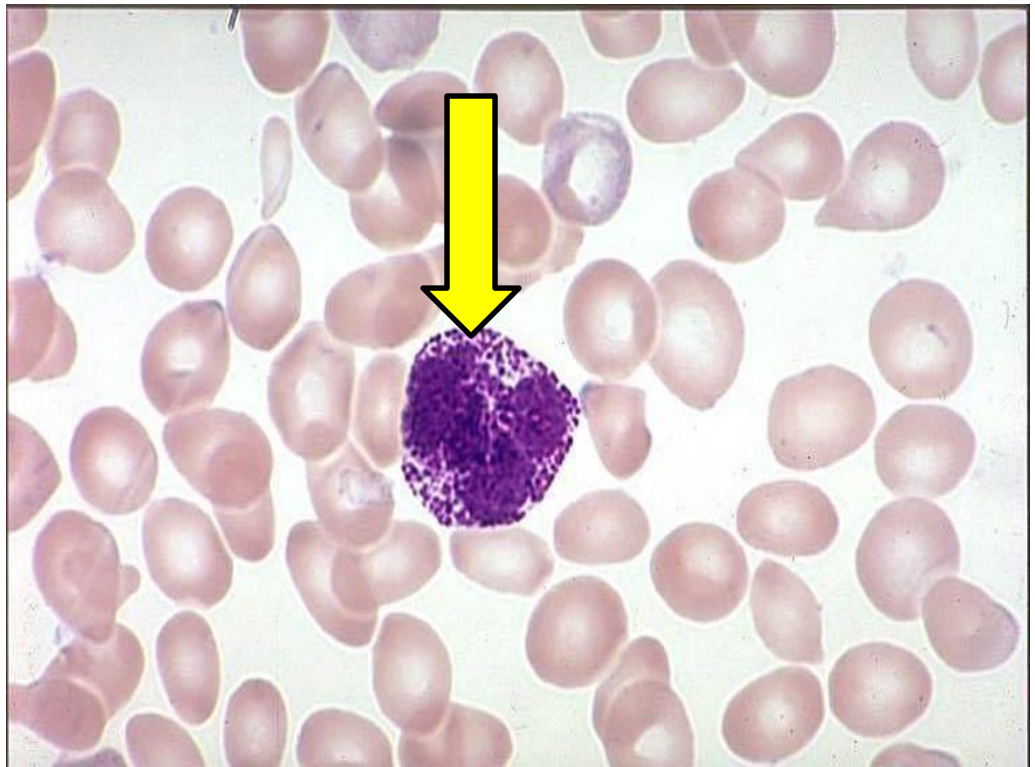
Eosinophils and basophils require stain reaction to be identified, so they'll not be asked about in the exam.

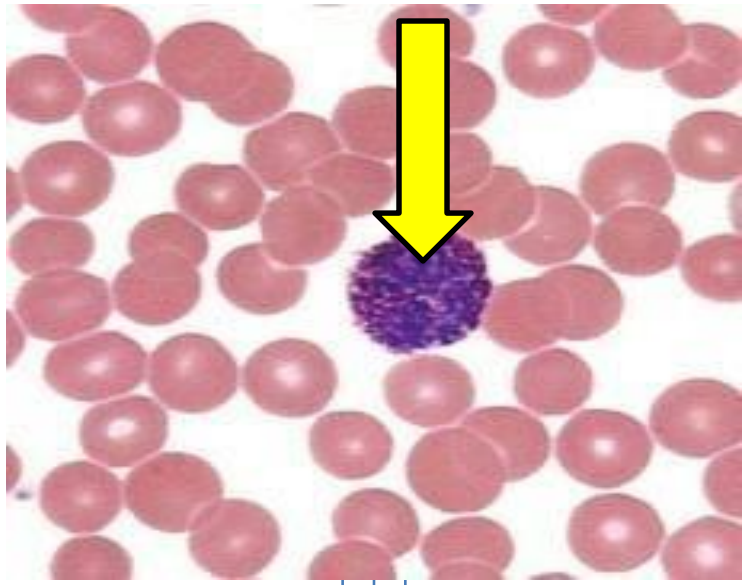


This shows an eosinophil in a blood smear. You can see that eosinophil has a bilobed nucleus.
These cells have large acidophilic specific granules - these stain bright red, or reddish-purple.



Basophils

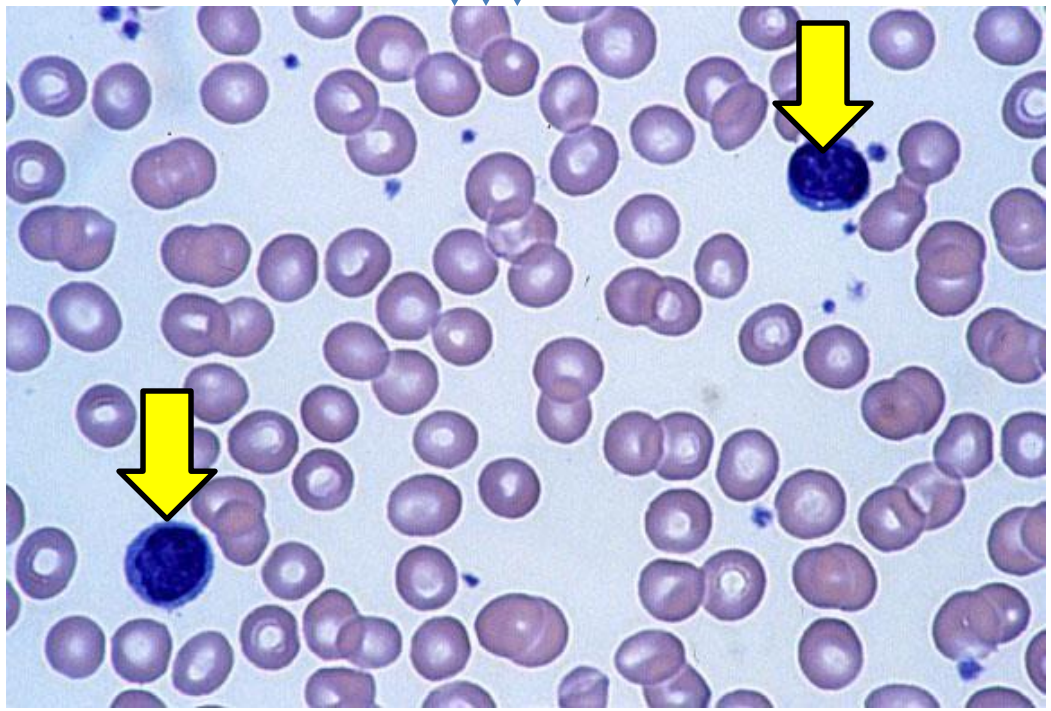


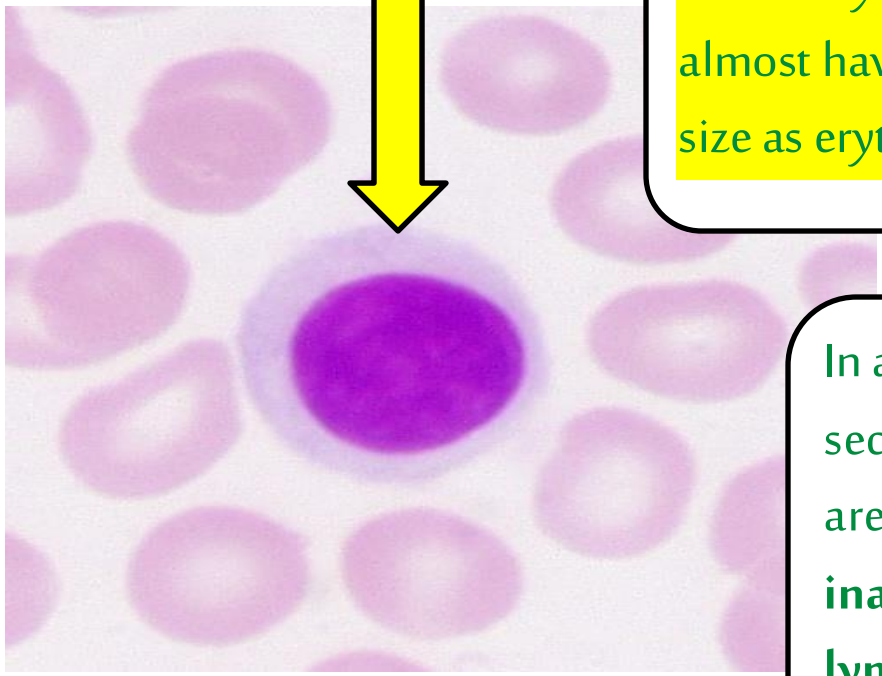


This shows a basophil in a blood smear. The basophil contains lots of deep blue staining granules (basic) and a bilobed irregular nucleus, that is often difficult to see because it's covered by the granules.

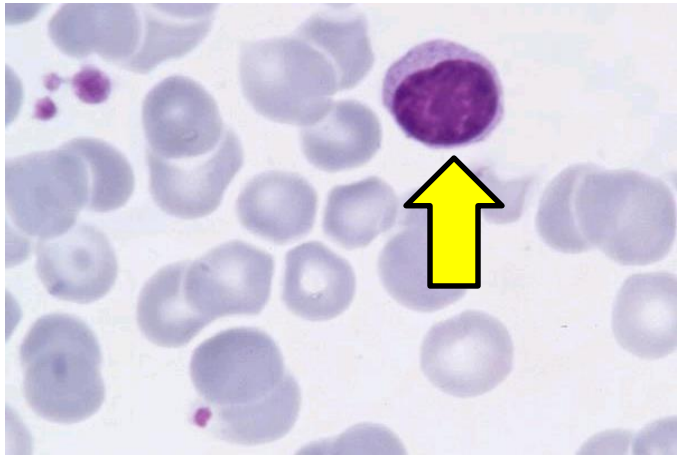
Lymphocytes

This shows lymphocytes in a blood smear. Most of the lymphocytes are spherical and small; a bit bigger than red blood cells, at about 6-9µm in diameter.
Lymphocyte has a small spherical nucleus (that is relatively large and occupies most of the cytoplasm) with dark staining condensed chromatin. Not much cytoplasm can be seen, and it is basophilic (pale blue/purple staining).



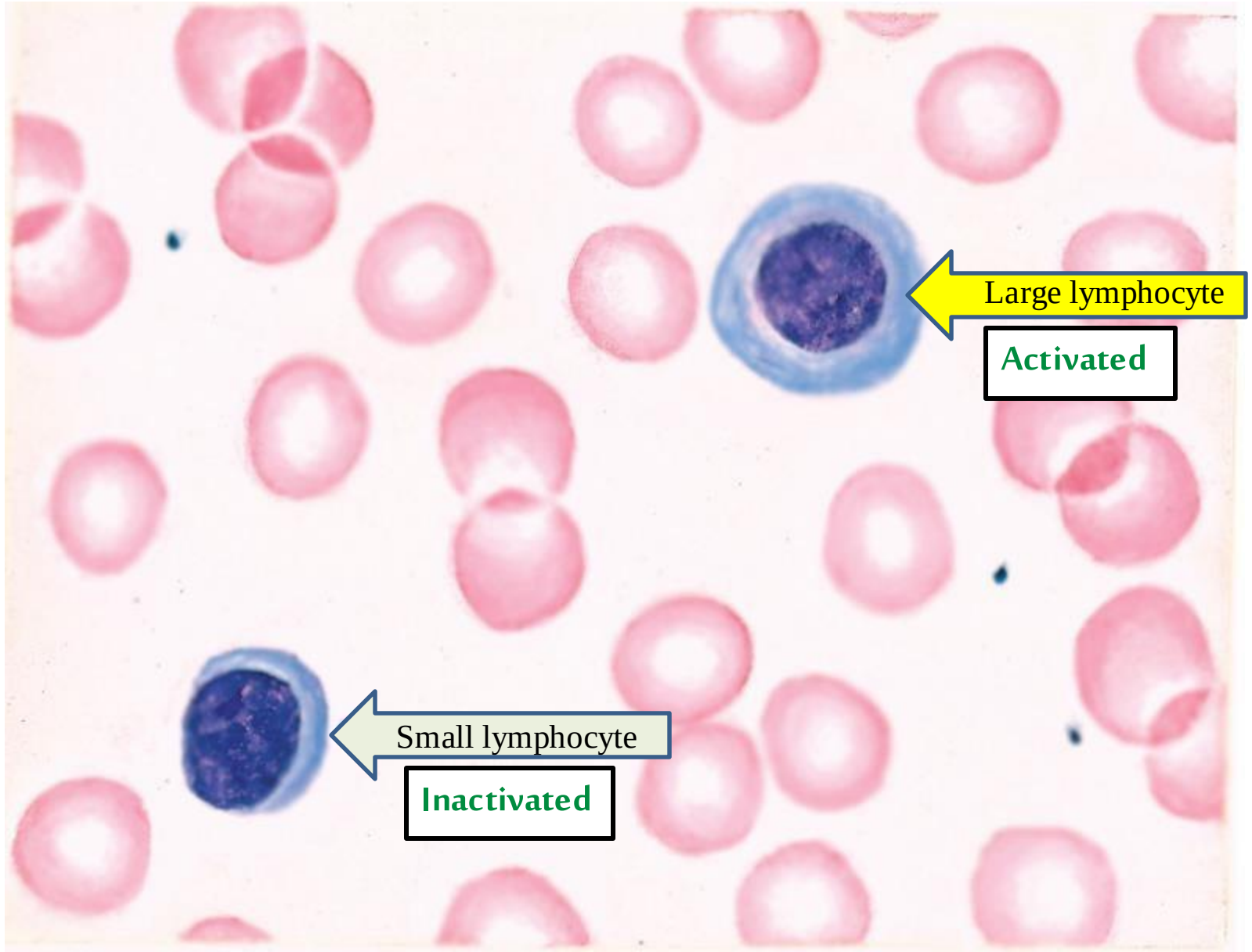


Inactive lymphocytes almost have the same size as erythrocytes



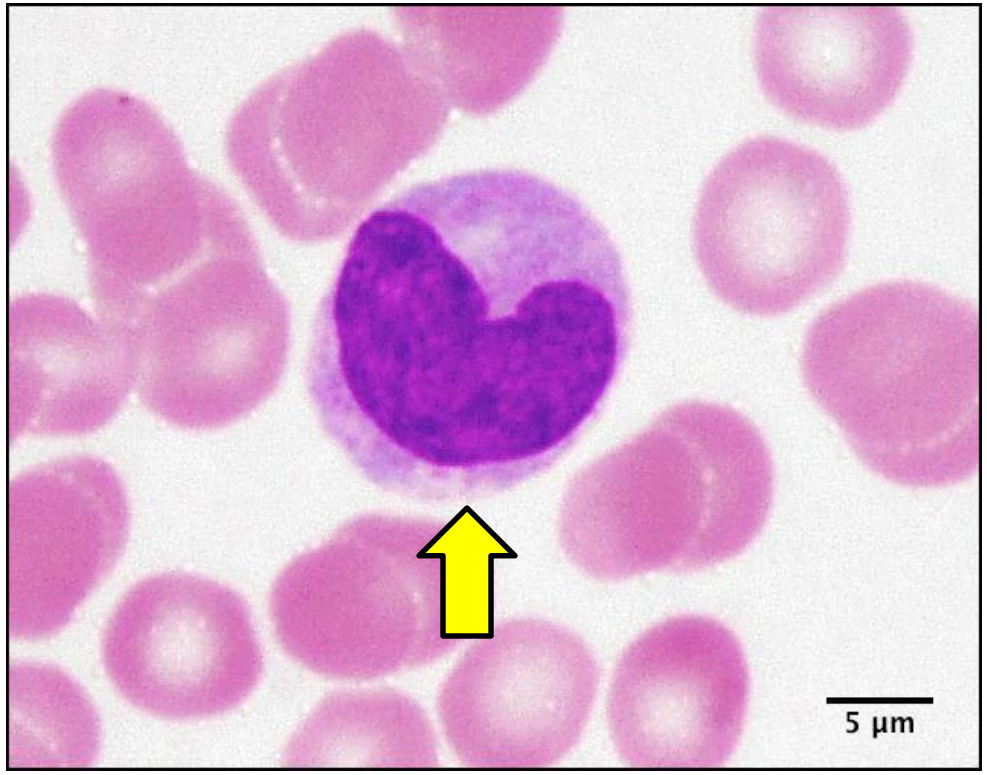
In all of these sections, these are small, **inactive lymphocytes.** So, if there's an option like: "B cell" or "T cell" it must be excluded.

The rest of lymphocytes (around 10%) are larger. These larger cells have more cytoplasm and more euchromatic nucleus. Larger lymphocytes are commonly activated lymphocytes.

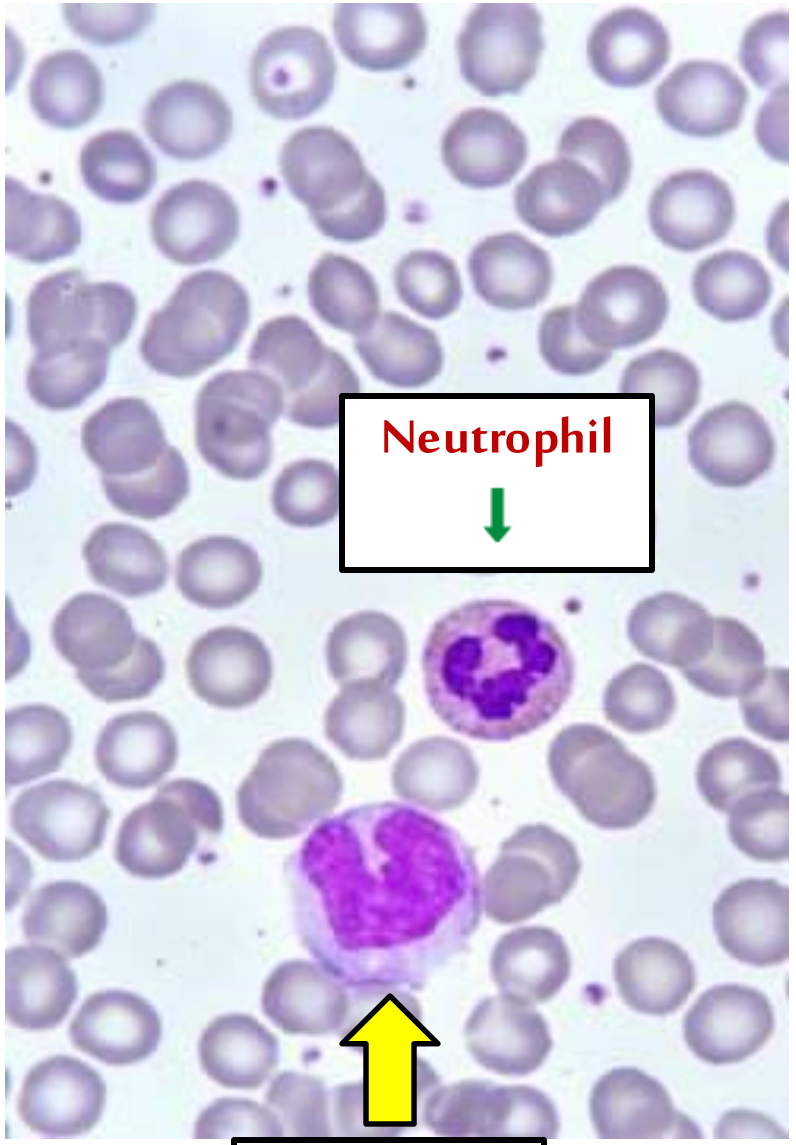


Monocytes

❗ Will probably be present in the exam because of its characteristic, obvious appearance



This shows a monocyte in a blood smear. Monocytes are the largest type of white blood cells, and can be up to 20µm in diameter. They have a large eccentrically placed nucleus, which is C or kidney bean shaped. They have abundant cytoplasm, and some fine purple granules in cytoplasm (frosted glass appearance).



🚨 **Don't confuse monocytes with neutrophilic band cells!!**

Because in that case:

1-The cytoplasm would be fine-granular.

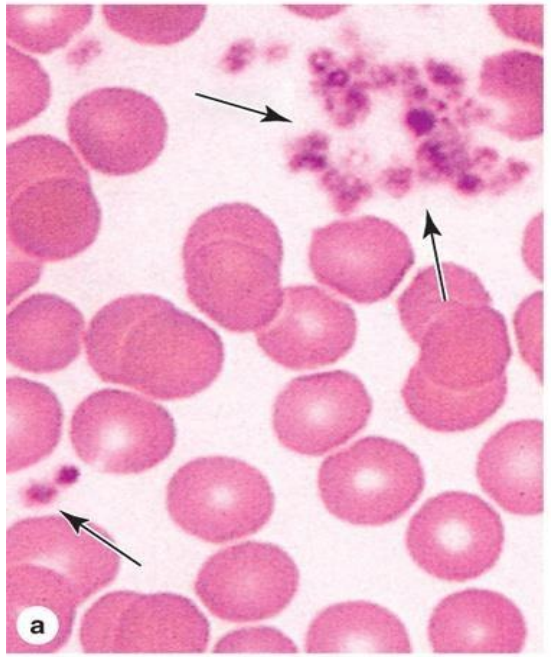
2-Its size wouldn't be bigger than the adjacent neutrophil.

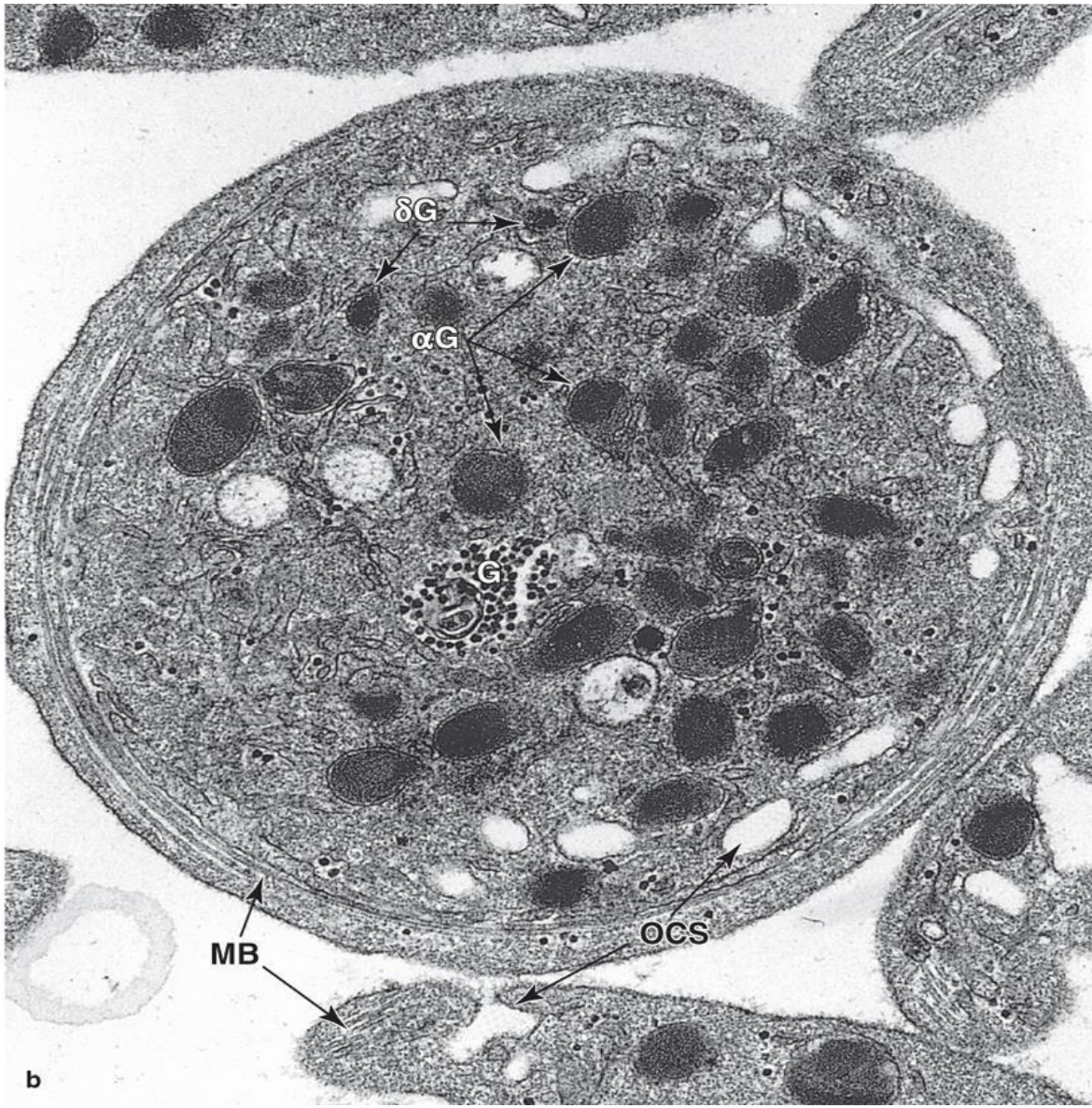
3- Check its staining rxn.

Platelets

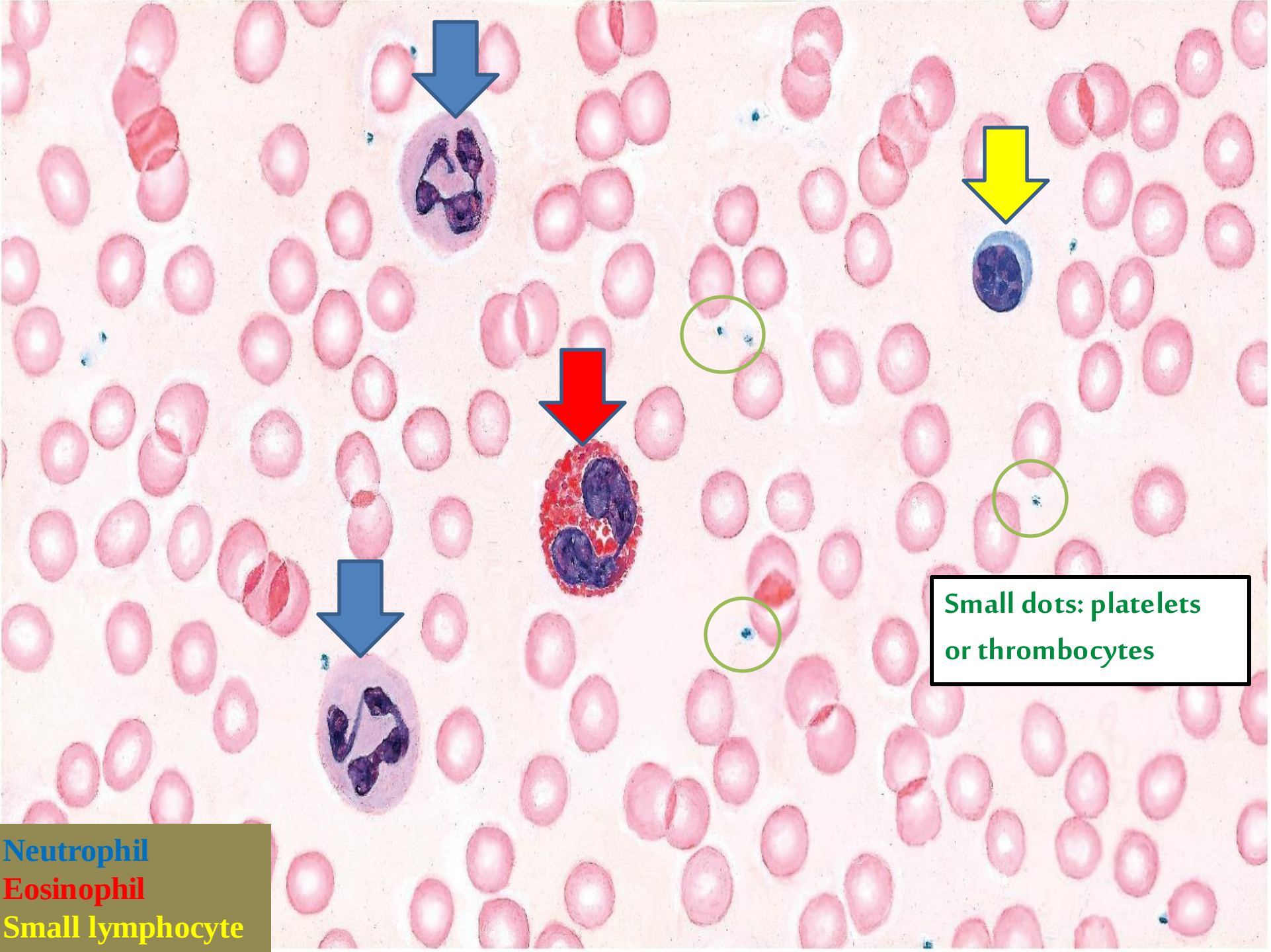
⬇ This B&W pic is taken from an electron microscope

You must be able to tell that it's a thrombocyte since there's no nucleus. It's also rounded with central granular area. Under light microscopy, it appears as aggregations of cells.





Identify



Q: All of the following are found in this section, except:

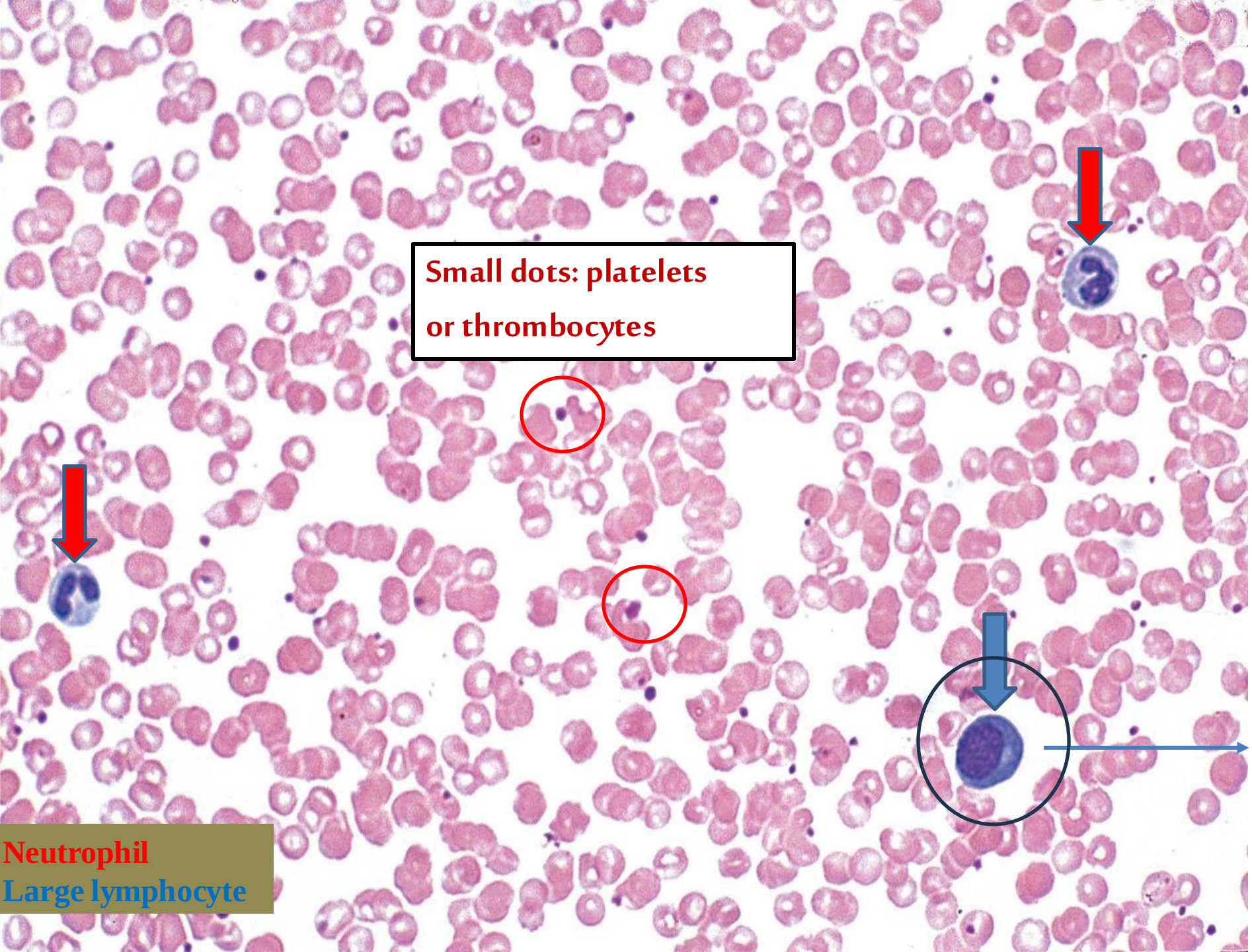
A- Erythrocytes

B- Thrombocytes

C- Neutrophils

D- Monocytes

Ans: D



Q: All of the following can be found in this section, except:

A- Erythrocytes

B- Thrombocytes

C- Neutrophils

D- Large lymphocyte

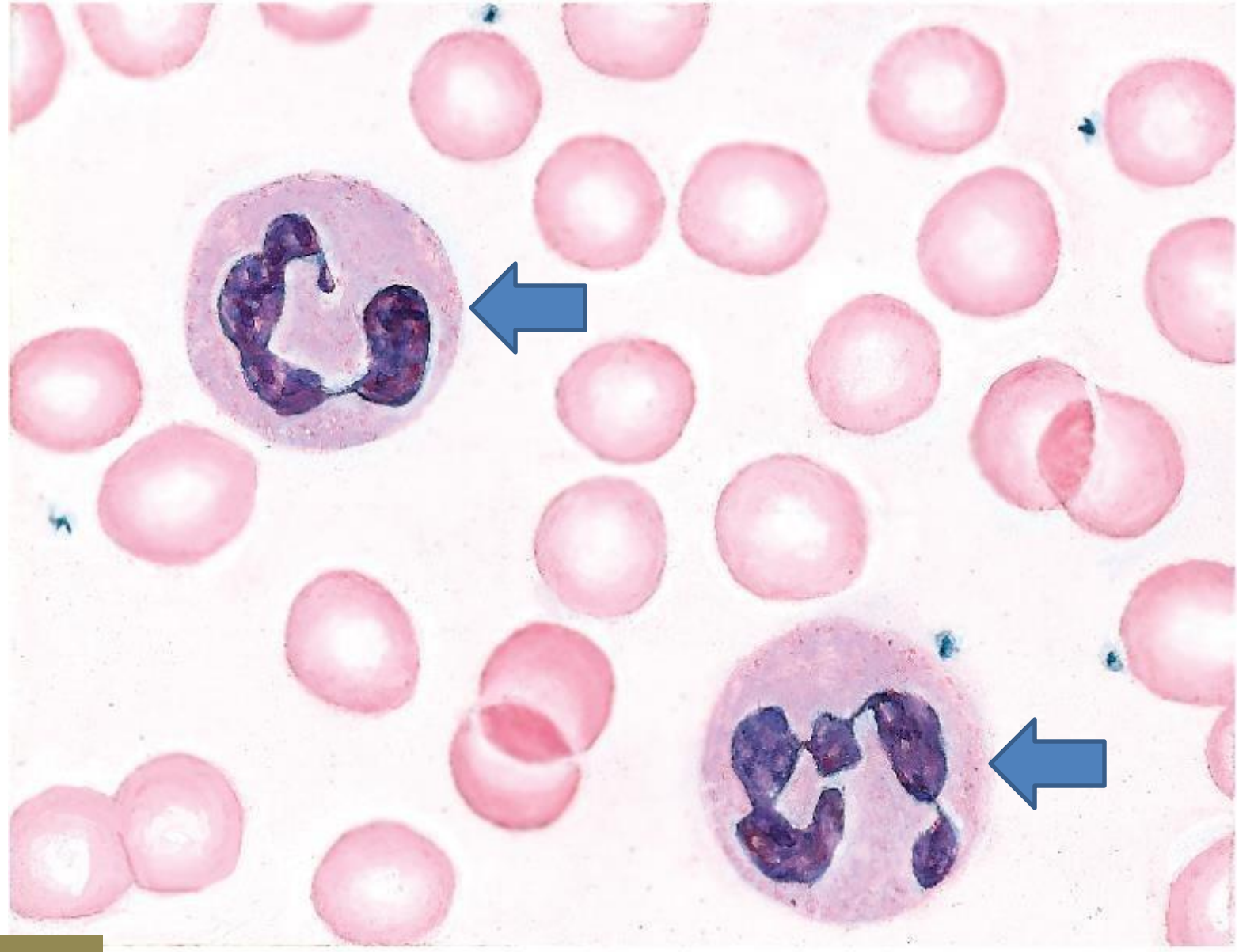
E- Small lymphocyte

F- Eosinophil

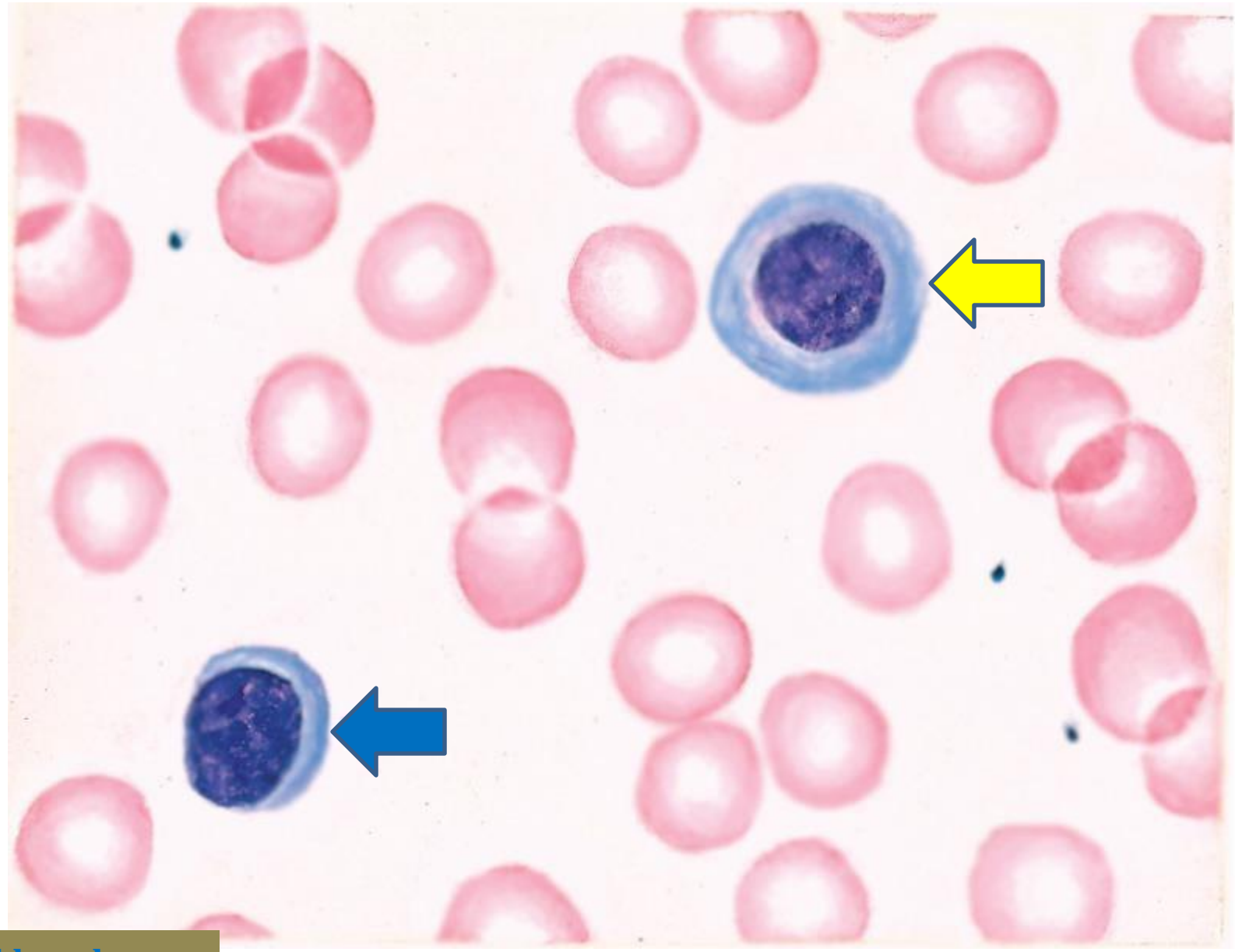
Ans: E&F

Active lymphocytes are Large in compare to surrounding erythrocytes

Female blood film

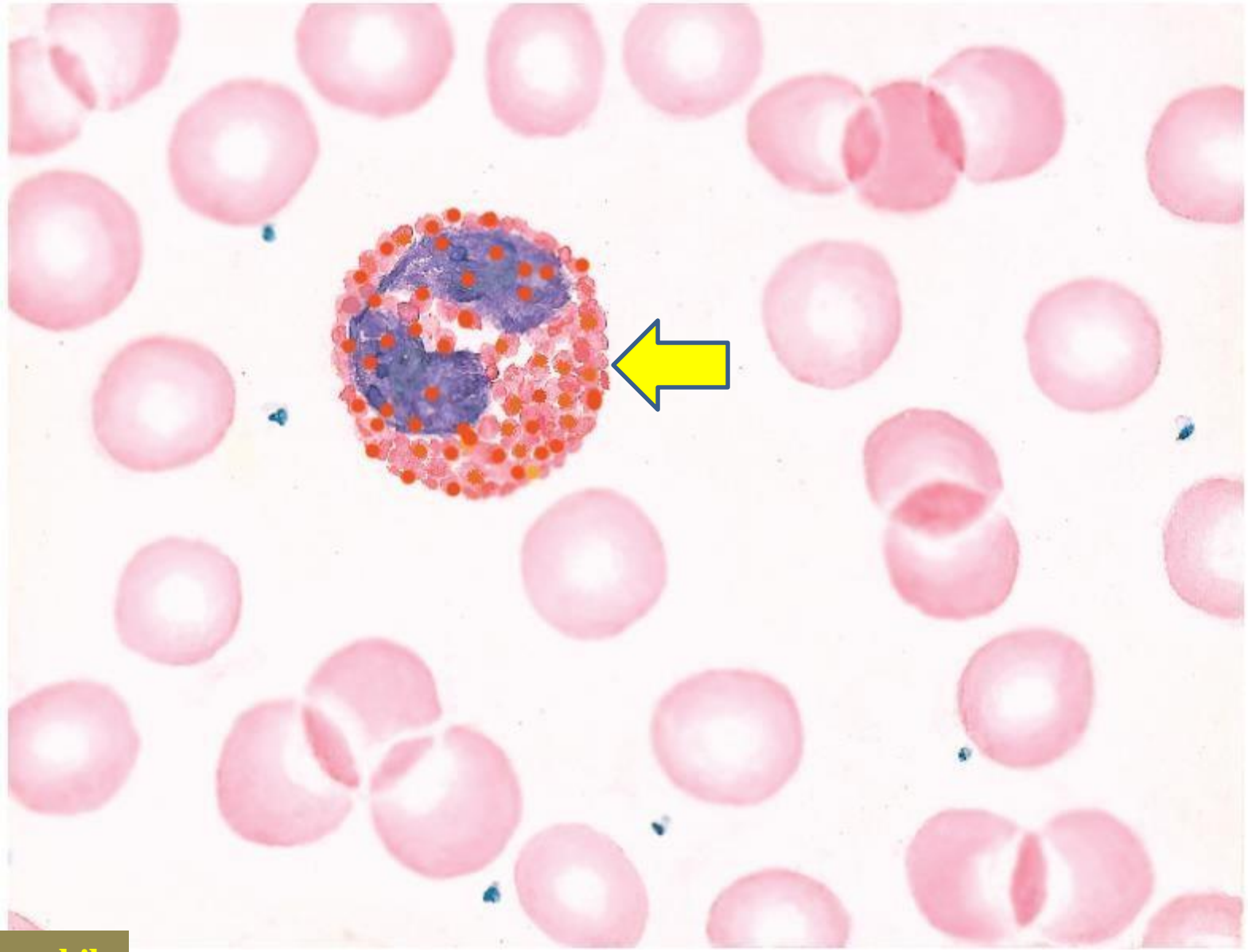


Neutrophil



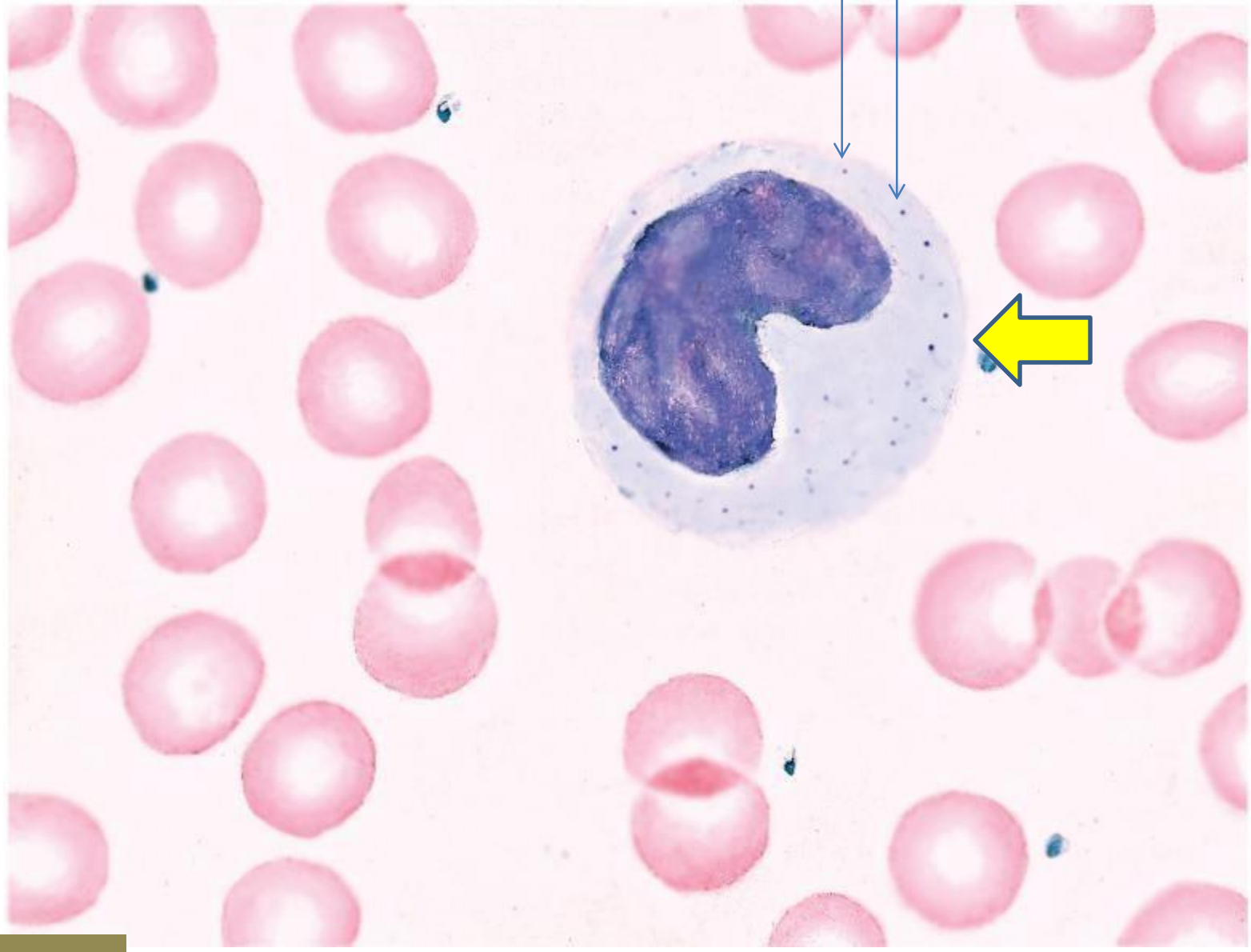
Small lymphocyte — Inactive

Large lymphocyte — Active



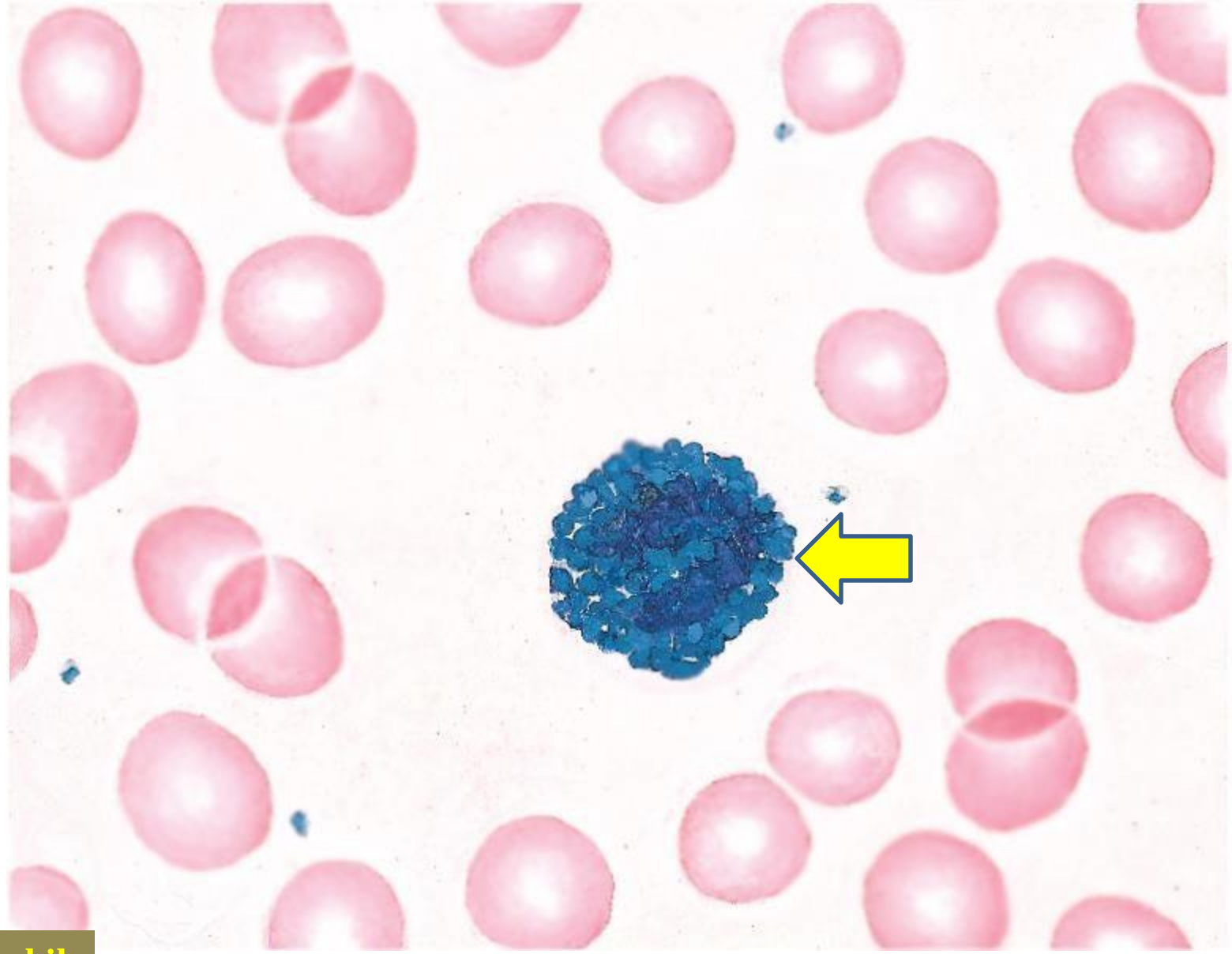
Eosinophil

These are azurophilic non specific granules not specific granules



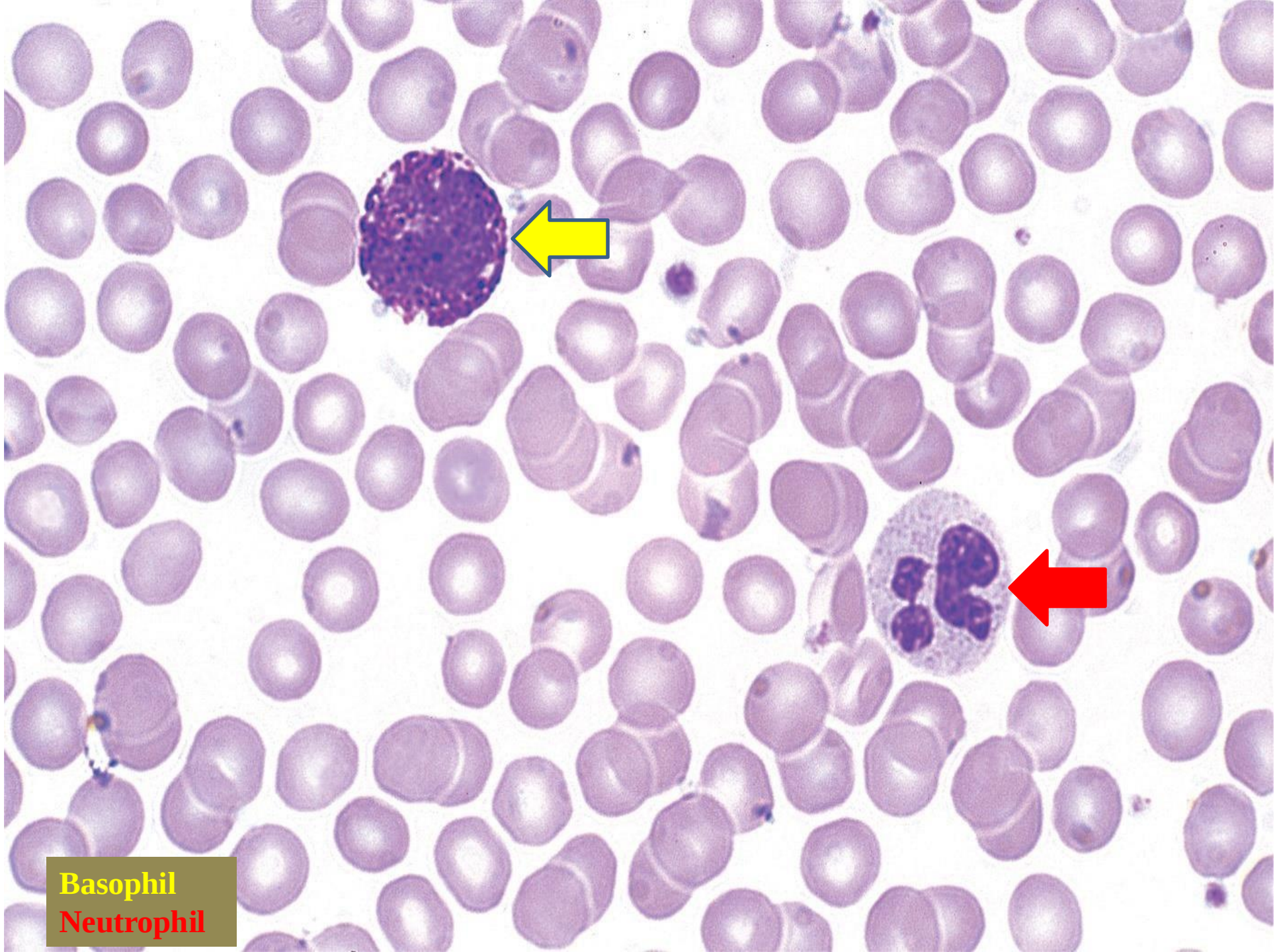
*C-shaped nucleus with cytoplasm containing small basophilic dots.
*Here, we used a special stain for azurophilic granules.

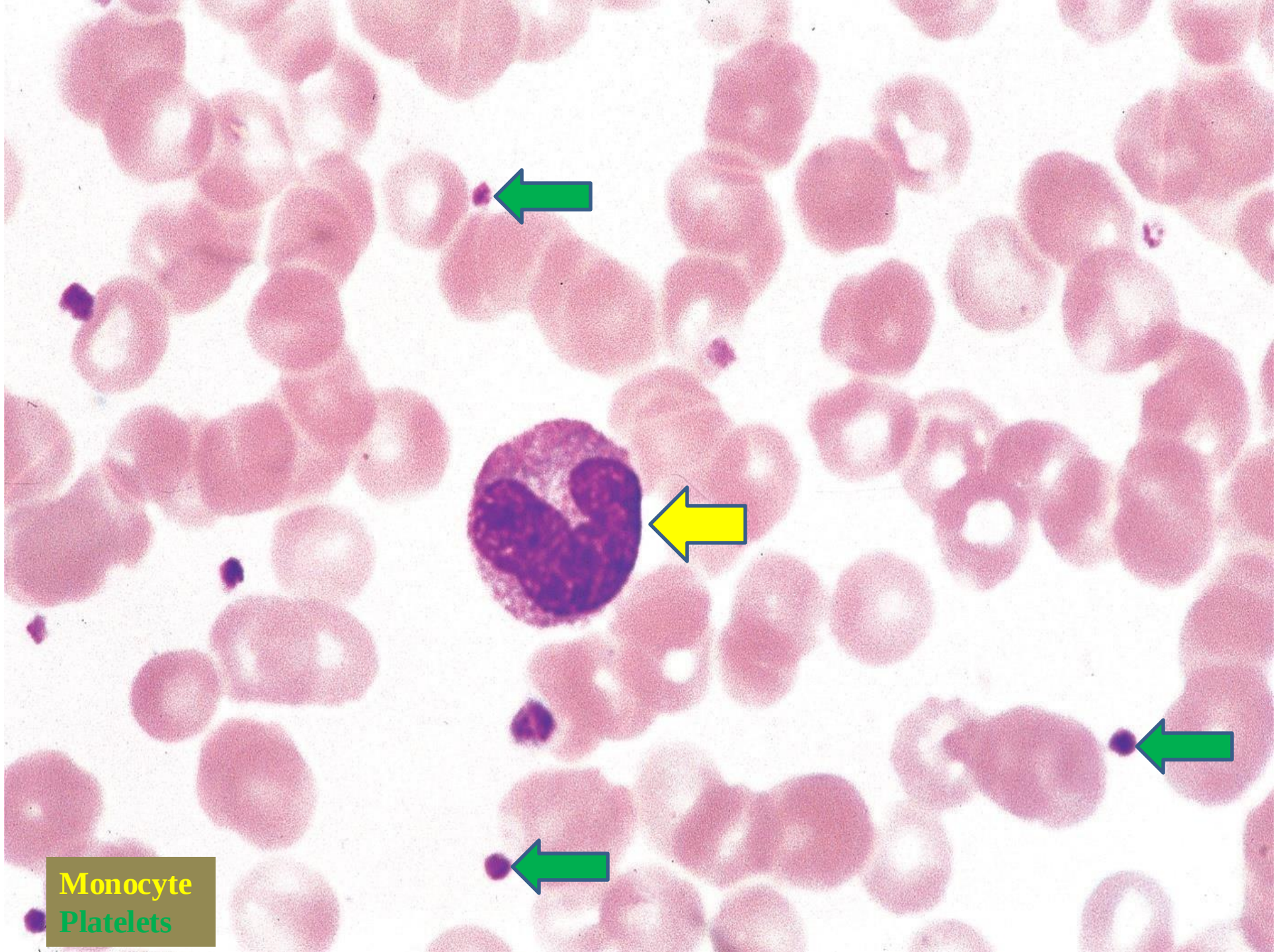
Monocyte

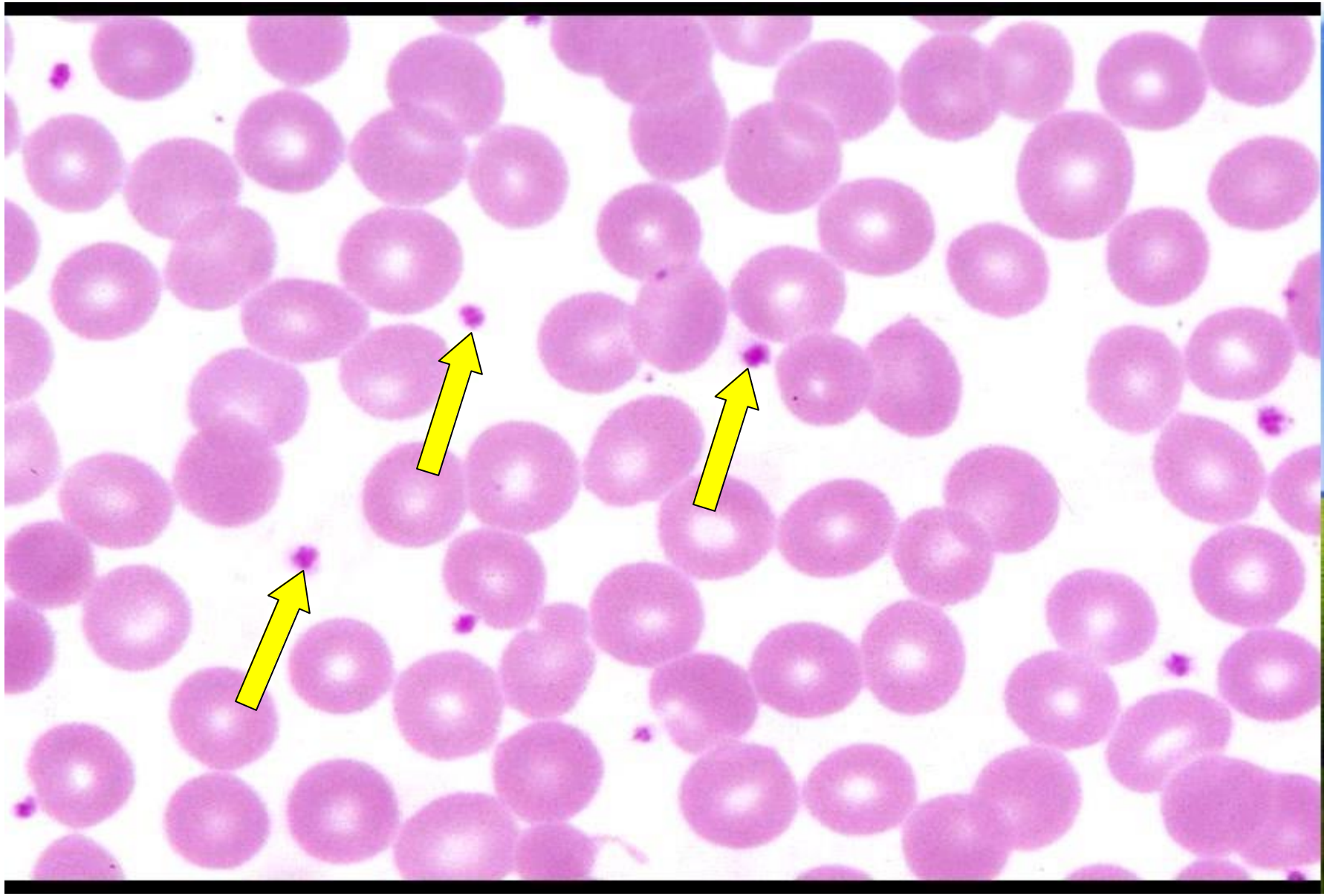


Can’t define its nucleus

Basophil


Basophil
Neutrophil

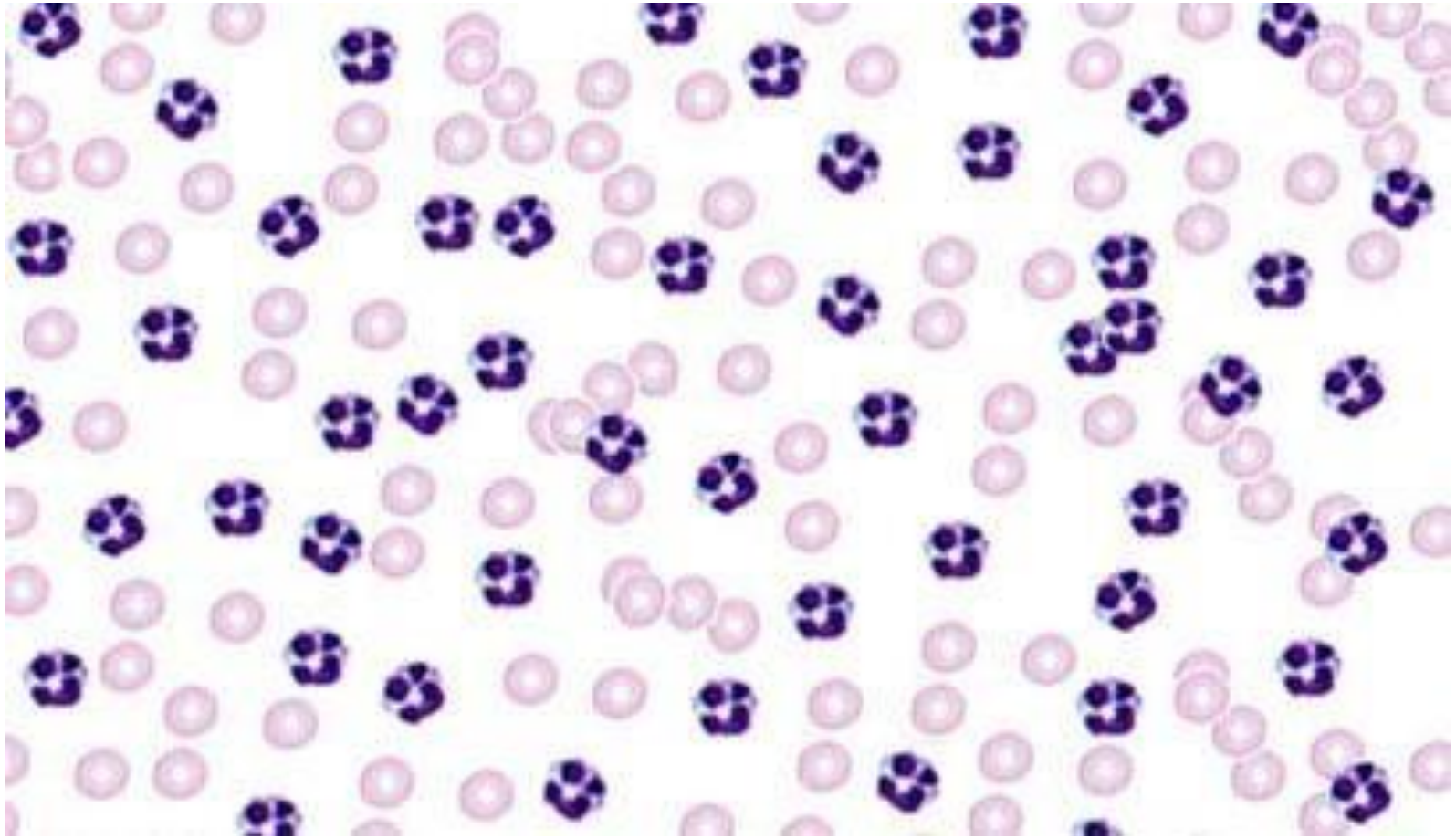

Monocyte
Platelets



Platelets

*Neutrophilia

*This is an indication that the immune system is well-functioning, dealing with the infection very well.

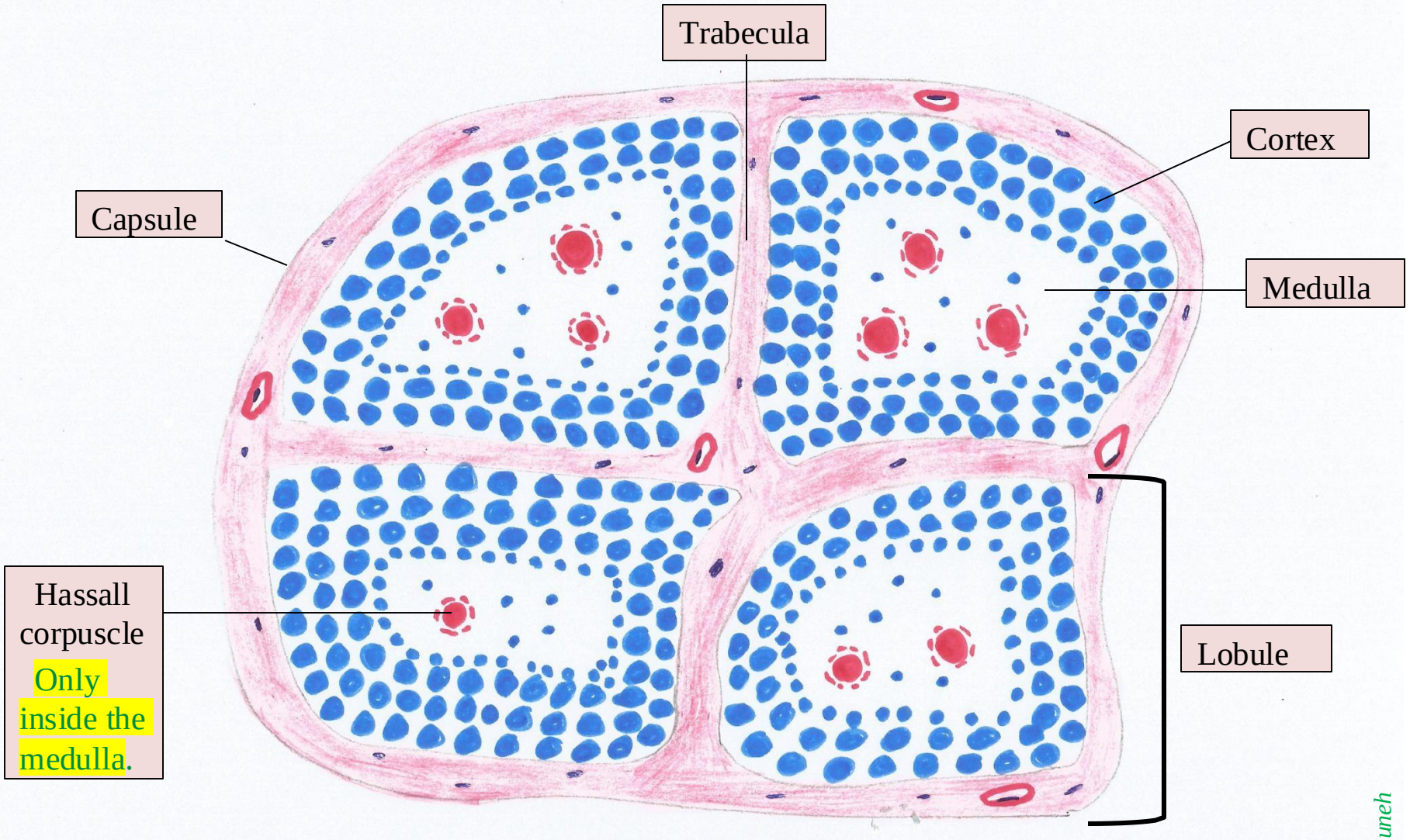


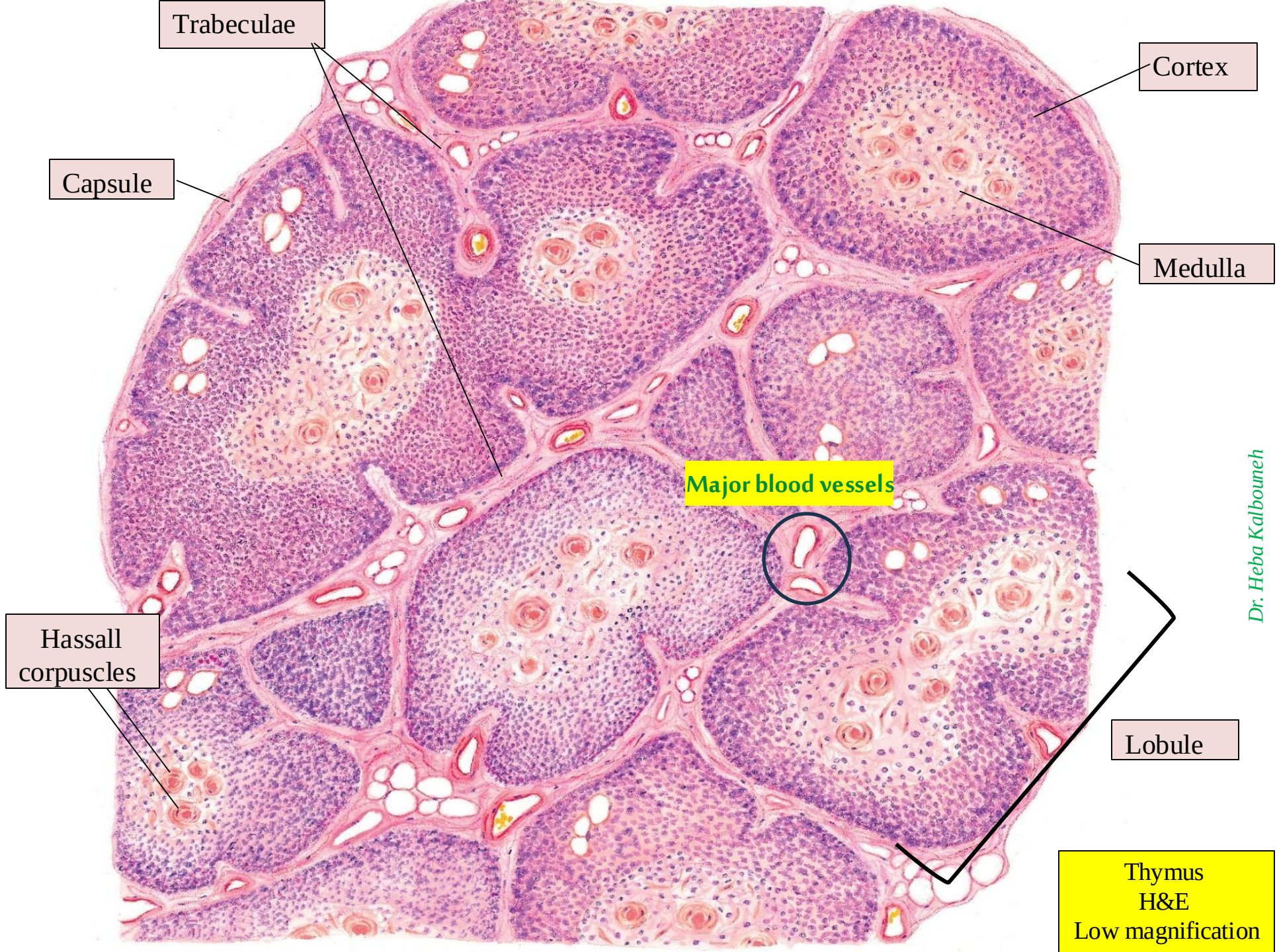
Don’t Worry, Be Happy! ☺

Thymus

A capsulated structure that sends trabeculae that divide it into smaller lobules, each lobule is further divided into outer cortex and inner medulla.

Note that the gland is organized into numerous lobules. Each lobule contains a dark-staining outer cortex and inner medulla. Also note the capsule that extends into the thymus to form the interlobular septa (trabeculae) that separate the lobules. The capsule and septa contain blood vessels, lymphatics and nerves.
Note also that thymus has no lymphoid follicles




Trabeculae
Capsule
Cortex
Medulla
Major blood vessels
Hassall corpuscles
Lobule
Dr. Heba Kalboumeh
Thymus
H&E
Low magnification

- **The previous structure is not a lymph node (so there are no follicles)** because there's an outer cortex and an inner medulla.
- Look at the shape of the lobules, **they don't share the same shape as follicles in a lymph node.**
- **They aren't splenic follicles either;** because in the spleen, the follicles would be scattered with red pulp in between them.
- **Note the presence of Hassal corpuscles in the medulla.**
- **All of these lobules are separated by septa/trabeculae originating from the capsule** that has major blood vessels running through.

Q: Which of the following can be found in the previous section:

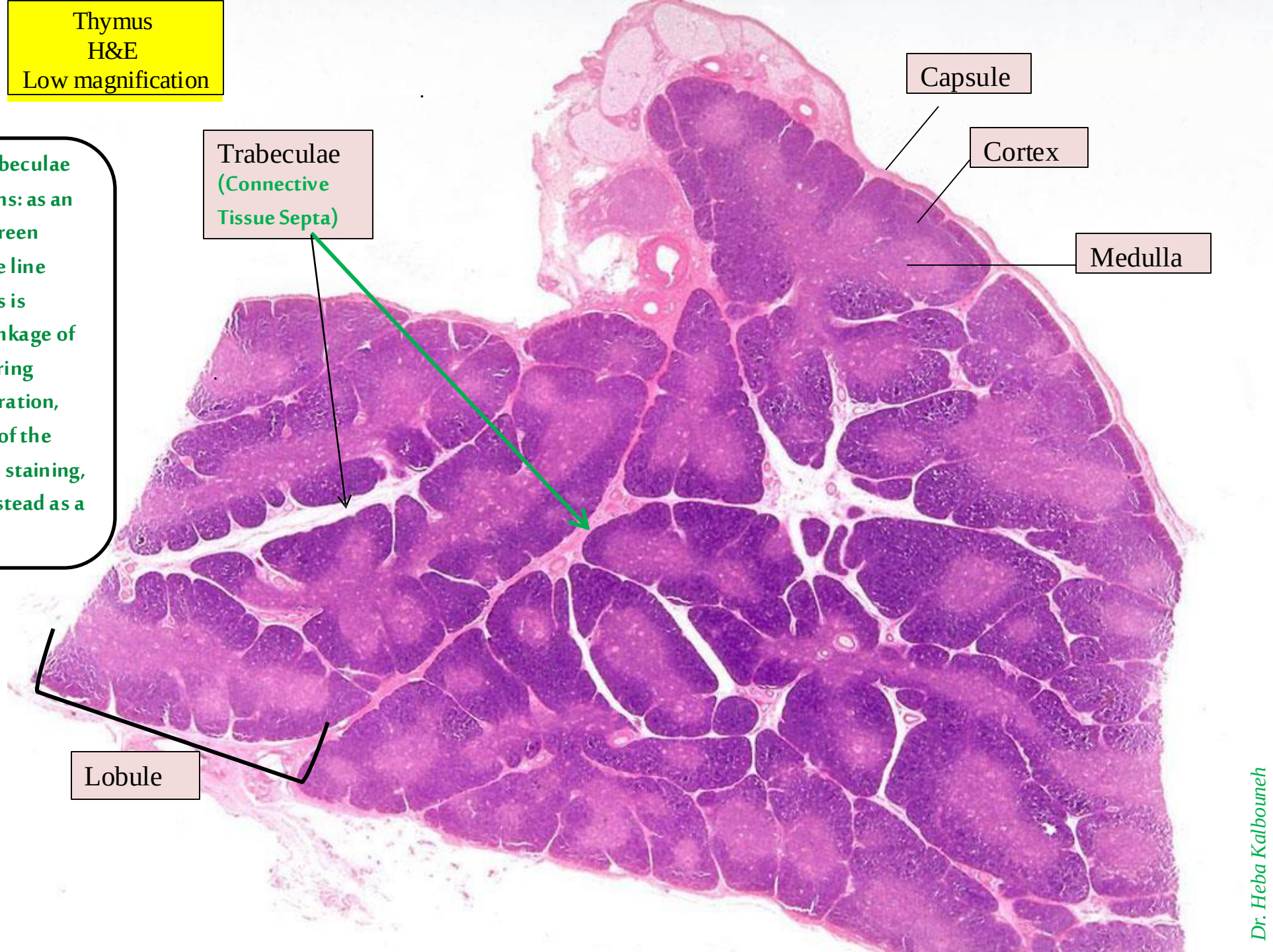
A- Primary follicle [there are no follicles in thymus]

B- Lymphatic sinus [this is not a lymph node in the first place]

C- Central arteriole [specific to spleen]

Ans: None of the above can be found.

Thymus
H&E
Low magnification



In this section, trabeculae appear in two forms: as an acidophilic line (green arrow) and a white line (black arrow). This is attributed to shrinkage of collagen fibers during histological preparation, resulting in a loss of the typical acidophilic staining, which presents instead as a white line.

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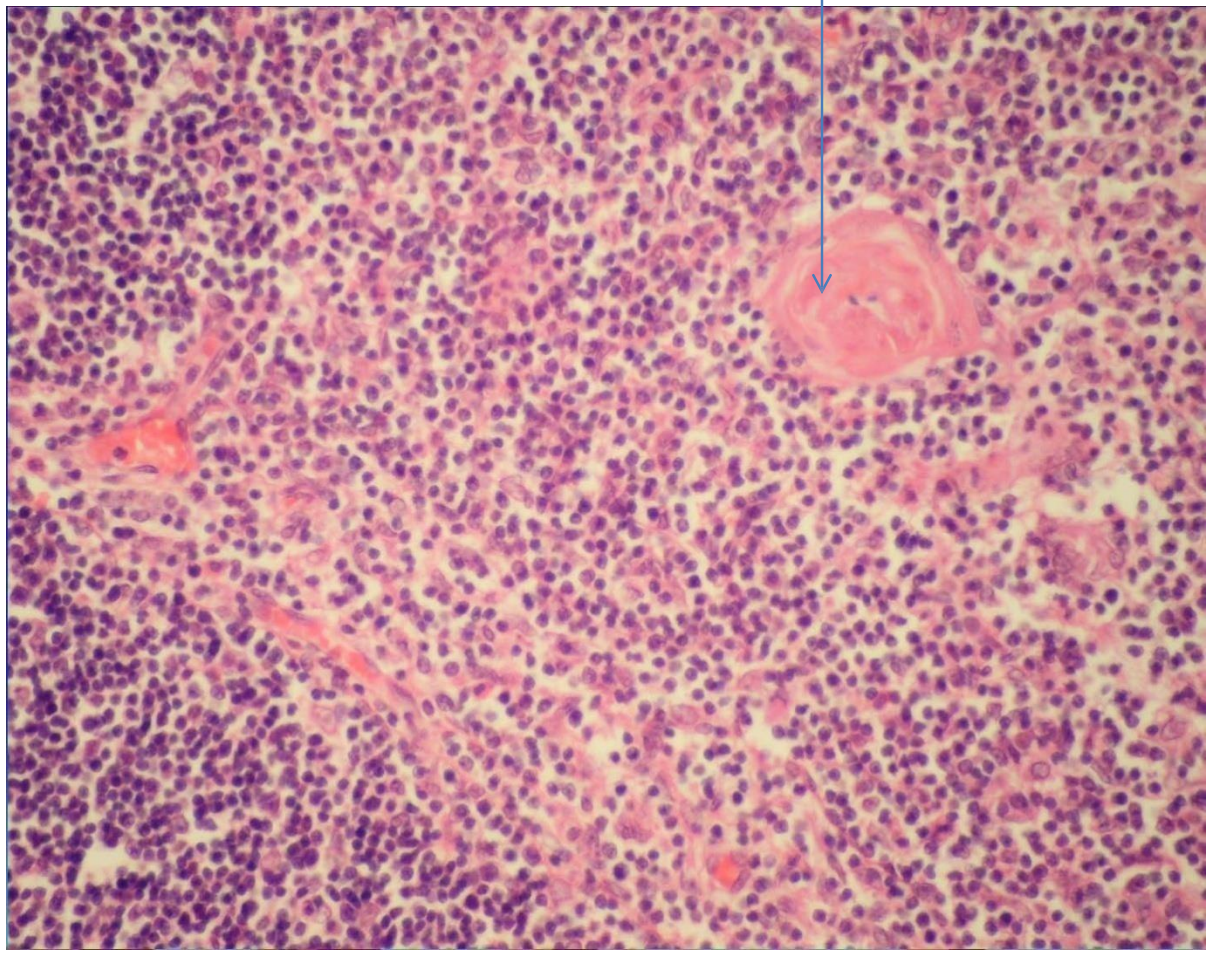
Thymic medulla
H&E
High magnification

Hassall
Corpuscle in medulla



The cells surrounding the corpuscle are MATURE T-Cells

-Remember:

Immature T-Cells: Cortex

Mature T-Cells: Medulla

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Involuted Thymus
H&E
Low magnification

-In this picture we have an involuted thymus.
-Involution is the shrinkage of thymus with age due to the replacement with fatty tissue which changes the morphology of lobules.
-As you can see there is a high number of fatty cells in this section.

The whole outlined section is a lobule

Hassal Corpuscle

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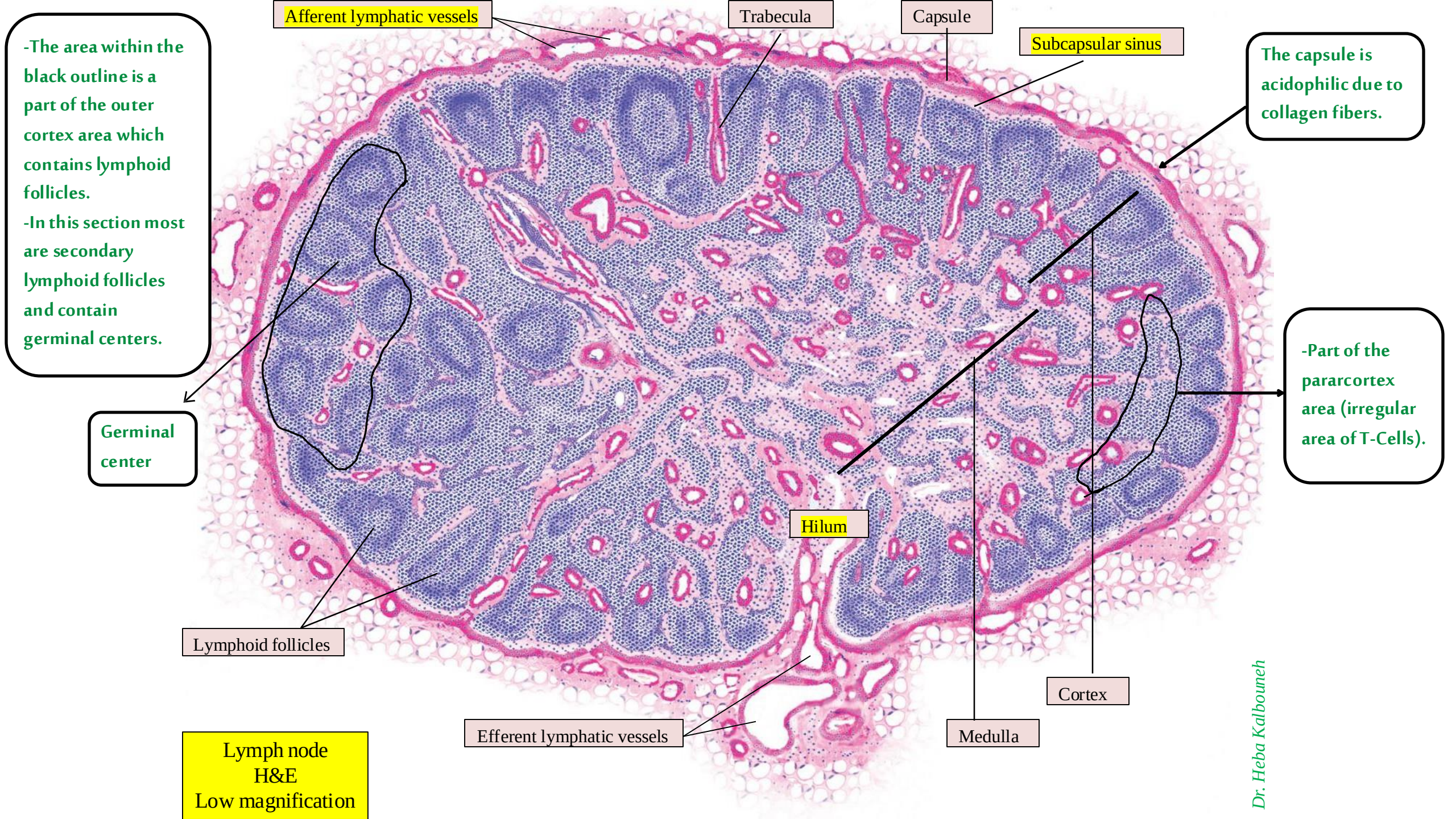
Lymph nodes

The lymph node is encased by a capsule. The lymph enters the node via afferent lymphatic vessels. The capsule and trabeculae, which extend into the node from the capsule, provide the main structural support. Note the B-cell containing lymphoid follicles located in the outer cortex. The medulla contains medullary cords (aggregates of lymphoid tissue) and medullary sinuses (lymphatic channels). Between the outer cortex and medulla lies an ill-defined region called the paracortex (inner cortex). The hilum of the lymph node is the location where blood vessels enter and exit the node. It is also where the medullary sinuses merge into efferent lymphatic vessels, which carry the lymph away from the node.


Afferent lymphatic vessels
Trabecula
Capsule
Subcapsular sinus
-The area within the black outline is a part of the outer cortex area which contains lymphoid follicles.
-In this section most are secondary lymphoid follicles and contain germinal centers.
The capsule is acidophilic due to collagen fibers.
Germinal center
-Part of the pararcortex area (irregular area of T-Cells).
Hilum
Lymphoid follicles
Cortex
Efferent lymphatic vessels
Medulla
Lymph node
H&E
Low magnification
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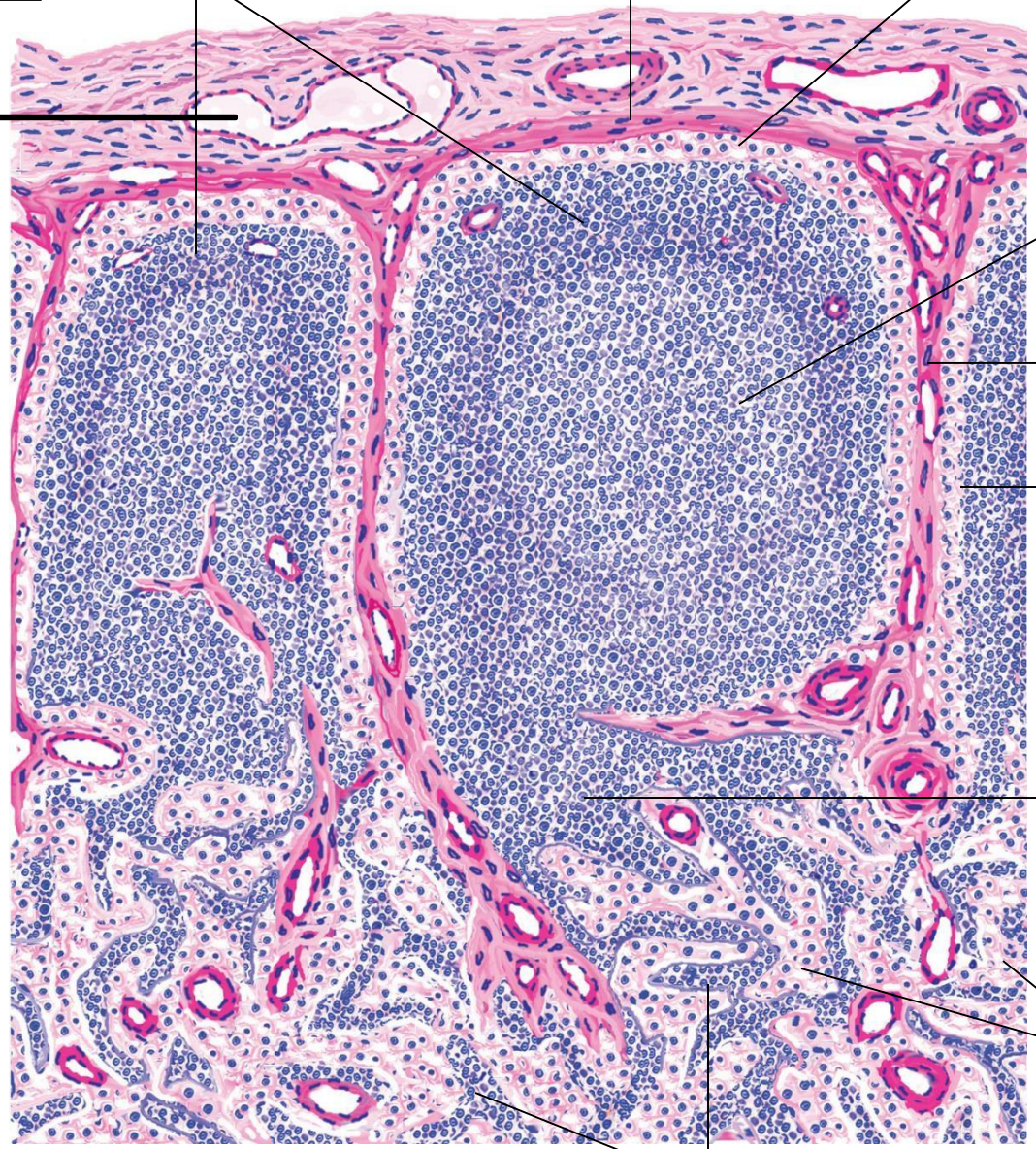
Lymph node
H&E
Higher magnification



Capsule

Subcapsular sinus

Lymphoid follicles

-Afferent lymphatic vessel inside the capsule.

Germinal center

Trabecula

trabecular sinus

Cortex

Paracortex

Medulla

What type of cells would you expect to find in the paracortex? It's an irregular area of T-lymphocytes.

Medullary sinuses

Medullary cords

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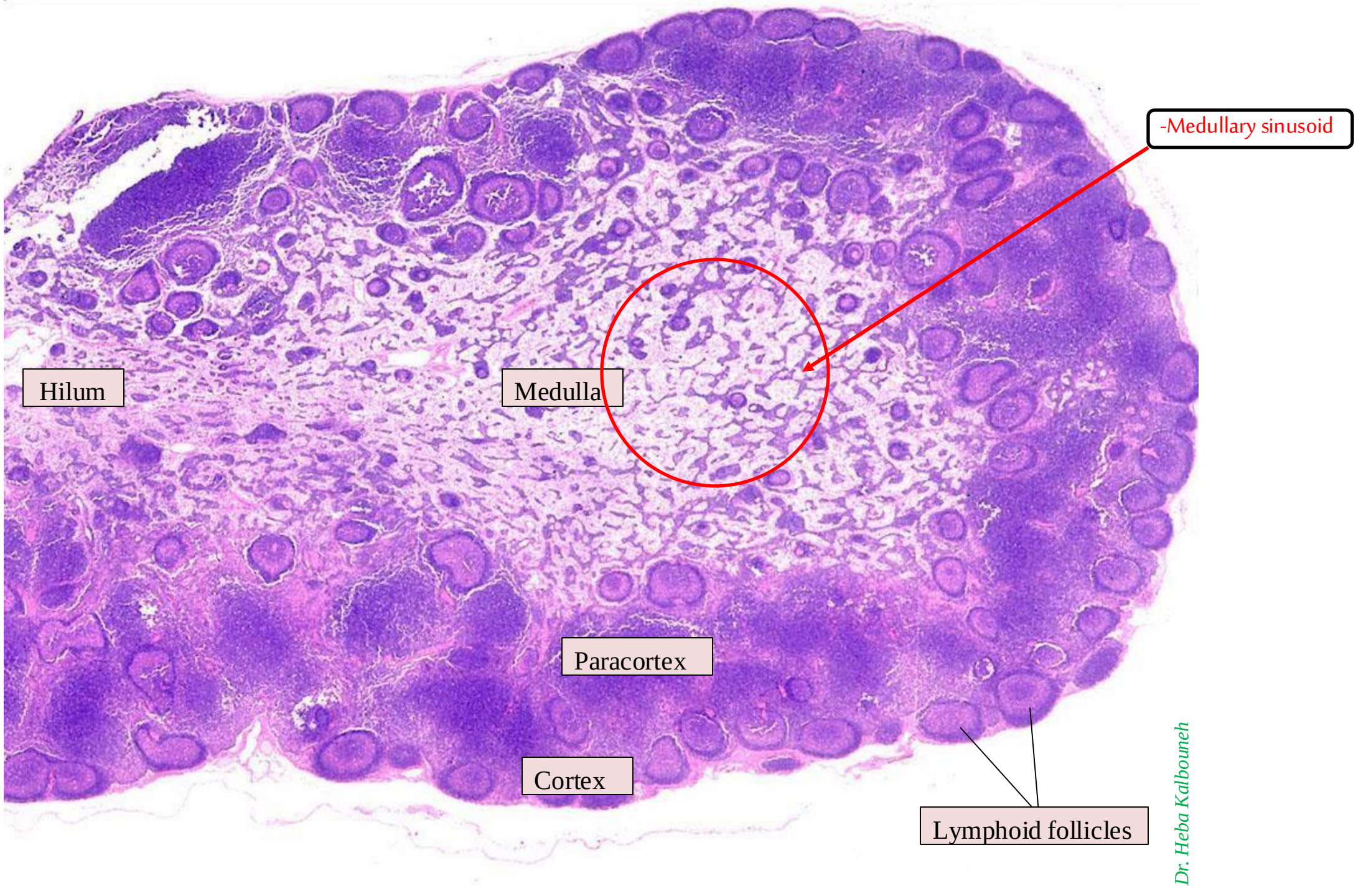

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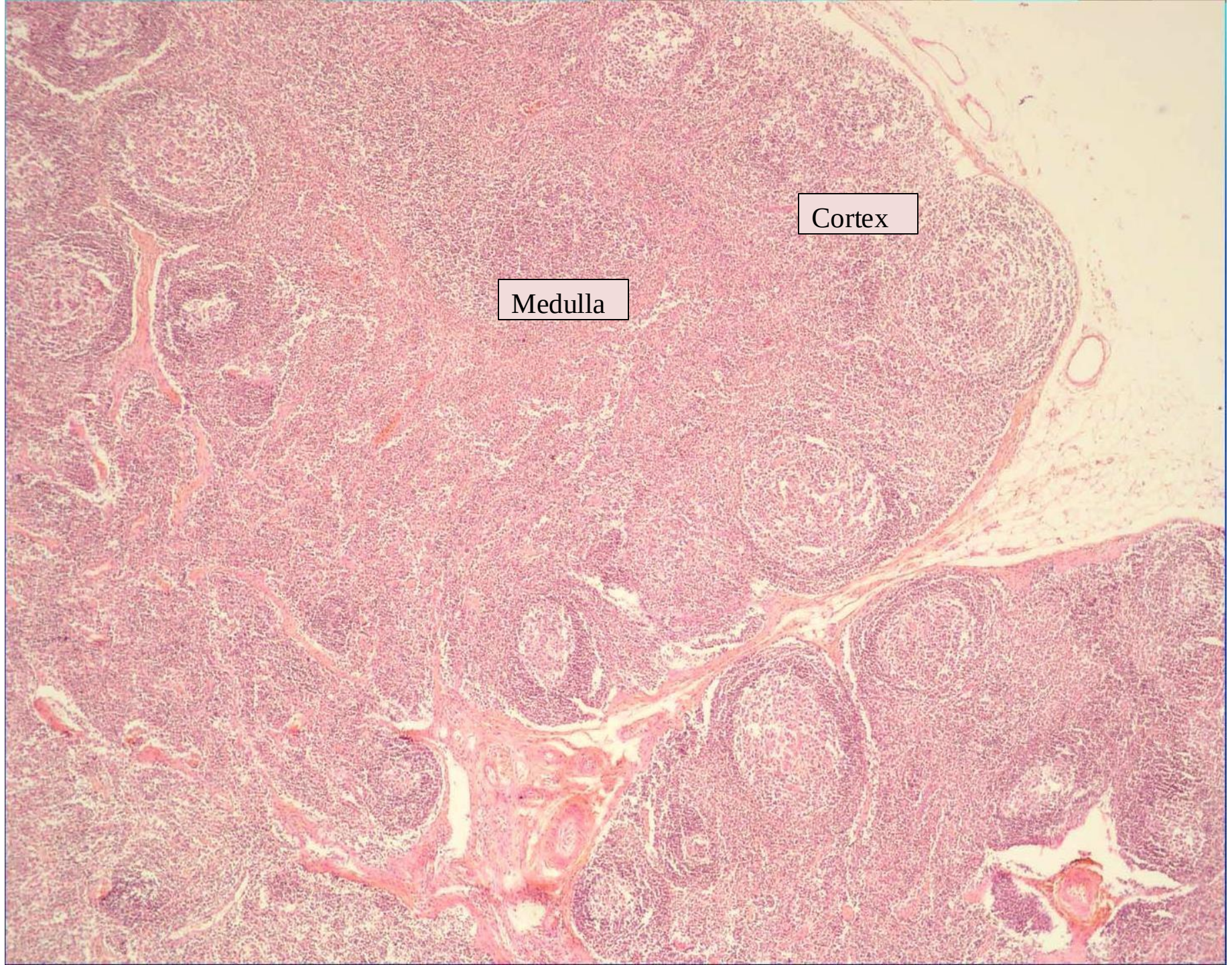

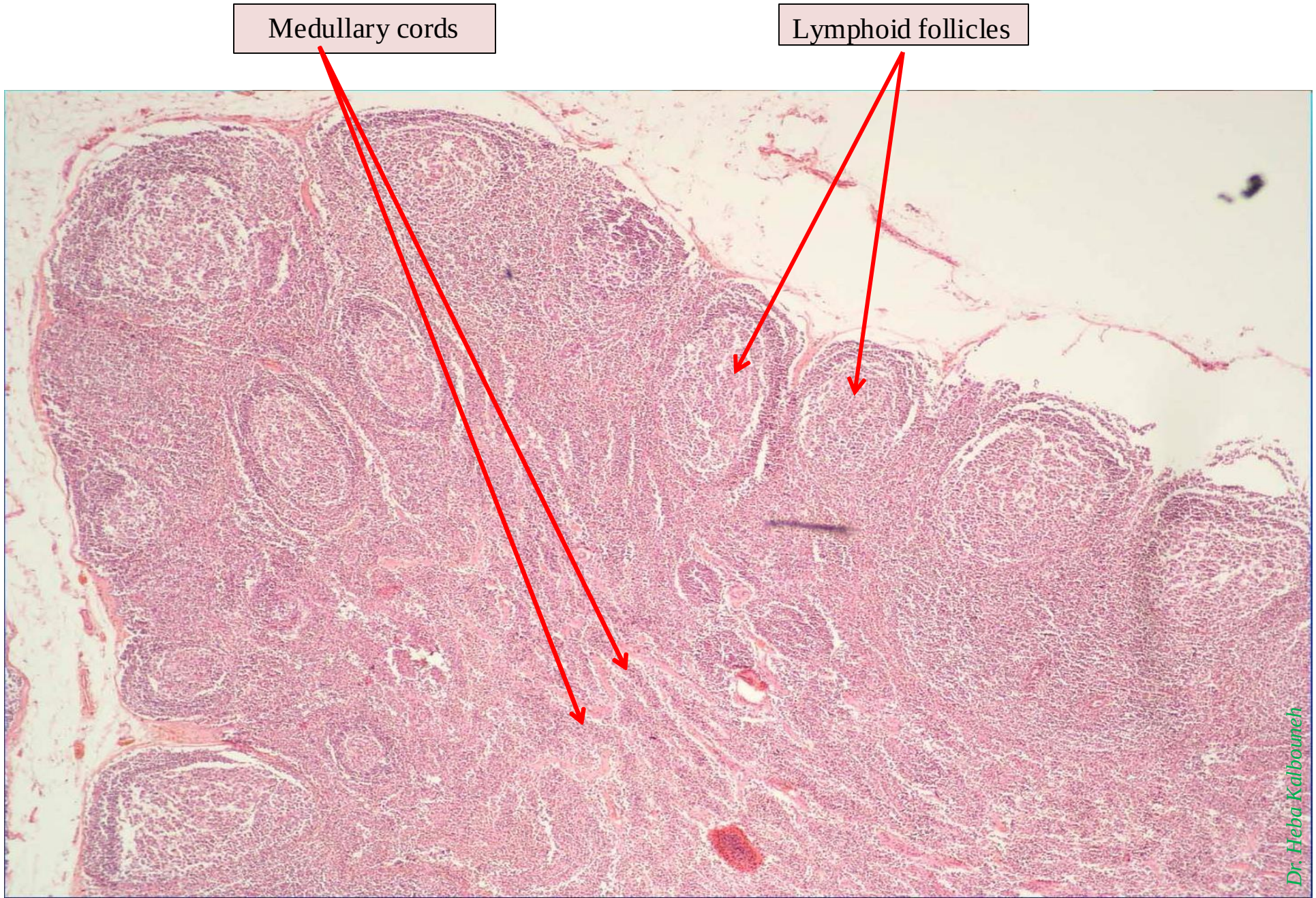
Medullary cords
Lymphoid follicles
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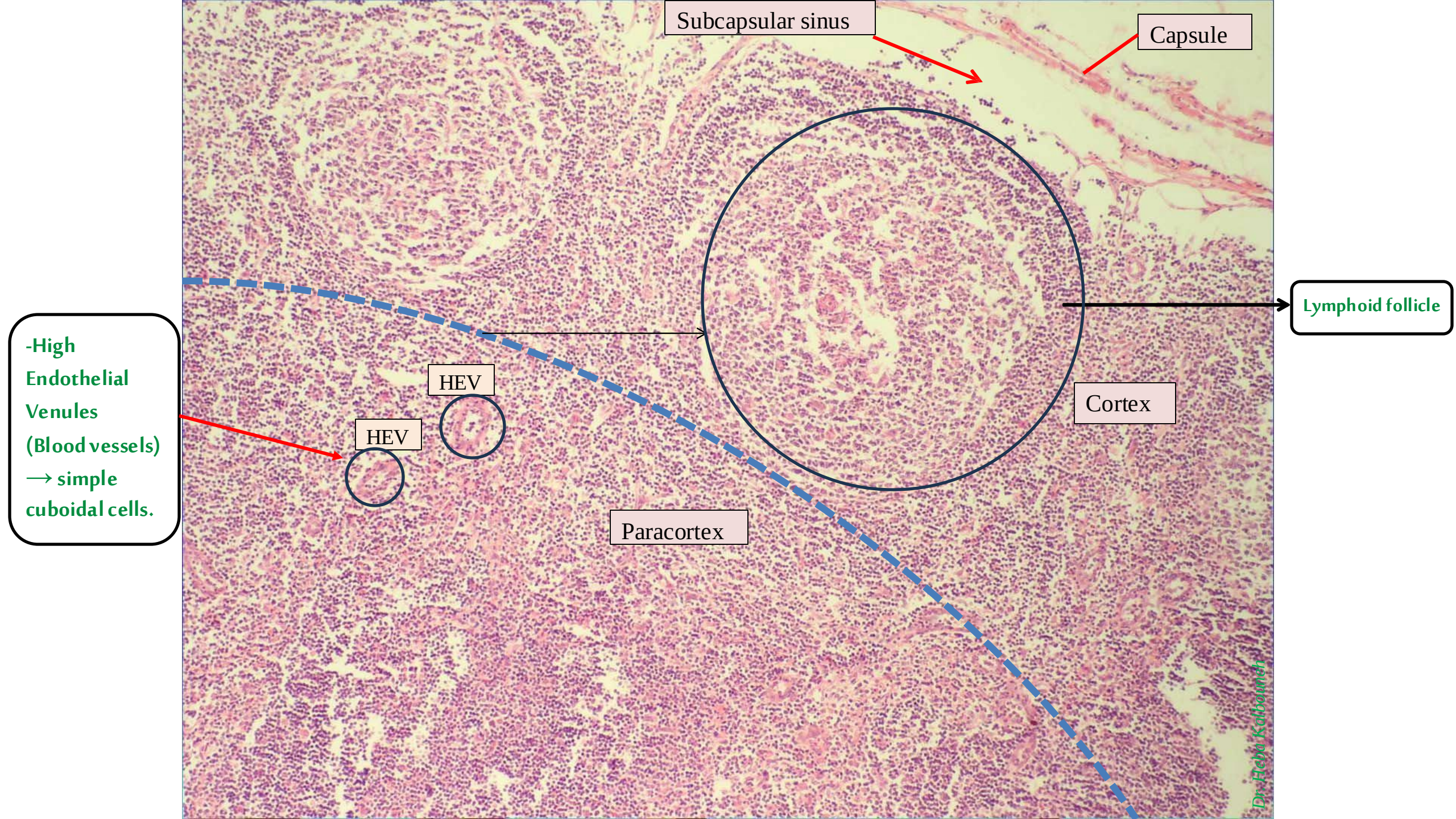
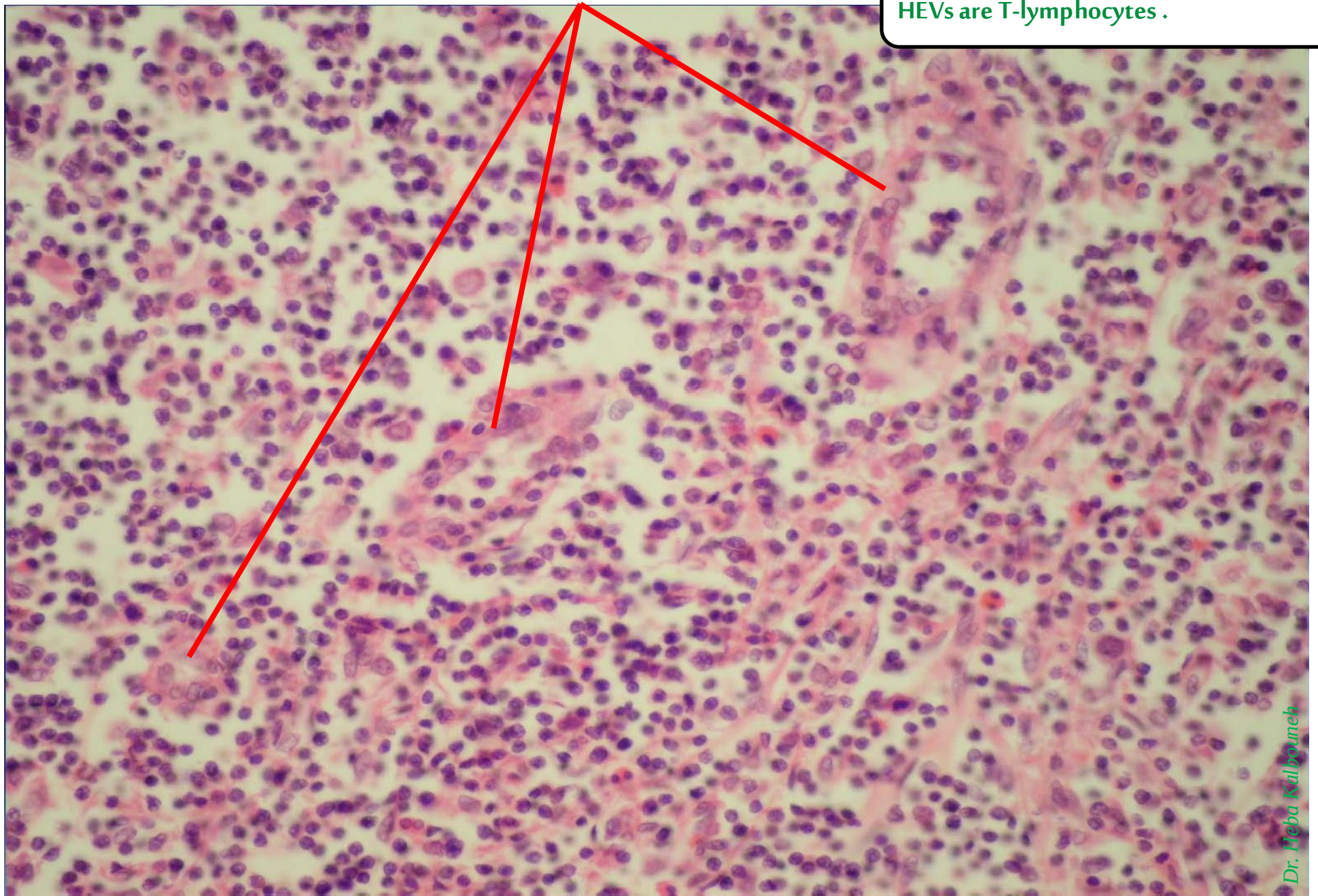

Subcapsular sinus
Capsule
Lymphoid follicle
-High Endothelial Venules (Blood vessels) ⟶ simple cuboidal cells.
HEV
HEV
Cortex
Paracortex
Dr.Heba Kalboush

HEVs in paracortex

-Most of the Basophilic cells surrounding HEVs are T-lymphocytes .

Higher magnification of the previous section.

HEVs look like ducts due to its cuboidal epithelium.



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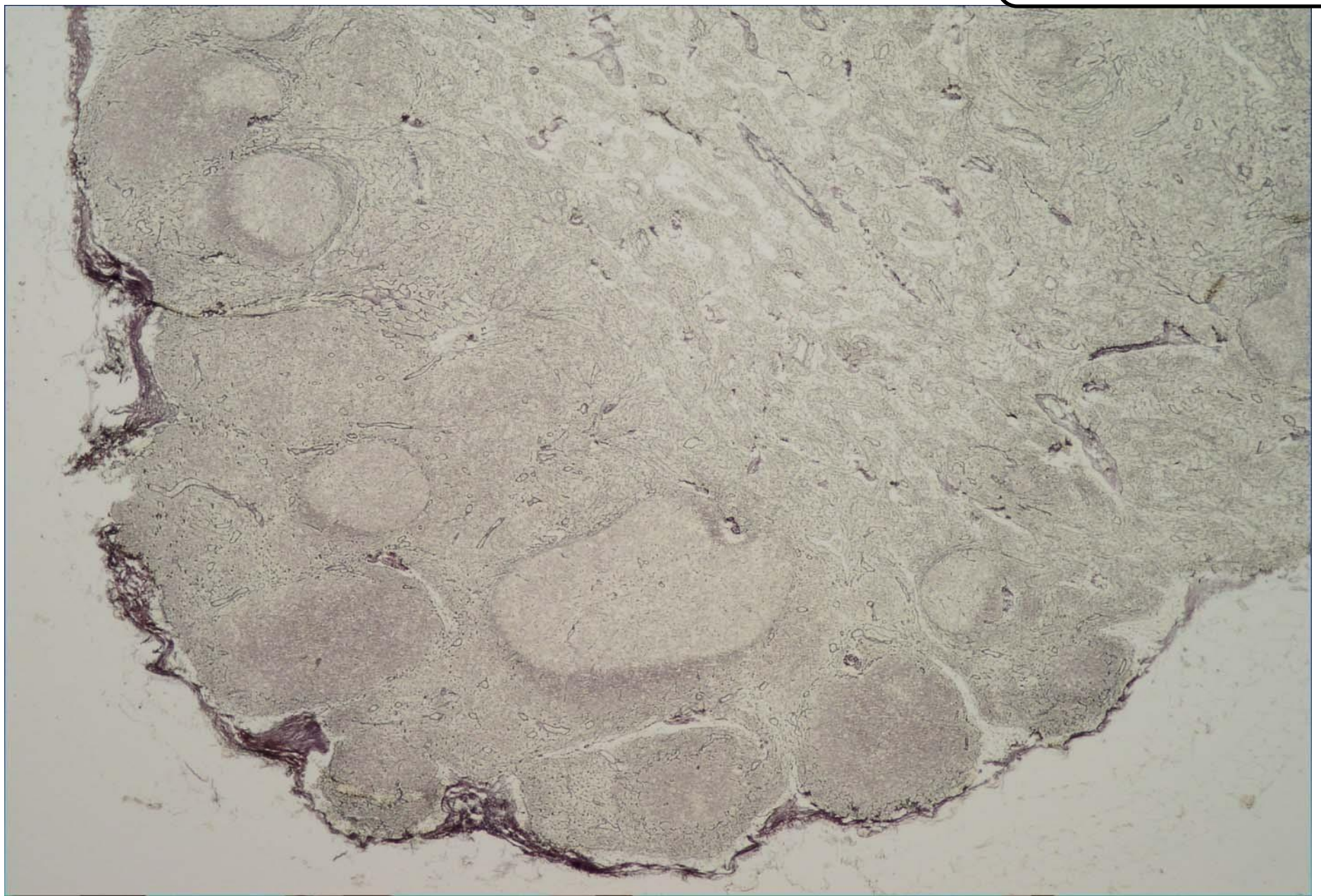
Lymph node- Silver Stain

Remember: reticular fibers are argyrophilic

-Reticular fibers form the stroma

-"Argyrophilic" means having an affinity for silver



Dr. Heba Kalbouneh

Spleen

1) Capsulated
2) Hilum on medial surface
3) Spleen filters blood not lymph so it doesn't have afferent lymphatic vessels

On the outer edge of spleen, note the presence of a capsule from which short trabeculae (containing a trabecular artery and trabecular vein) extend into the parenchyma. In contrast to lymph nodes and thymus, the spleen is not arranged into cortex and medulla. Instead, the majority of the spleen consists of well-vascularized red pulp (pale-stained due to lower cell density) with white pulp (lymphoid aggregations) scattered throughout. Note the presence of sinuses within the red pulp.

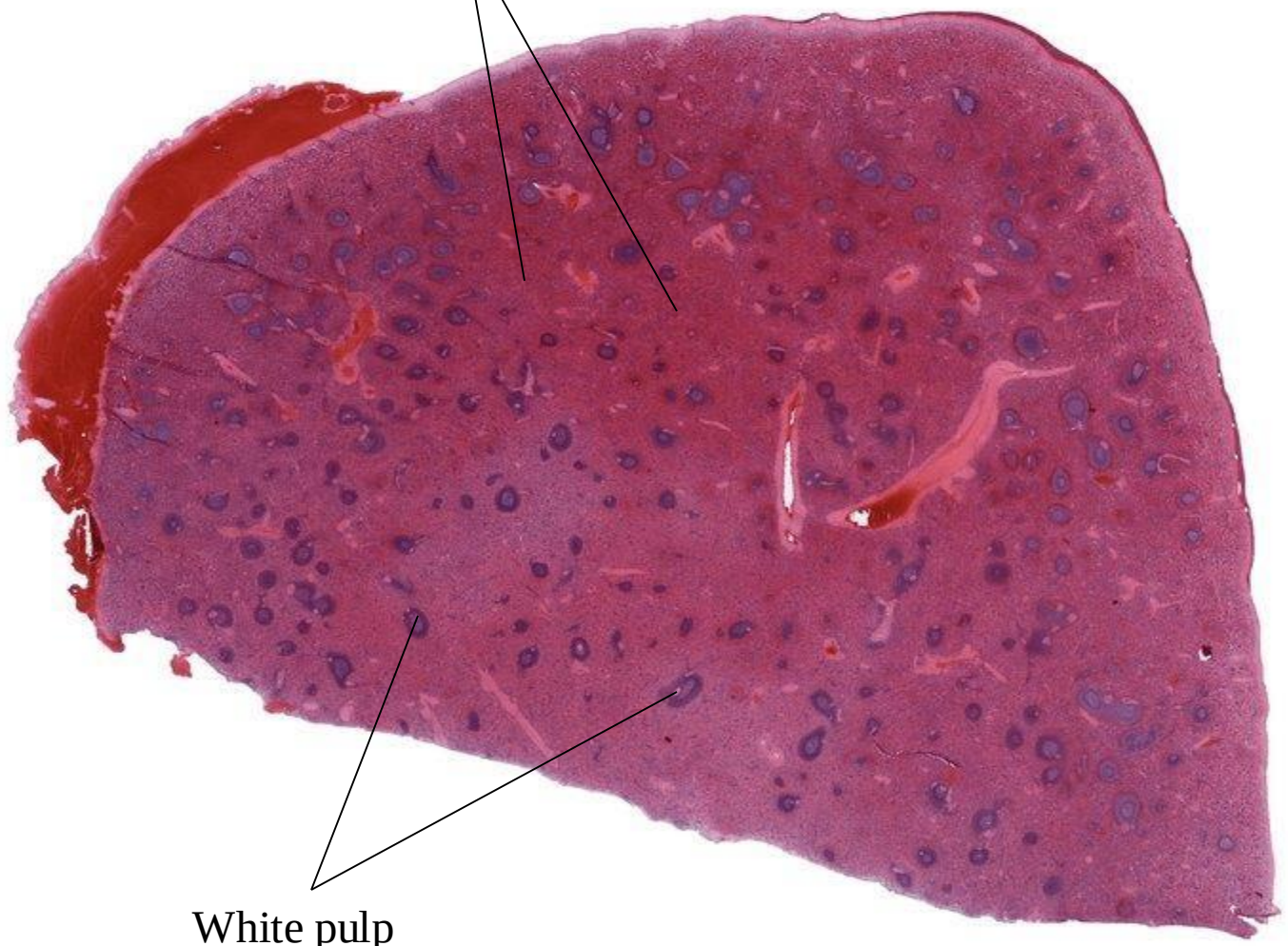
Reminder of splenic artery course to help us understand histology of spleen:

-Splenic artery enters hilum ⟶ Trabecular artery ⟶ Center arterioles ⟶ Penicillar arterioles ⟶ Terminal capillaries ⟶ either Splenic cord (open circulation) or Splenic sinusoid (closed circulation).

- From Splenic sinusoid ⟶ Trabecular vein ⟶ Splenic vein.

Spleen
Gross observation

-Macroscopically (Gross), the white pulp appears whitish in color. However, under a light microscope with standard stains (e.g., H&E), it appears bluish due to the dense aggregation of basophilic lymphocytes.



Capsule

Central arteriole

Germinal center
(Malphigian corpuscle)

Spleen
H&E
Low magnification

Check the next slide

Trabecula

The basophilic cells in the capsule are fibroblasts

PALS

Trabeculae containing trabecular artery and vein

Trabeculae containing trabecular arteries.

Lymphoid follicle (white pulp)

Red pulp

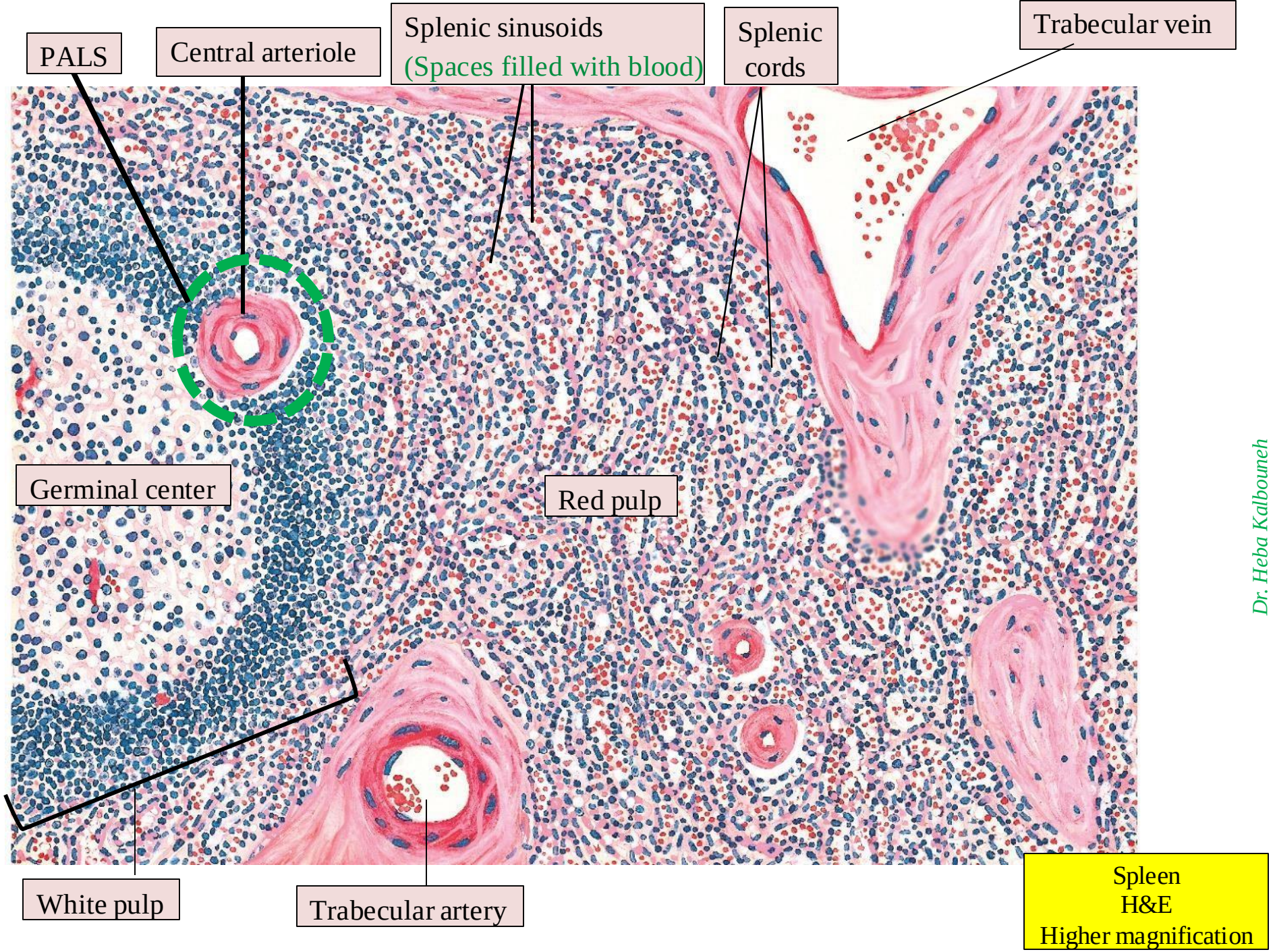
Dr. Heba Kalbouneh

-Regarding the previous section, the doctor said that the spleen can be identified by two key features:

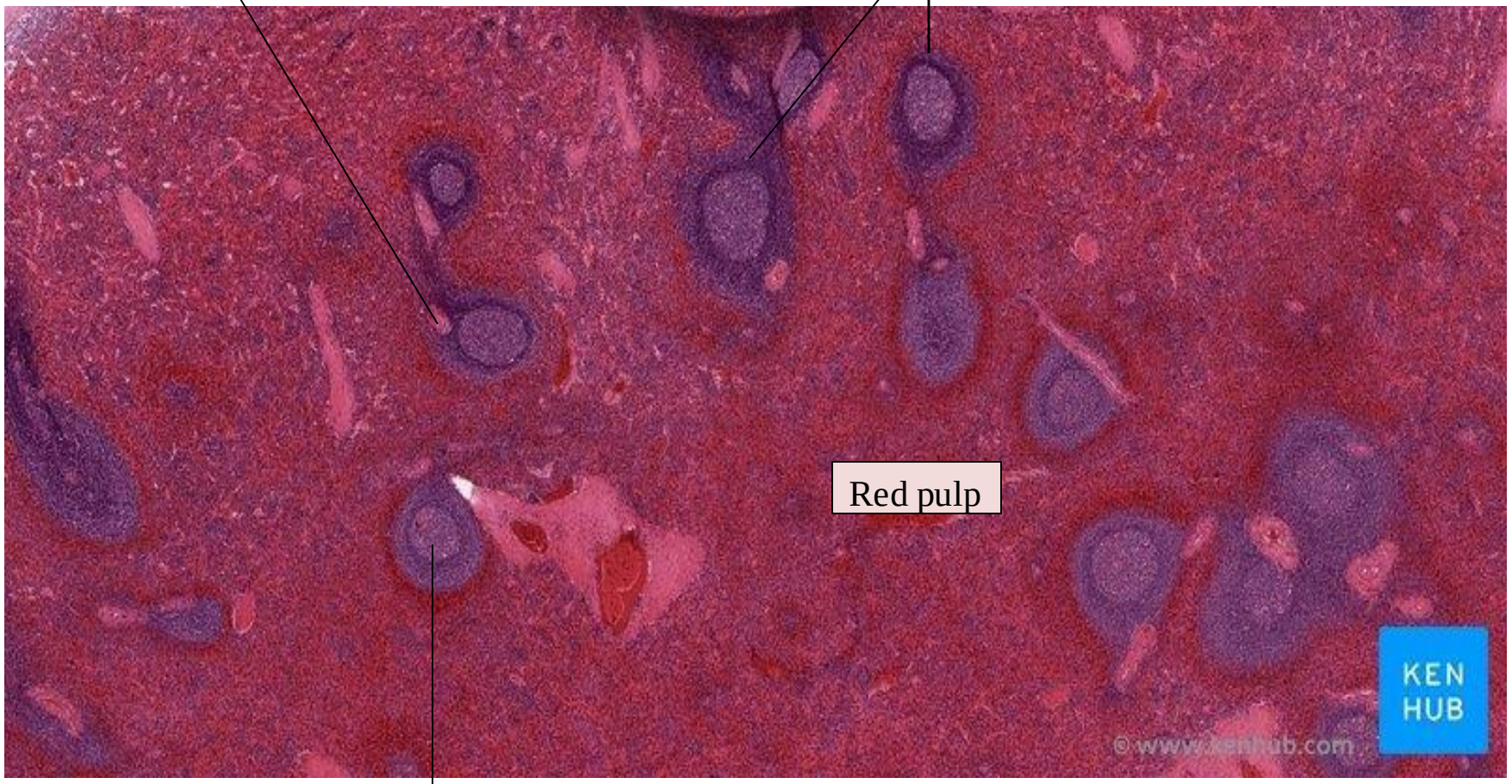
1) Scattered follicles, which lack organized arrangement like the ones seen in lymph nodes.
2) Central arterioles.

-Due to the expansion of periarteriolar lymphatic sheath (PALS) to incorporate a lymphatic follicle, the central arteriole gets displaced to one side and acquires an eccentric (peripheral) position to the follicle but is still called a central arteriole.

-The central arteriole remains centrally located in PALS area, So its peripheral relative to the follicle and central relative to the PALS area.


PALS
Central arteriole
Splenic sinusoids
(Spaces filled with blood)
Splenic cords
Trabecular vein
Germinal center
Red pulp
White pulp
Trabecular artery
Spleen
H&E
Higher magnification
Dr. Heba Kalbouneh

Scattered follicles



Stimulated follicle

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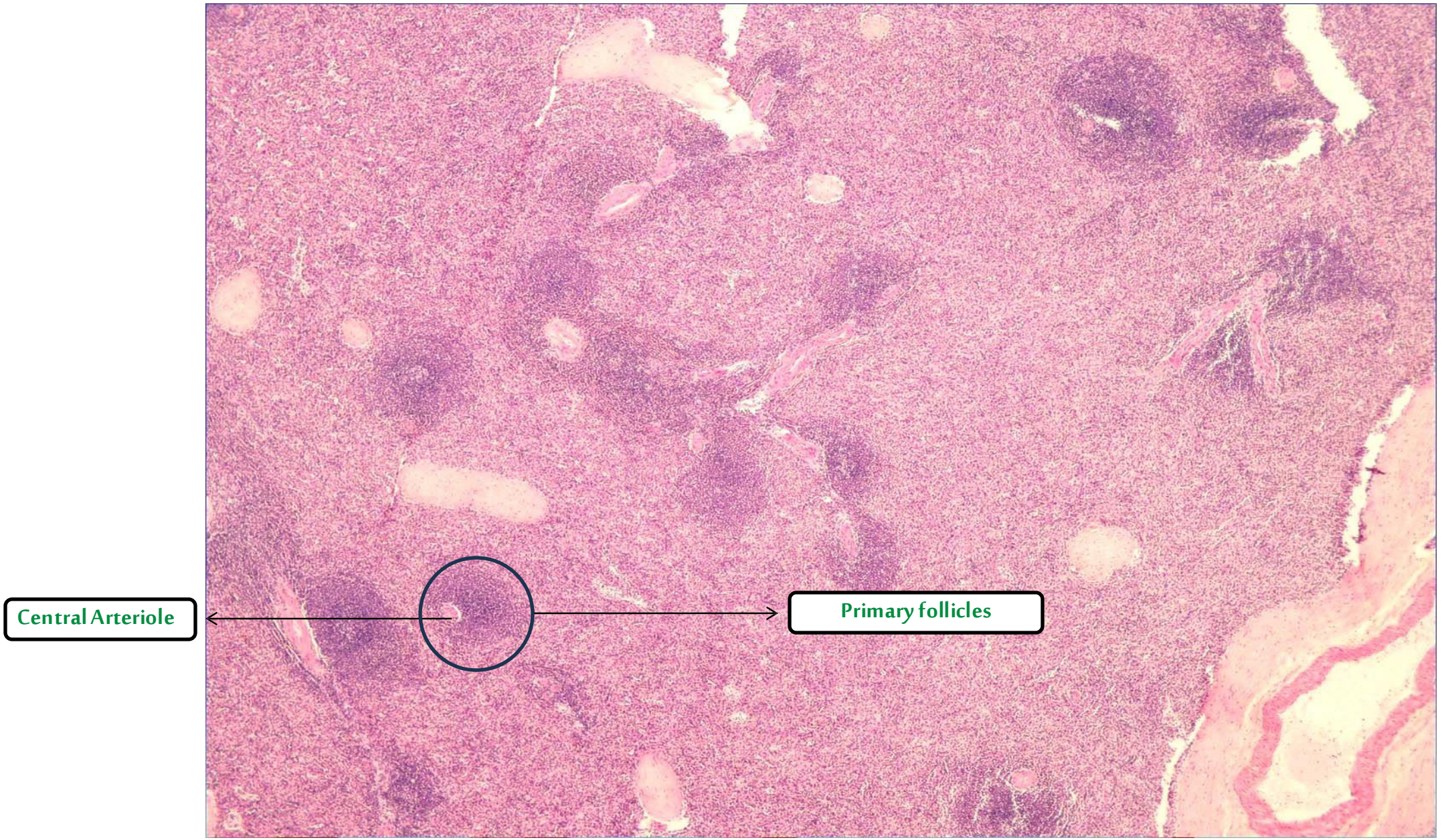

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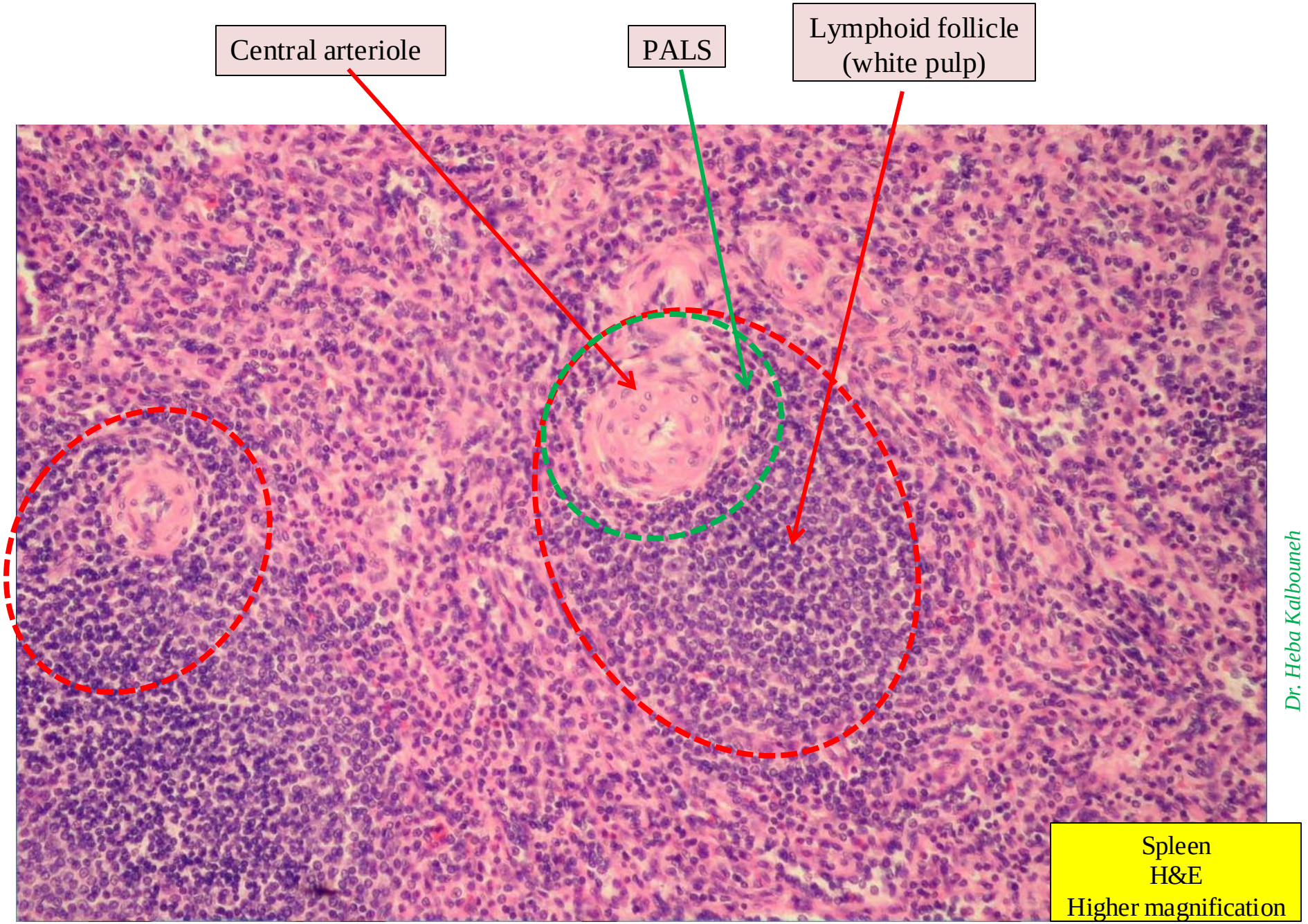
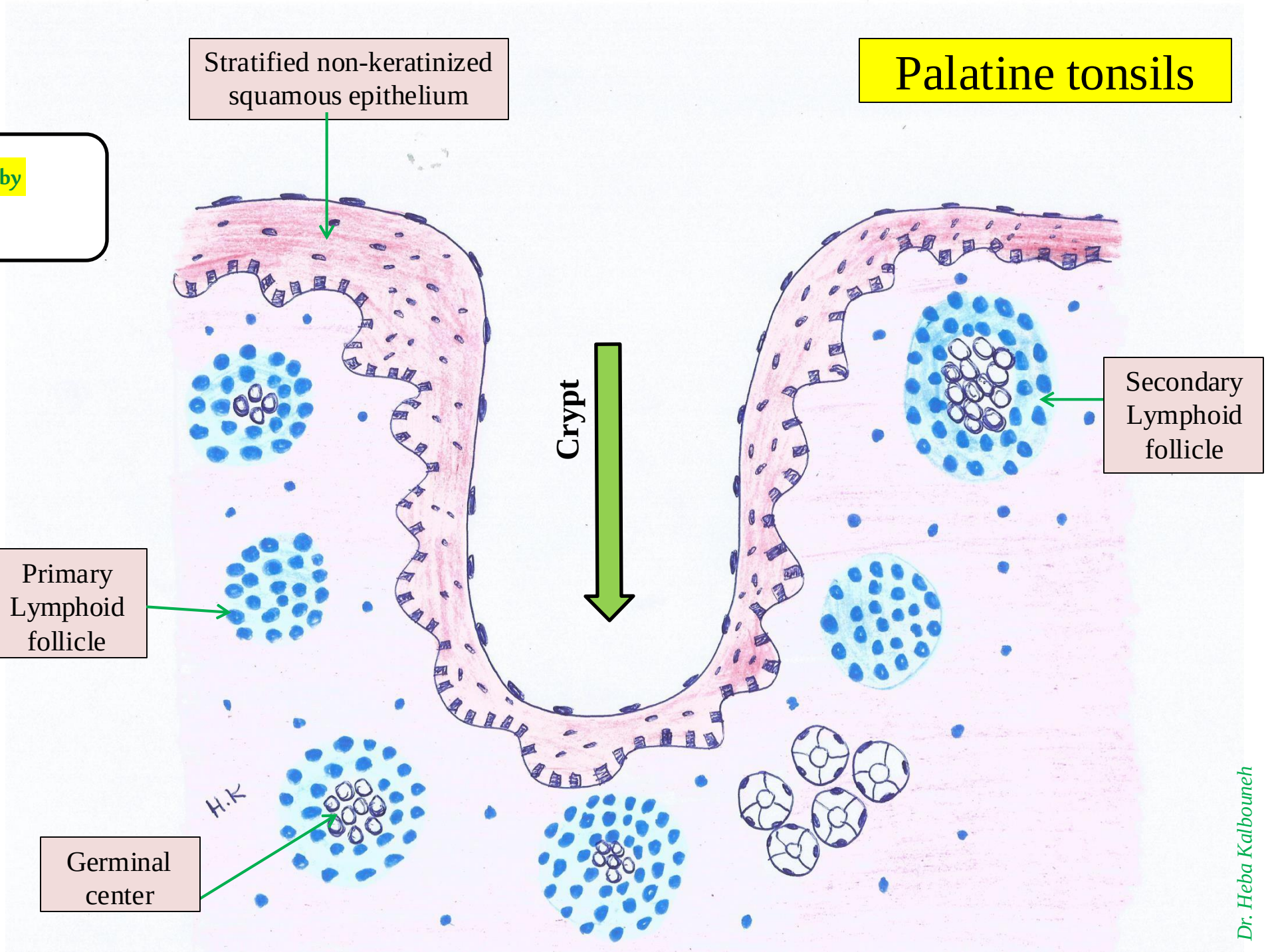

Central arteriole
PALS
Lymphoid follicle
(white pulp)
Dr. Heba Kalbouneh
Spleen
H&E
Higher magnification

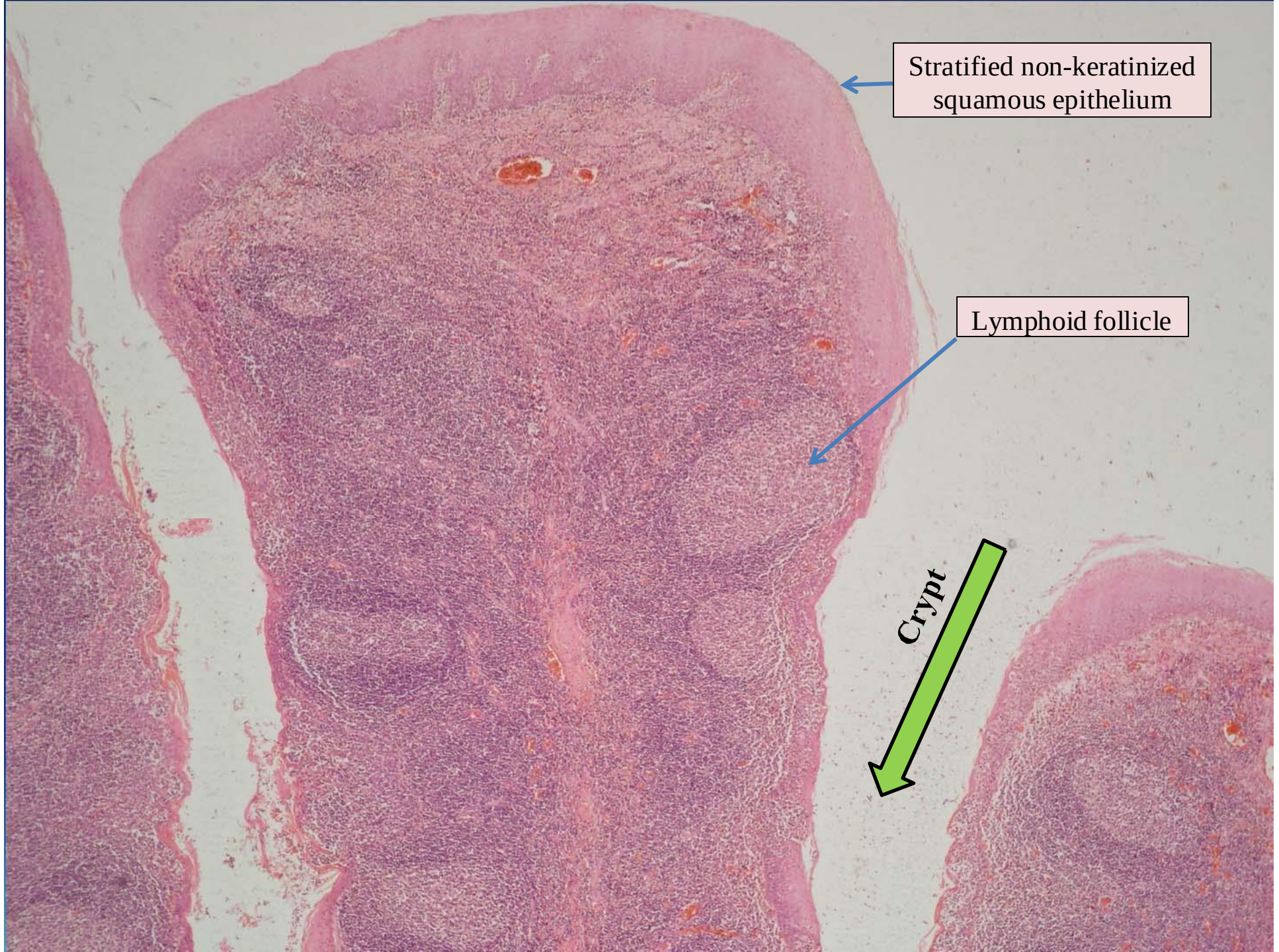
Palatine tonsils

Palatine tonsils can be identified by noticing an invagination (crypt) surrounded by lymphoid follicles

Palatine tonsils

#Crypt surrounded by follicles




Stratified non-keratinized squamous epithelium
Lymphoid follicle
Crypt


Dead squamous epithelium
(Desquamated cells)
Crypt
(base)
Lymphoid follicles
Dr. Heba Kalbouneh

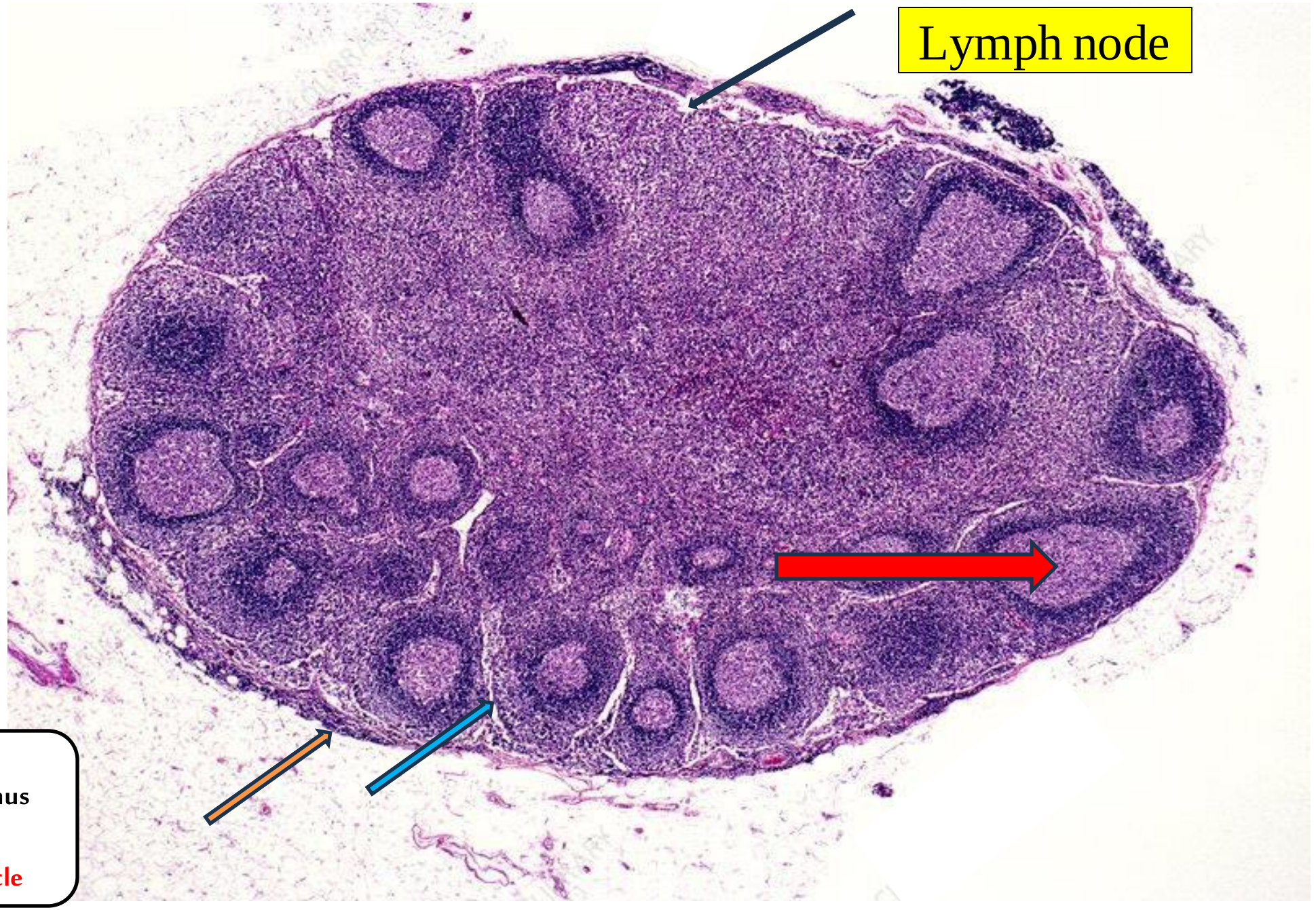
Dr. Heba Kalbouneh

Identify

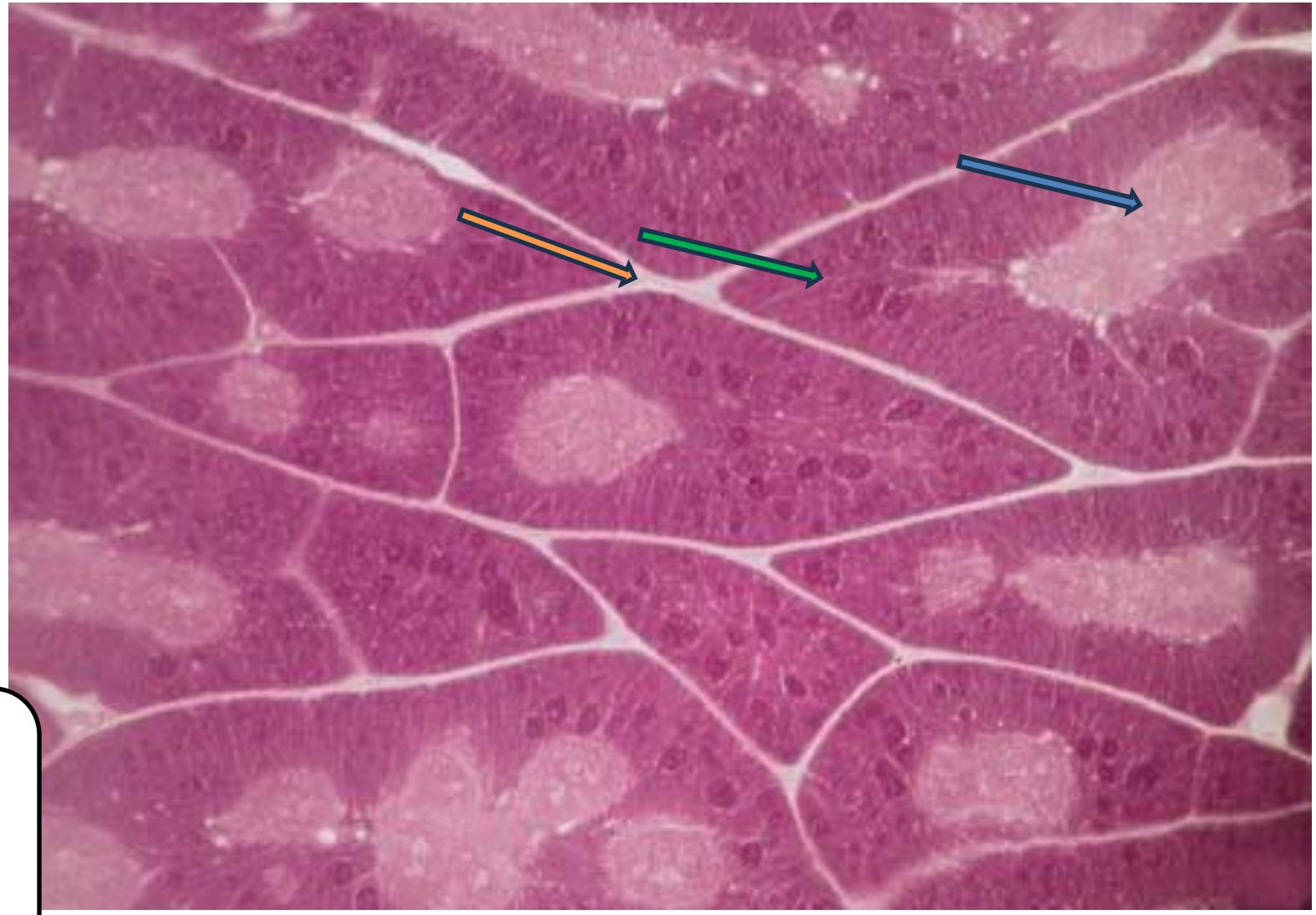
-The following images may appear in the exam

-Examples of question types in exam include:

1) Identification questions
2) Which of the given matches is correct or incorrect
3) Which of the following is not found in the provided section


Lymph node
Capsule
Subcapsular sinus
Trabecula
Lymphoid follicle

Thymus

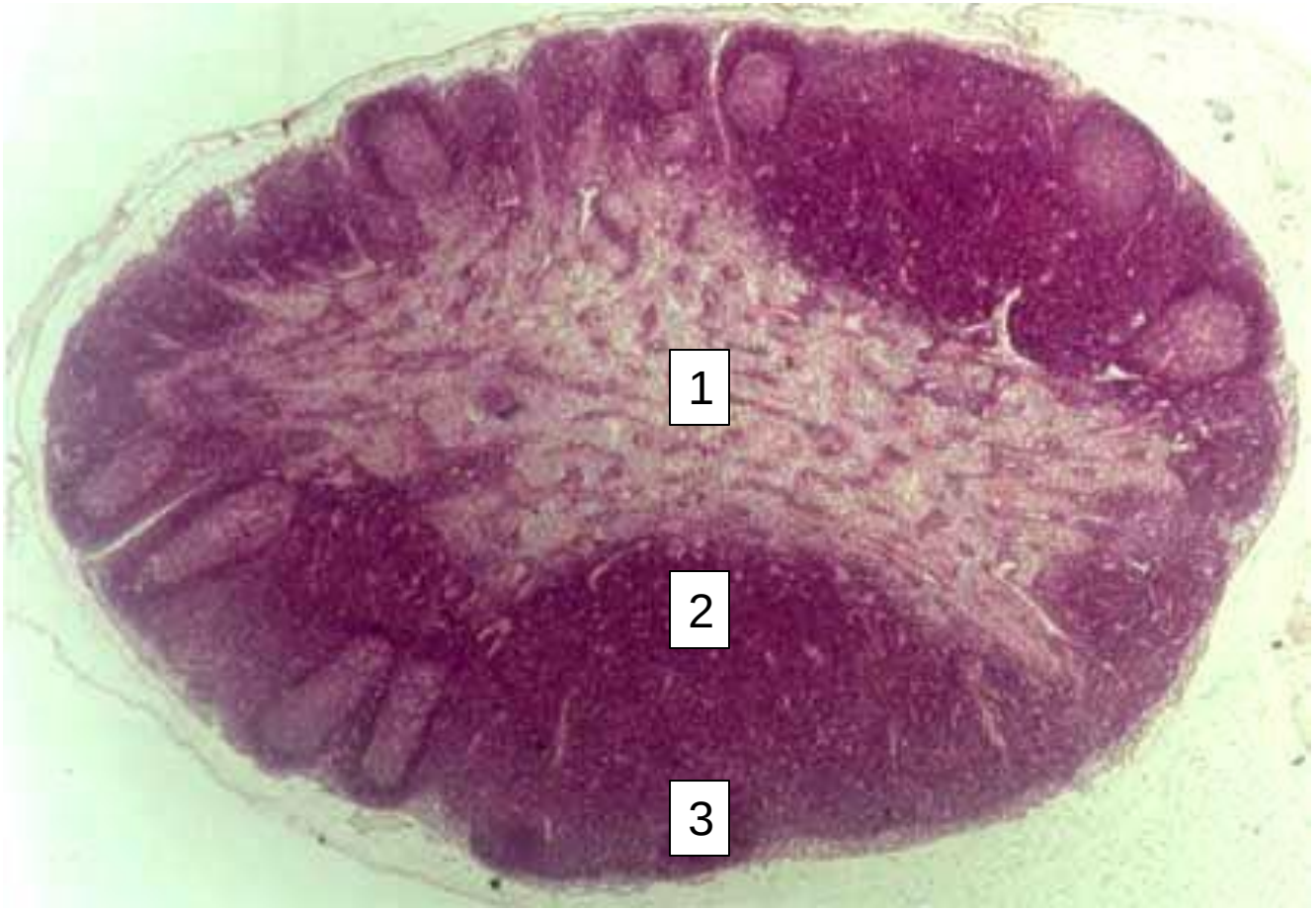


Medulla

Cortex

Shrunken trabeculae (TECs)

Lymph node



1) Medulla
2) Paracortex
3) Outer cortex

Palatine tonsils

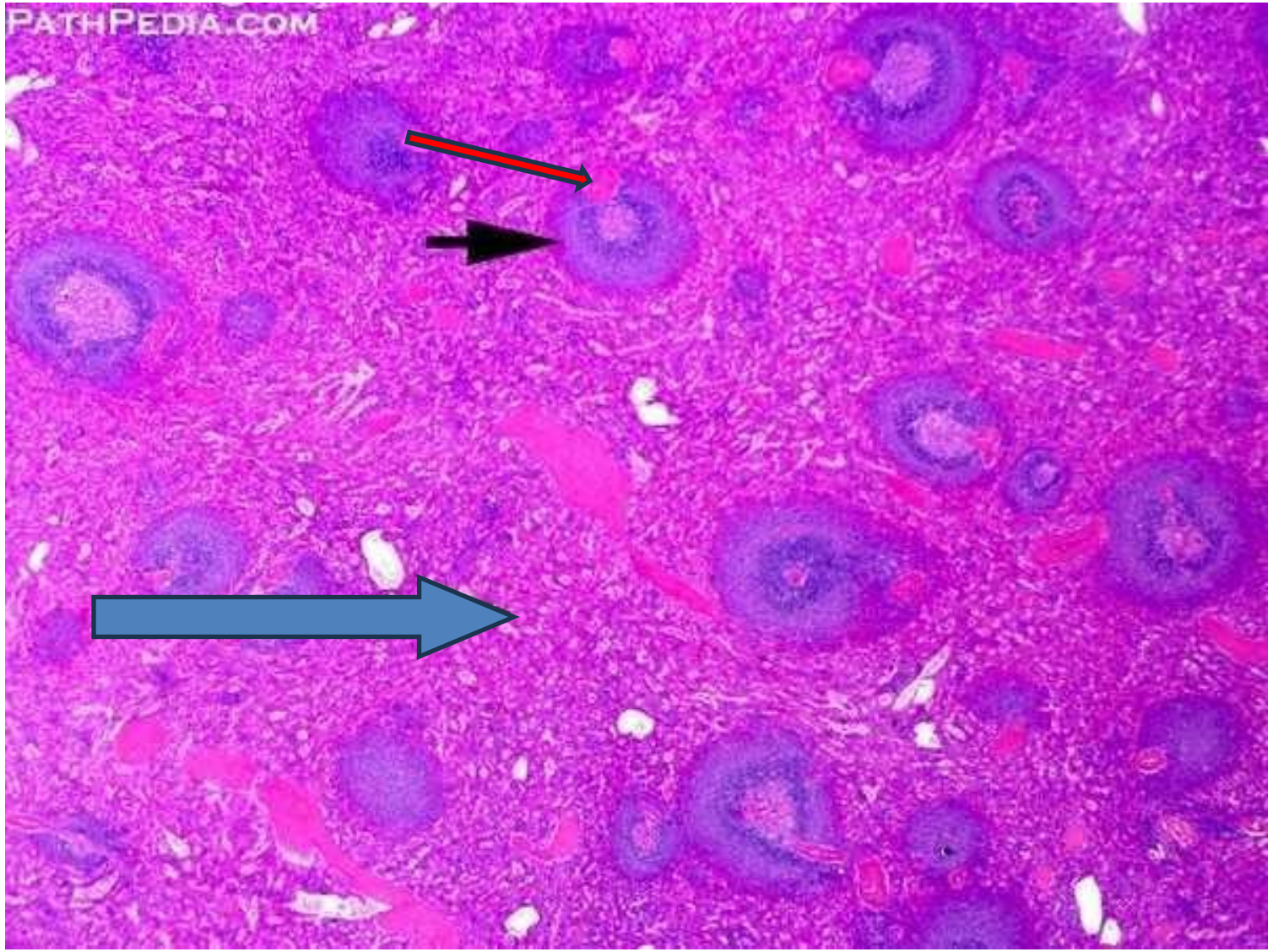
Crypts

Lymphoid follicle

Stratified non-keratinized squamous epithelium

The doctor said this picture won't be in the exam

Spleen

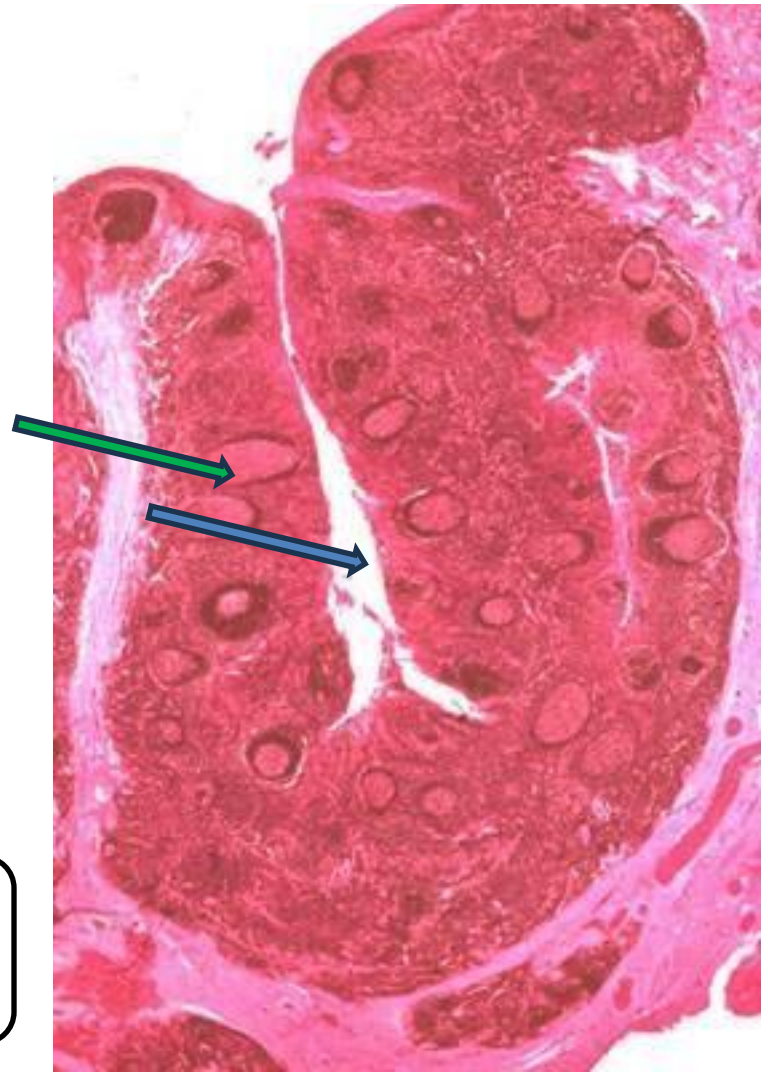


Central arteriole

White pulp

Red pulp

Palatine tonsils



Crypt

Lymphoid follicle

Thymus

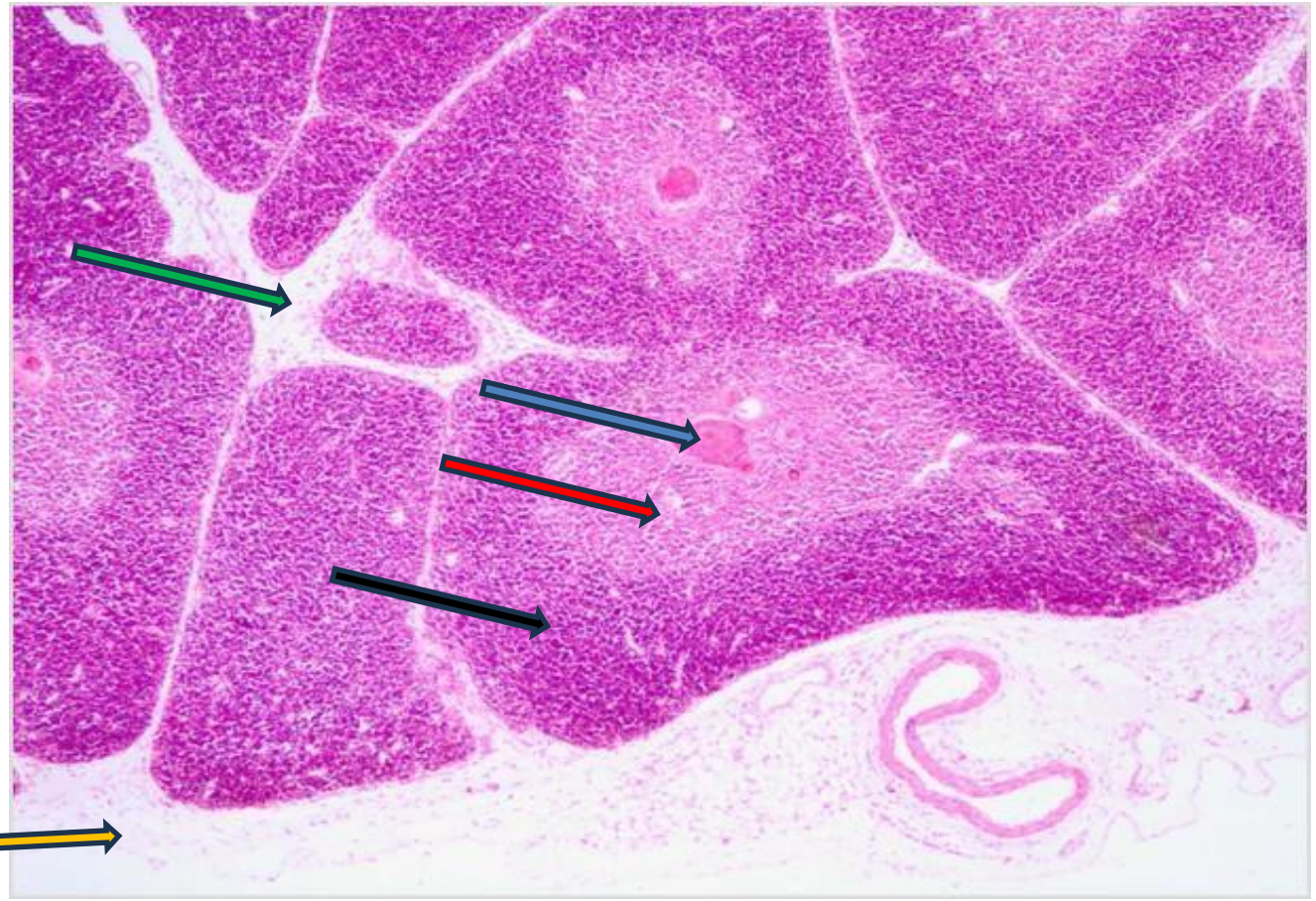
Trabeculae (TECs)

Medulla

Cortex

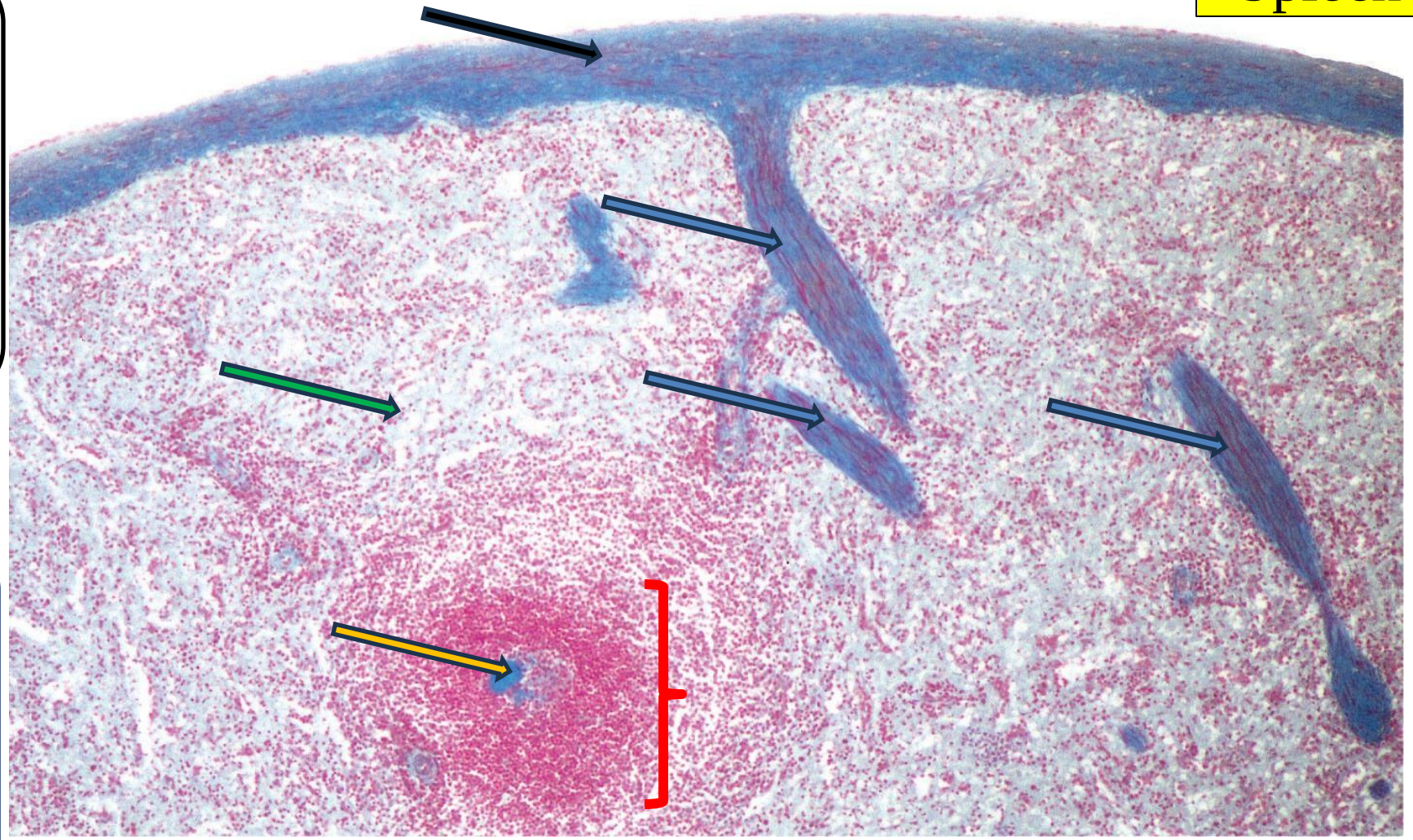
Hassal corpuscle

Capsule



Spleen

Connective tissue is blue in color due to staining by a special stain: Mallory Trichome stain



Capsule

Trabeculae

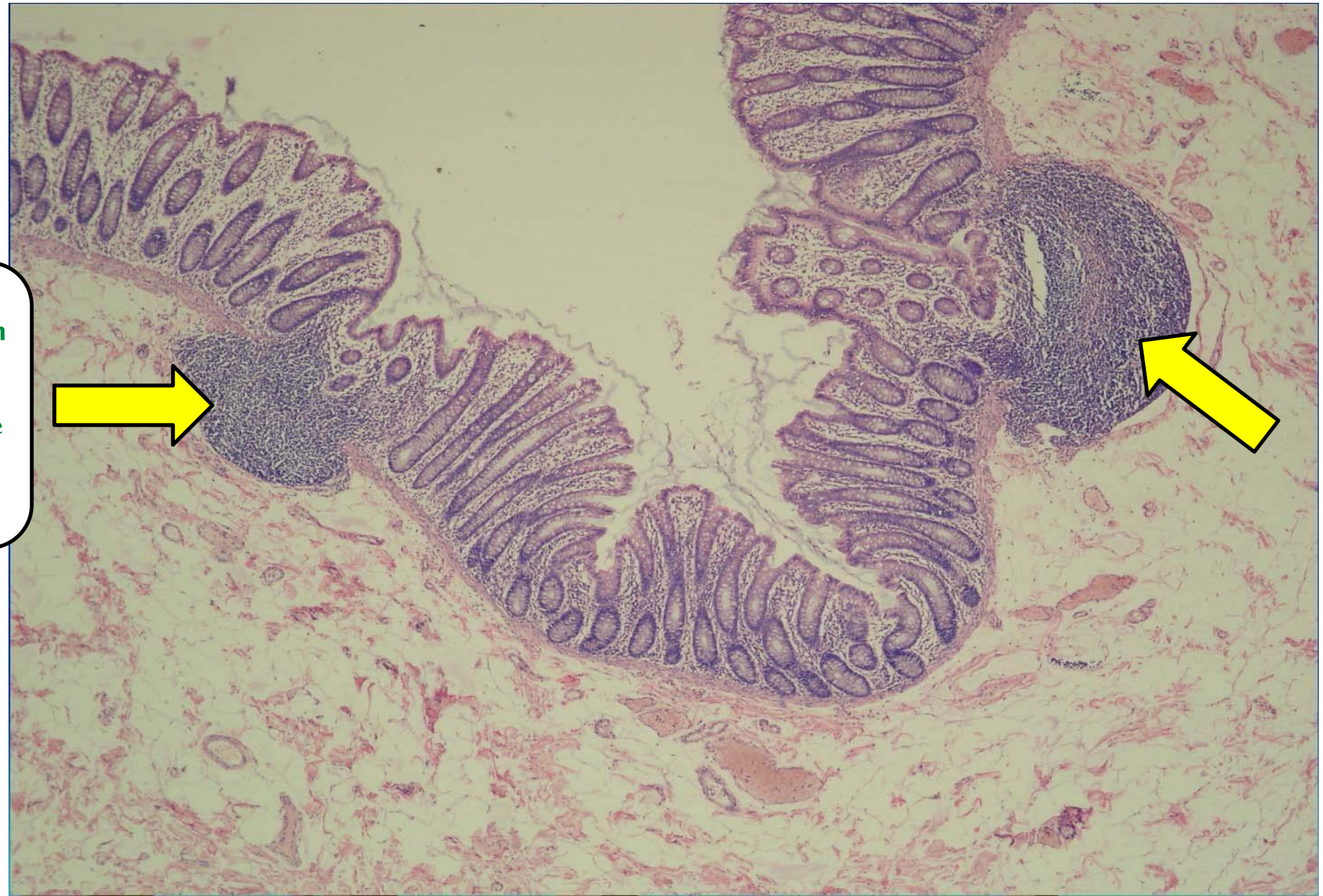
Central arteriole

White pulp

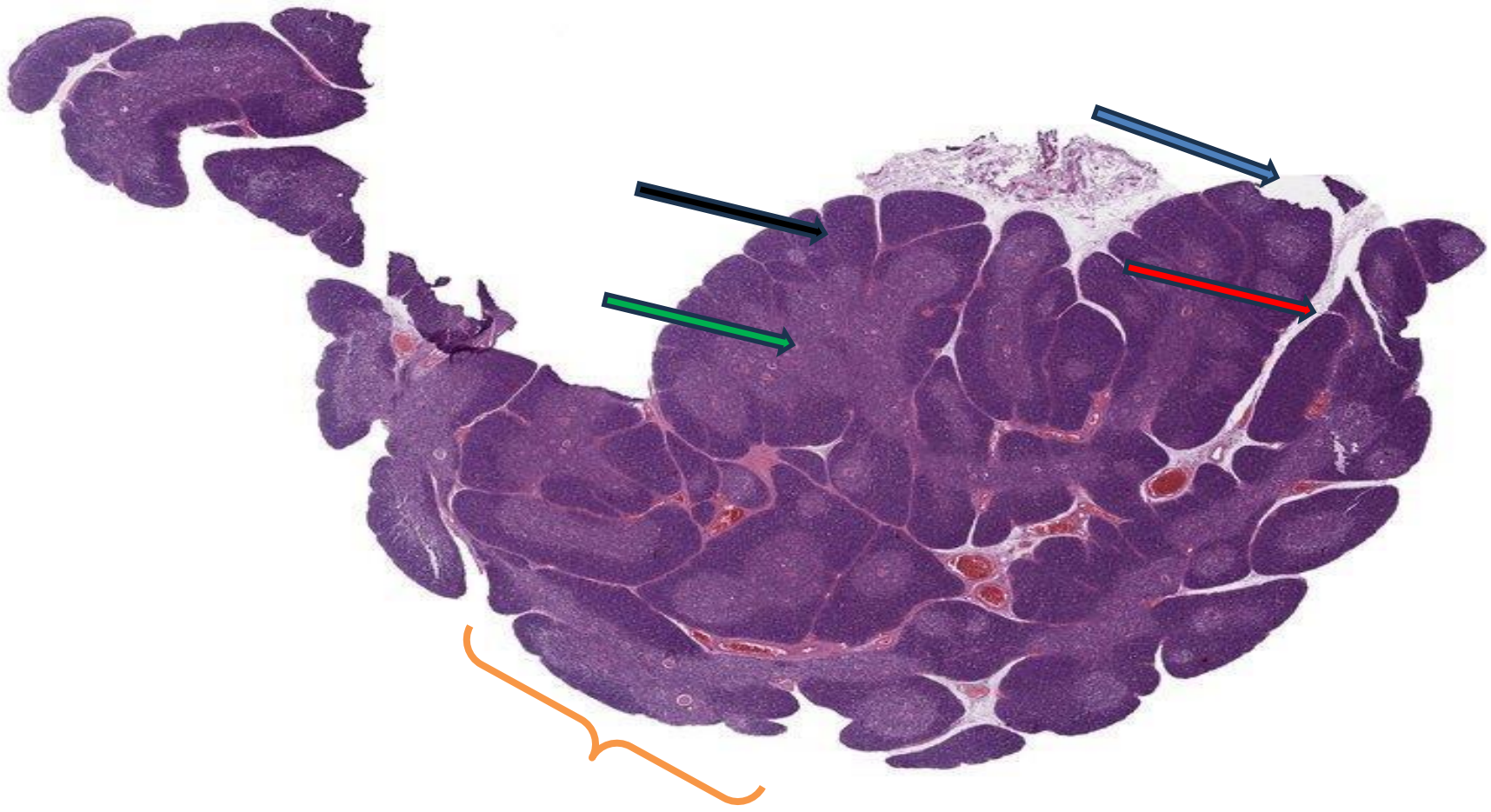
Red pulp

Solitary Lymphatic nodules (diffuse lymphatic tissue)

Usually beneath mucous membranes like in the GI tract.



Thymus



Capsule

Trabeculae

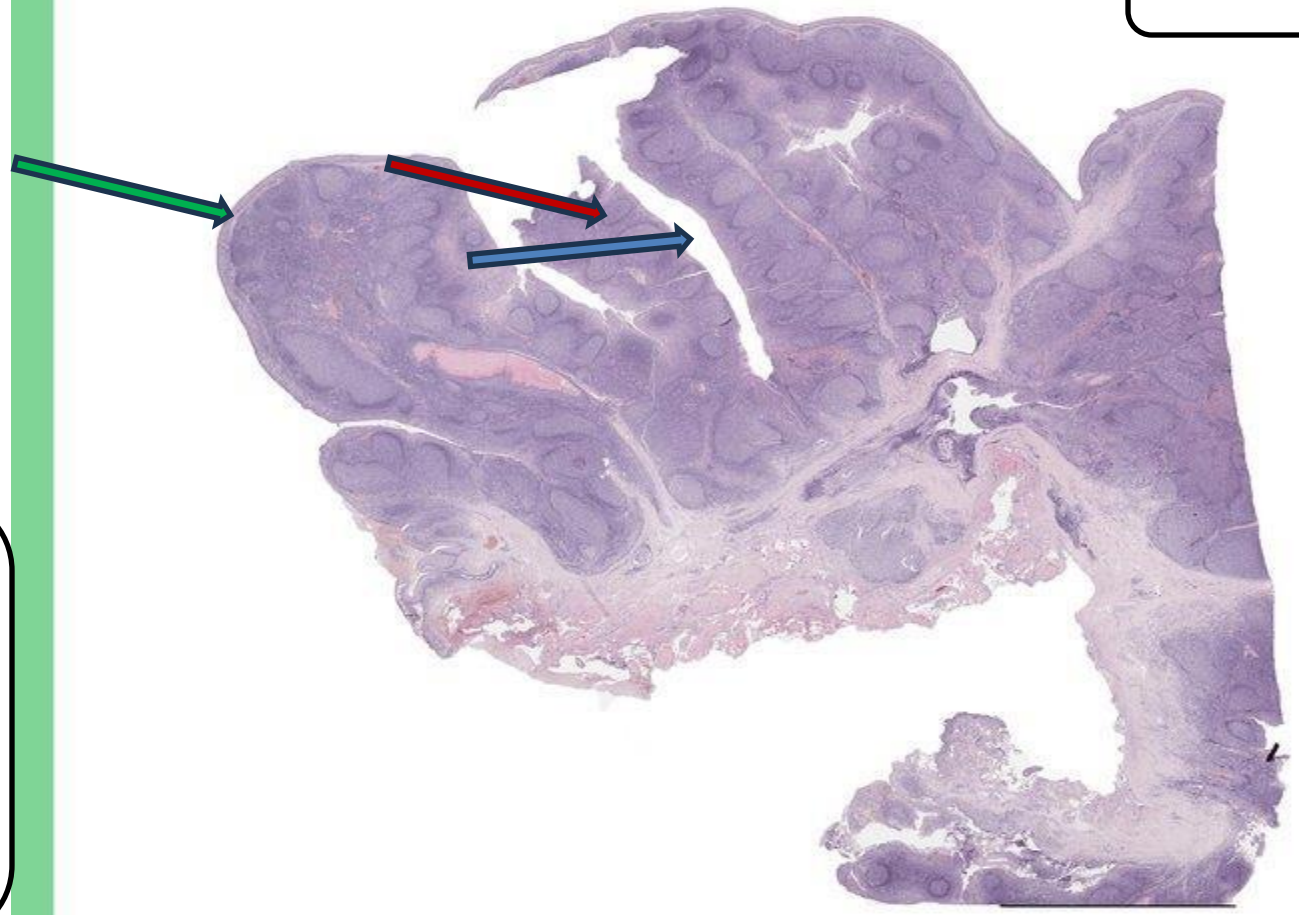
Cortex

Medulla

Lobule

Palatine tonsils

Crypt surrounded by follicles

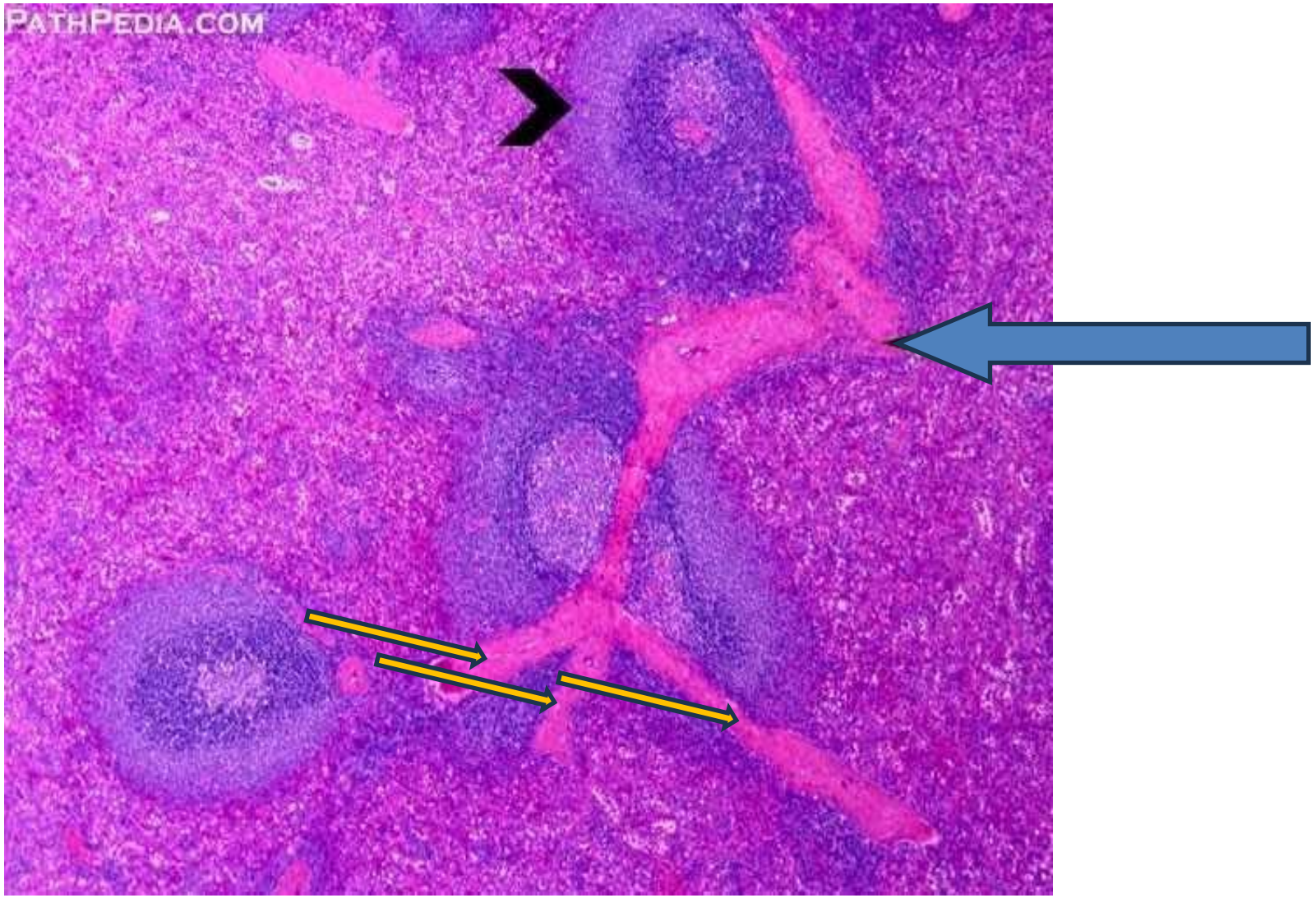


Crypt

Lymphoid follicle

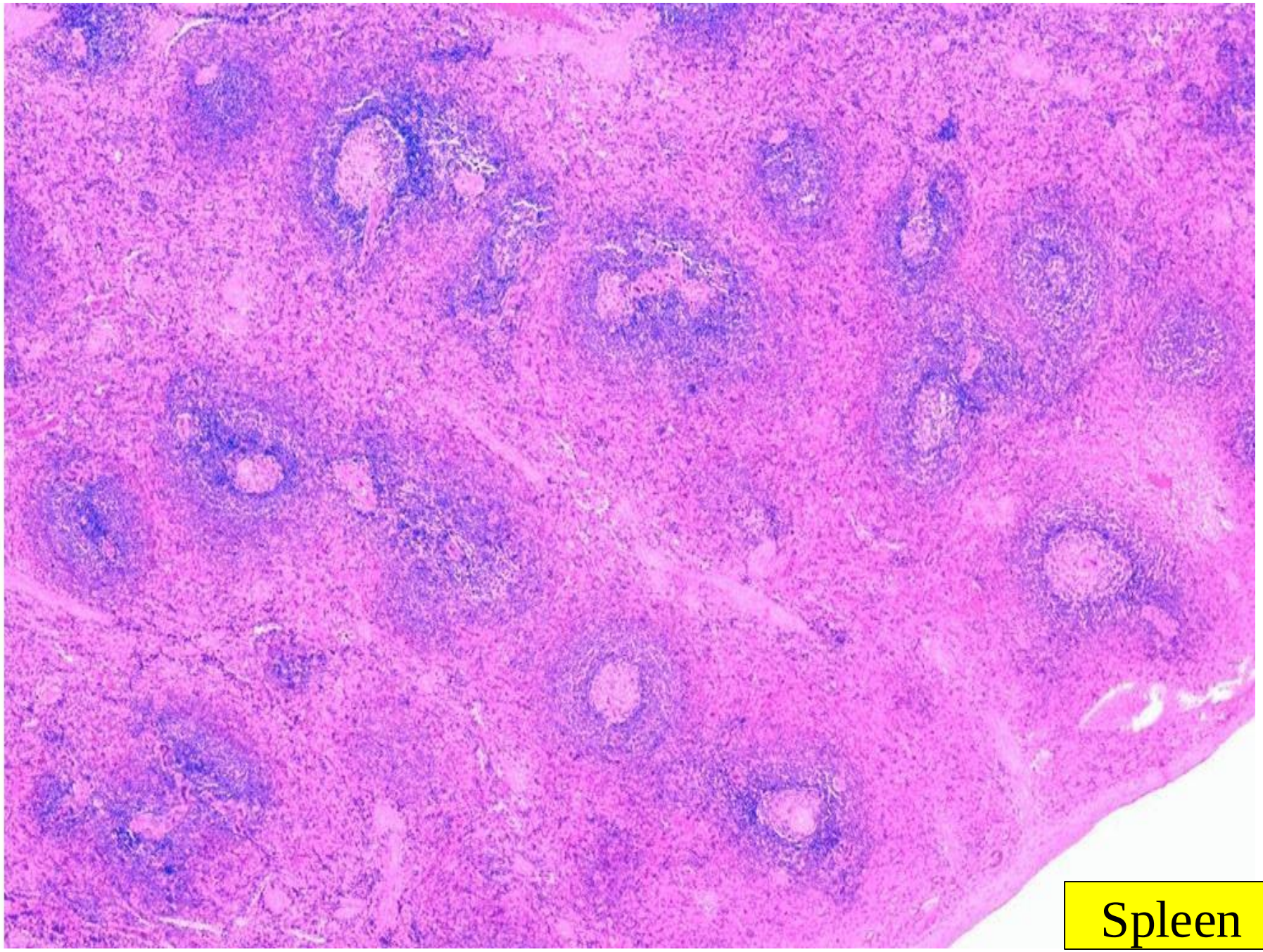
Stratified non-keratinized squamous epithelium

Spleen



Central arteriole

Penicillar arterioles



Spleen

This table is very important

	Lymph node	Spleen	Thymus
Cortex/ medulla	Present	Absent	Present
Lymphatic follicles (nodules)	Present (in cortex only)	Present (in white pulp only)	Absent
Lymphatic vessels	Afferents at capsule, emptying into subcapsular sinus; efferent at hilum	No afferents; efferents in trabeculae	No afferents; few efferents in septa
Unique features	Thin paracortical region between cortex and medulla, with high endothelial venules (HEV); medullary cords and sinuses	Minor white pulp component, with central arterioles; major red pulp component, with many sinusoids	Hassall (thymic) corpuscles in medulla; epithelial- reticular cells in cortex and medulla