

Teacher guide

Course Clinical Pathology

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VQuest tasks

Pathology-UG case 1

Patient history:

This 52-year-old male patient has undergone laparoscopic cholecystectomy due to chronic cholecystitis. The surgical preparation shows a fibrotic gallbladder with a 3 cm diameter gallstone in the neck of the gallbladder. Outside the gallbladder an enlarged lymph node is seen.

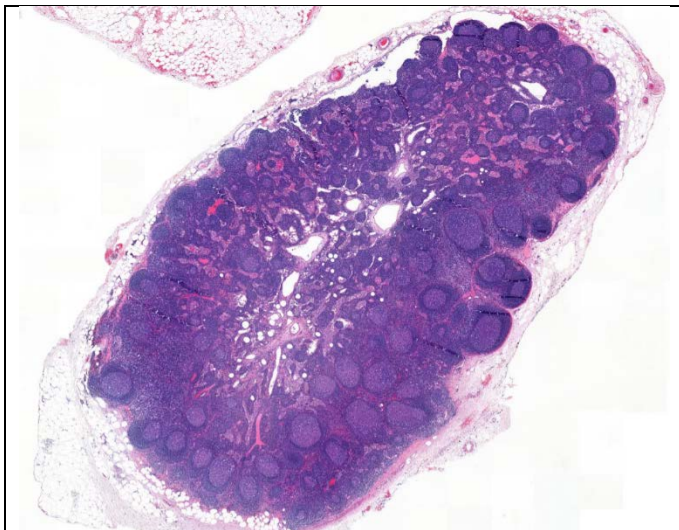
Histopathological report (PAD):

Macroscopic examination reveals a 9 x 3 cm, thick-walled gallbladder and an *enlarged 15x10mm lymph node*.

Histological examination

Gallbladder: The cross-section of the gallbladder shows that the remaining columnar epithelium is normal. The wall of the bladder is thickened with mild chronic and focal active inflammation. Also, sinuses of Aschoff-Rokitansky are identified indicating a long-term inflammation of the gallbladder.

Lymph node: The enlarged lymph node has a regular capsule and subcapsular area and shows follicular hyperplasia: numerous lymphatic follicles with germinal centres and well-defined mantle zones. Many germinal centres are polarized, displaying centrocytes (light zones) and proliferating centroblasts (dark zones). The lymphocytes show a normal cellular structure and between the germinal B centers 'tingible body' macrophages can be seen.



Lymph node: chronic cholecystitis (WSI)

Questions

Here we show the section of the lymph node described in histopathological report

Marker question 1

Find and mark a primary follicle in the cortical area of the lymph node

Mark one of the many secondary follicles.

Instruction: identify the mantle, centrocytes and centroblasts of this follicle.

Longlist questions

The mantle zone of a lymph follicle is mainly composed of resting:

Long list of celltypes (B-lymphocytes)

The dark zone of a secondary follicle is mainly composed of:

Long list of celltypes (centroblasts)

Corresponding free-text question

Describe what processes take place in these secondary follicles and explain the role of the different cells involved. What do you think will be the main goal of these processes in this particular case?

[*Answer:* Processes: The mantle zone contains small resting naive B cells. The fate of B cells in a mantle zone can go one of two ways. The B-cells either remain in the lymph node and mature into antibody secreting plasma cells, or they transform into memory B cells that re-enter the systemic circulation.

The light zone contains centrocytes that interact with follicular dendritic cells that express intact antigen on their surface. Centrocytes with high affinity binding to the follicular dendritic cell antigen will persist, while those with weak binding undergoes apoptosis. While resident macrophages help to clean up apoptotic B cells, helper T cells support the remaining B cells and foster the class switching phase of the cellular maturity.

In the dark zone of the germinal centre, the centroblasts are highly mitotic and have a strong likelihood of producing mutated antibodies. These are the source cells for the light zone.

Goal: The regional lymph nodes of the gallbladder filter the continues drainage of debris and pathogens from the chronic focal inflammation of the gallbladder wall and are therefore in a continues reactive state.]

Marker question 2

Mark an area in the lymph node where you expect to find a lot of macrophages.

Mark one of these macrophages.

Longlist question

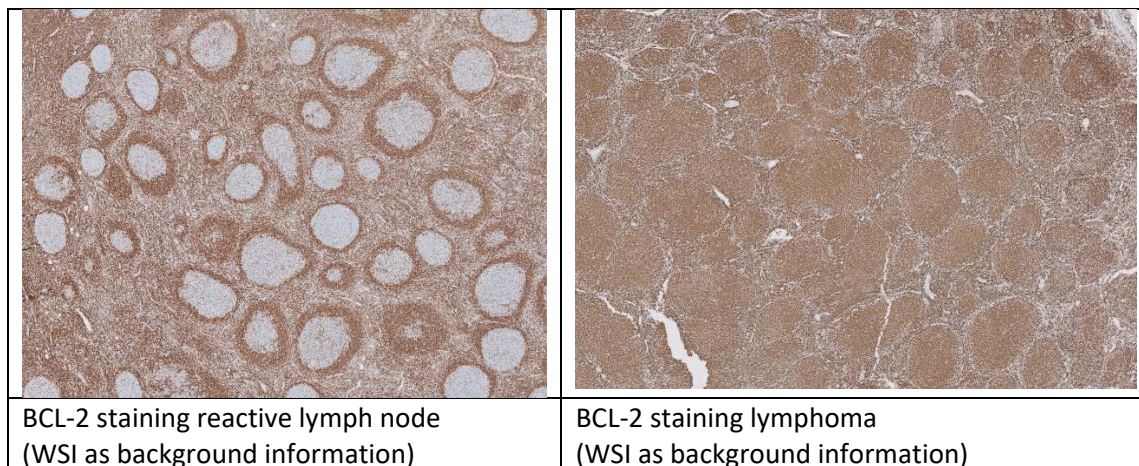
Tingible body macrophages contain the nuclear debris of:

Long list of celltypes (B-lymphocytes)

Corresponding free-text question

Why do you expect macrophages especially in this area? Describe the role of the macrophages in the processes that take place in these areas.

[*Answer:* The role of macrophages is to phagocytose debris and apoptotic cells, which can lead to conversion to a wound healing or reparative macrophage. Reparative macrophages, along with fibroblasts, resorb debris and begin to lay down new matrix, which along with factors released to promote angiogenesis, form granulation tissue. The last phase is the remodeling phase which involves remodeling of the granulation tissue to form the mature tissue or scar through the work of matrix metalloproteinases and their respective tissue inhibitors. Prolonged remodeling may occur in the presence of a foreign body and may lead to exuberant tissue fibrosis and scarring.]



Comparison question

Here we show two still images\WSIs of tissue samples, each from of different patient. Both lymph nodes are immunohistochemically stained to detect expression for BCL-2.

Free-text question: Compare both images and describe the staining pattern for each sample. What is the reason for the difference in the immunoexpression?

[*Answer:* The bcl-2 gene is commonly described as an ‘apoptosis suppressor’ gene. The gene is responsible for the production of the anti-apoptotic protein bcl-2 that prevents cells from undergoing apoptosis.

In cancer cells, overexpression of bcl-2 protein, in combination with other mechanisms, may block or delay the onset of apoptosis. The bcl-2 protein normally selects and supports the maintenance of long-living cells by arresting these cells in the G0 phase of the cell cycle.

In case of malignant lymph node, overexpression of bcl-2 protein increases the lifespan of B cells. In follicular lymphoma for instance this overexpression is caused by a chromosomal t(14;18)-translocation, placing the bcl-2 gene from chromosome 18 next to the immunoglobulin heavy chain locus on chromosome 14. This fusion gene gets deregulated, leading to increased levels of Bcl-2 protein and, thus, decreased apoptosis.

Immunohistochemistry can be used to detect overexpression of bcl-2 protein. In non-neoplastic lymph nodes, bcl-2 antibody reacts predominantly with B-cells in the mantle zone, as well as with some individual T-cells. In malignancies with overexpression of bcl-2 protein the immuno-reactions with bcl-2 and therefore the staining is increased.

For example, bcl-2 immunoexpression is commonly used as a diagnostic aid in clinical pathology to distinguish follicular hyperplasia of lymph node (germinal centers are bcl-2 immunonegative) from follicular lymphoma (germinal centers are bcl-2 immunopositive). However, it is good to bear in mind that also some follicular lymphomas are bcl-2 negative.]

Multiple choice question

Which of the two images is of a reactive lymph node?

- The left tissue sample (t)
- The right tissue sample
- Both tissue samples

Summary

The gallbladder shows chronic and mildly active cholecystitis. The lymph node shows reactive follicular hyperplasia. No signs of malignancy.

Pathology-UG case 2

Patient history:

The 41-year old female was examined for changes in her right breast and axillary region. Her anamnesis revealed that she underwent surgery for breast implants approximately 10 years ago. In a current MRI scan, the implants show superficial lacerations and are surrounded by collections of fluid. In addition, the axillary lymph nodes are rounded and slightly increased in size.

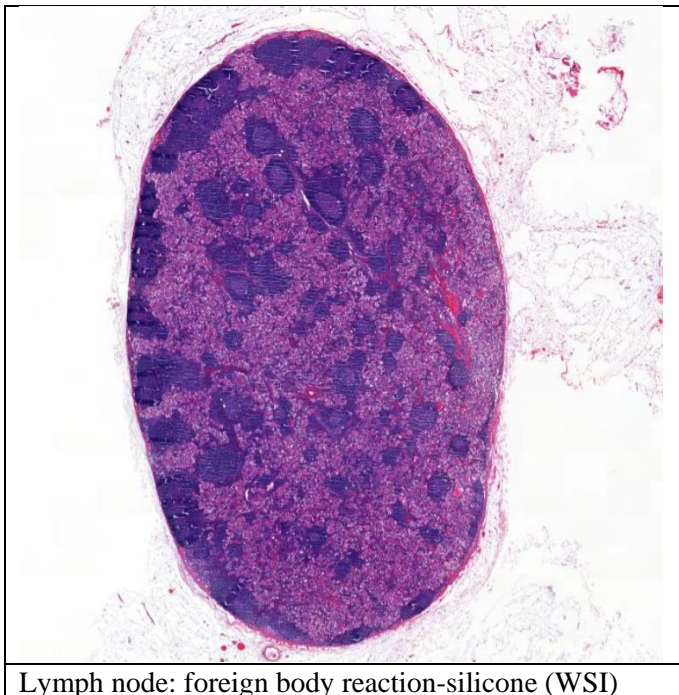
Histopathological report (PAD):

Macroscopic examination of the specimen from the axilla shows two enlarged lymph nodes (45 and 15 mm in diameter) embedded in axillary fat.

Histological examination

Lymph node: The enlarged lymph node as a normal fibrous capsule and normal subcapsular sinus. The general structure of the lymph node however is distorted. Only focally, regular lymphocytes can be seen, and rarely they form follicular structures. These rare follicular areas also show occasional germinal centers and mantle zones. The cellular structure of the lymphocytes is normal.

The major part of the lymph nodes consists of multi-nucleated giant cells. These giant cells are filled with a various number of nuclei. In addition to the nuclei, the giant cells show small rounded open spaces that represent foreign body material.



Lymph node: foreign body reaction-silicone (WSI)

Questions

Here we show the section of the lymph node described in histopathological report

Marker question 1

Mark an area where the general structure of the lymph node is clearly distorted.

Corresponding free-text question

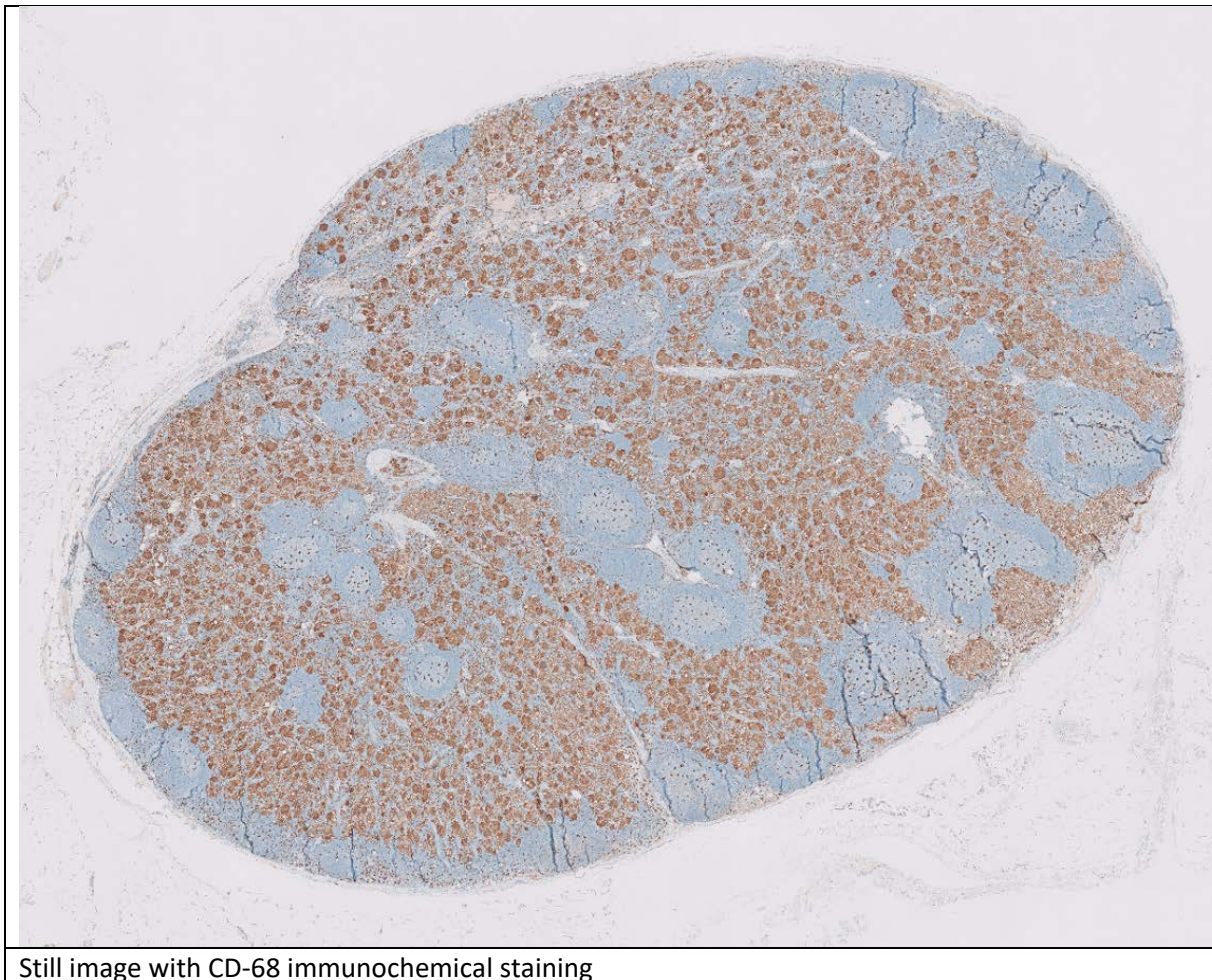
Describe why this area is different from the structure of a normal lymph node.

Answer:

Marker question 2

Mark a multi-nucleated giant cell

Multiple choice question



This specimen of the axillary lymph node demonstrates immunohistochemical expression of CD68.

Giant-cells like macrophages express CD-68 antibody. The reason for this is that giant cells:

- are formed by macrophages that fuse in immune responses (t)
- enclose macrophages in an auto-immune process
- present the antigenetic material to the macrophages in a humoral immune response
- are coloured as an artifact of the staining technic

- fagocyte the same foreign body material as the giant cells

Multiple choice question

Which processes are most likely the main cause of lymph node enlargement in foreign body lymphadenopathy?

- accumulation of foreign body materials (t)
- hyperplasia of macrophages (t)
- hyperplasia of T and B-lymphocytes
- proliferation of malignant cells
- congestion of small blood vessels

How are the precursors of giant cells called?

- basophilic granulocytes
- eosinophilic granulocytes
- lymphocytes
- monocytes (t)
- neutrophilic granulocytes
- histiocytes (t)

Corresponding free-text question

Describe the path and manner in which foreign body material will be transported from the interstitial fluid of the breast to the multi-nucleated giant cells in the axillary lymph node.

[*Answer:* Monocytes leave the bloodvessels to enter the tissue of the breast. The monocytes become histiocytes (phagocytes) and enclose the foreign body material that is present in the interstitial space. The phagocytes are taken up by the lymphatic capillaries and transported to regional axillary lymph nodes. The afferent lymph vessels pierce the surface of the lymph node and bring the lymph with phagocytes into the subcapsular space. The afferent lymph vessels extend from here to the deeper areas of the lymph node by way of the trabecular extensions of the cortex. The fluid travels from here to the cortical sinuses; which are branches of the subcapsular sinus.]

Multiple choice question

Which three causes among the following options would you consider when you find an unilateral enlargement of inguinal lymph nodes in a patient?:

- Hip joint prosthesis at the same side (t)
- Infectious mononucleosis
- Melanoma of the leg at the same side (t)
- Shoulder joint prosthesis at the same side
- Rheumatoid arthritis
- Receiving a ankle tattoo at the same side (t)

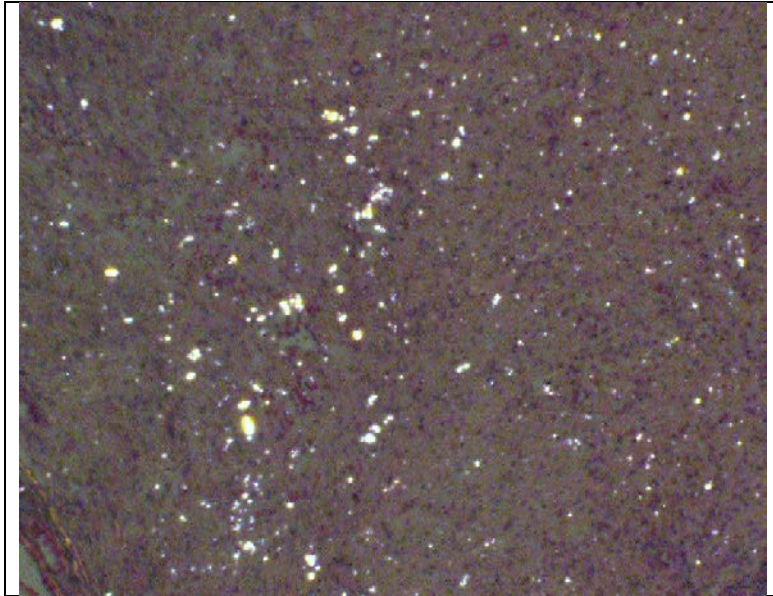
Which three causes among the following options would you consider when you find an bilateral enlargement of axillary lymph nodes in a patient?:

- Breasttumor at one side
- Infectious mononucleosis (t)
- Human Immunodeficiency Virus infection (t)
- Upper airway infection
- Rheumatoid arthritis (t)

- Vaccination for measles

Summary

The foreign material is silicone originating from the ruptured implant. In polarized light, the foreign particles show a typical bi-refringent reaction. This particular specimen does not show a granulomatous inflammation although it is common in foreign body reactions.



Still image with foreign body material in polarizing light-
(WSI as background information)

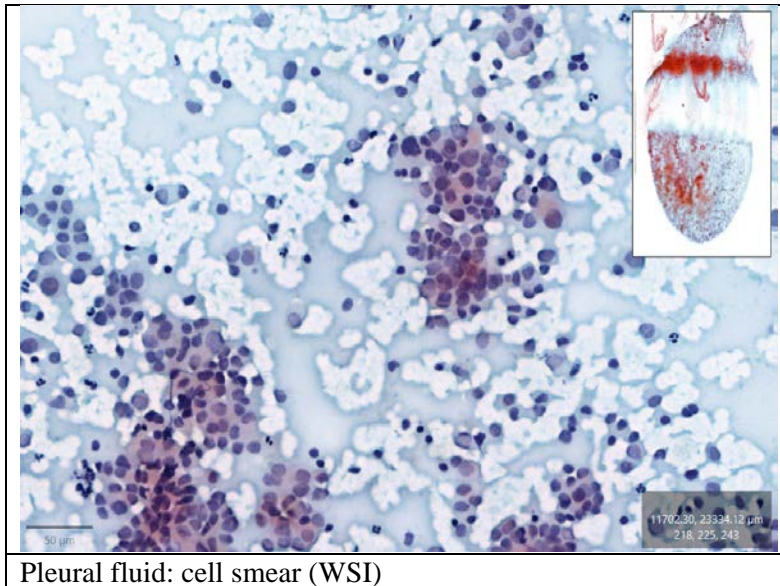
Pathology-UG case 3

Patient history:

The 39-year old, previously healthy male patient was rushed to the emergency department due to shortness of breath. His thorax X-ray revealed a striking pleura effusion. Pleural space was drained and aspirated fluid sent for cytologic examination.

Cytological report of the cell smear:

In addition to mononuclear inflammatory and single mesothelial cells, the specimen is composed of atypical cells with nuclear atypia and hyperchromasia. The finding is consistent with malignancy and represents either metastatic spread or primary pleural malignancy.



Questions

Here we show the slide of the cytological smear

Marker question 1

Mark a malignant cell/cellgroup.

Corresponding free-text question

Describe how microscopic features of the malignant and inflammatory cells differ from each other.

[*Answer:* size, shape (pleiomorf, anisokarisosis) and colour (pigmentations) of cells and the presence of mitotic figures. Normal: small regular cells.]

Patient history- continued

In further clinical examination, an enlarged lymph node was observed in axillary region. The node was resected and prepared for histological examination.

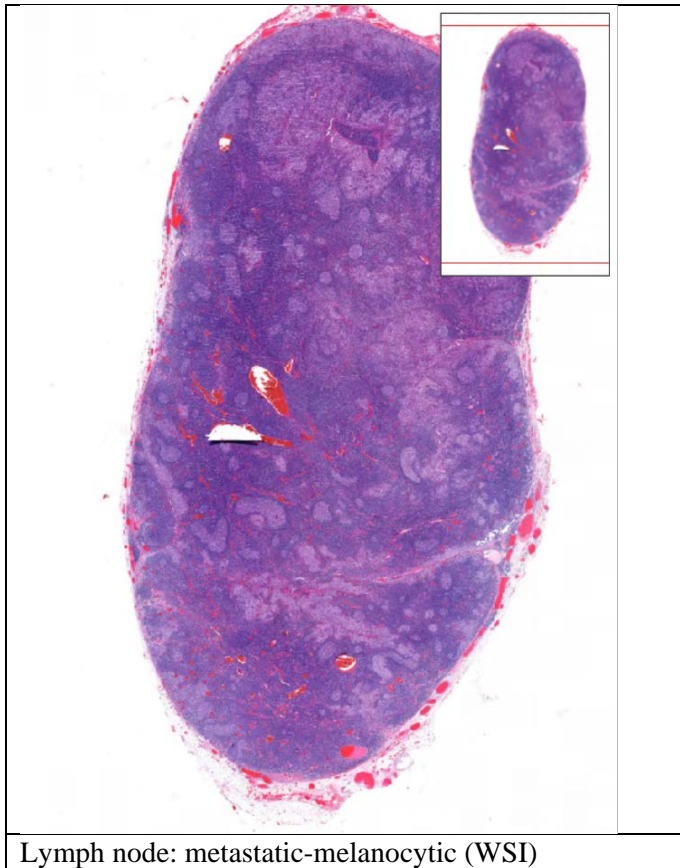
Histopathological report:

Macroscopic examination reveals an *enlarged 20x10mm lymph node* embedded in axillary fat.

Histological examination

Lymph node: The enlarged lymph nodes has a normal fibrous capsule and normal subcapsular sinus. The general structure of the lymph node is partly preserved. Focally, however, subcapsular and central areas with atypical cells can be seen. The finding represents malignant cells consistent with metastasis.

Immunohistochemical examination will be performed in order to determine the primary tumor



Lymph node: metastatic-melanocytic (WSI)

Questions

Here we show the lymph node described in histopathological report.

Marker question 2

Mark an area where the general structure of the lymph node is clearly abnormal.

Corresponding free-text question

Describe why this area is different from the structure of a normal lymphnode.

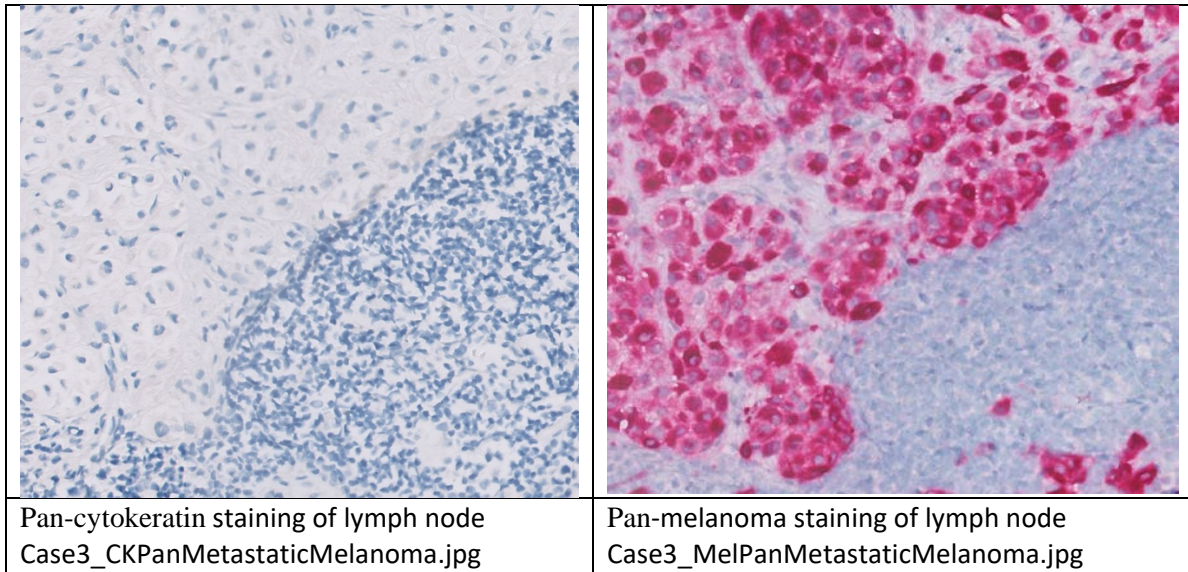
[Answer: recognition that it isn't normal histology]

Marker question 3

Mark an area with malignant cells

[Answer: example of region of interest]

Here we show images of the results of the immunohistochemical stainings of the lymph node: a Pan-cytokeratin staining (left) and Pan-melanoma staining (right) both counterstained with hematoxylin.



Multiple choice

Both immunohistochemical stainings show the same pathological area. Which staining is positive?.

- a) Pan-cytokeratin staining (left)
- b) Pan-melanoma staining (right) [r]

Marker question 4

Reconsider the former question with this additional information in mind and mark again an area with malignant cells.

[Answer: region of interest in OMERO]

Multiple choice question

Immunohistochemically, the atypical cells do not express cytokeratins. The reason for this is that the atypical cells:

- are inflammatory cells (f)
- represent lymphoma (f)
- are not epithelial by origin (t)
- represent carcinoma (f)
- suffer from poor fixation resulting in staining artifact (f)

Immunohistochemically, the atypical cells do express Pan-melanoma-antibody. The reason for this is that the atypical cells:

- are fagocytosing macrophages (f)
- are epithelial by origin (f)
- originate from malignant melanoma (t)
- represent malignant mesothelioma (f)
- suffer from cross-reaction of the antibody due to poor fixation (f)

Which of the follow diseases are most likely to cause lymph node metastasis in this anatomical site?

- glioblastoma (f)
- basal cell carcinoma (f)

- breast cancer (t)
- carcinoma of the tonsil (f)
- gastric carcinoma (f)

Corresponding free-text question

Describe pathways by which malignant neoplasms disseminate in the body.

[Answer: Robbins & Cotran Pathologic Basis of Disease, 10th Edition, page 195]

Patient history continued

Immunohistochemical staining pattern of the lymph node is consistent with metastasis of malignant melanoma. A wider lymphatic excision revealed three additional metastatic nodes. Clinical examination of skin and mucosal membranes did not reveal lesions suspicious for malignant melanoma. Instead, a scar was seen on his arm. In further anamnesis, the patient remembers that approximately 5 year ago a lesion was removed from this area. The lesion was not sent for microscopic examination at that time.

[show classification: https://vquest.nl/wp-content/uploads/2023/01/AJCC_Melanoma_of_the_Skin_Staging-scaled.jpg]

Please stage this tumor with the TNM classification

[Answer: pTXN2M1c]

DD_TNM_staging_T

0

is

X [r]

1

2

3

4

DD_TNM_staging_N

X

0

1

2 [r]

3

DD_TNM_staging_M

0

1a

1b

1c [r]

1d

free-text question

What are the prognostic implications of this classification?

[Answer: braf inhibitors]

[Summary: The patient has malignant melanoma pTXN2M1c. The primary tumor was most likely the melanocytic lesion non-radically removed 5 years ago, although no histological specimen was taken at that time. Additon of therapie: specific information on braf inhibitors.]

Option: summary and annotation in OMERO as extra question in Vquest that can manually be added during the online session.