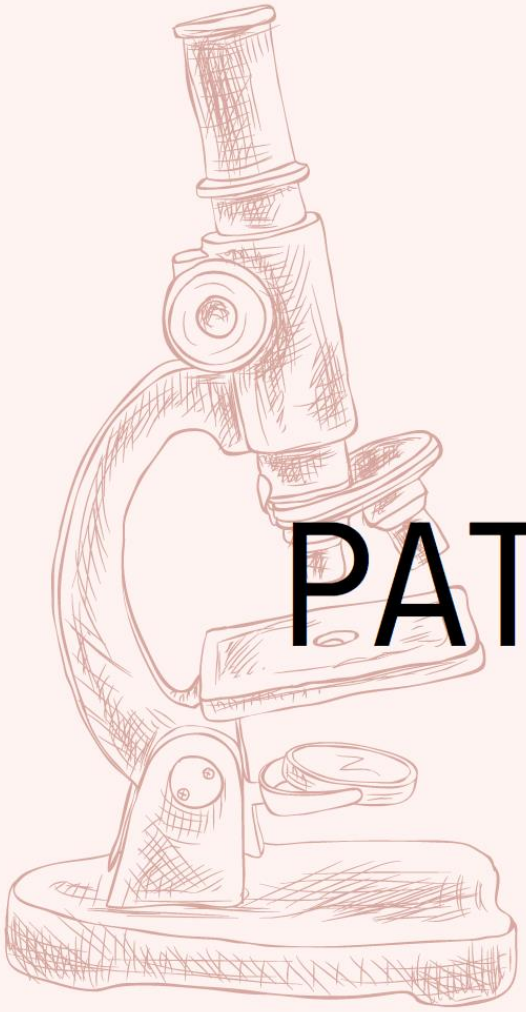
Sheet No. 5



PATHOLOGY

Central Nervous System

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1. diffuse (infiltrating) astrocytoma (WHO grade 2-4).
2. circumscribed astrocytic gliomas: PA, SEGA, pleomorphic xanthoastrocytoma (PXA).

Let's start with a quick revision

Diffuse (infiltrating) Astrocytoma:

- 80% of primary brain tumors in adults.
- **Age at diagnosis**: (40–60)year old.
- **Location**: **cerebral hemispheres** +/- cerebellum, brainstem, or spinal cord (less common locations).
- **Presentation**→ depends mainly on location

  1. **Seizures**, sudden onset is a common presentation for most brain tumors.

  2. **Headaches**; due to increase in intracranial pressure for example

  3. **Focal neurologic deficits** related to the anatomic site of involvement.

- Static for years

  or

- Progressive: such as rapid clinical deterioration, due to the appearance of higher-grade component and more rapid tumor growth (these tumors can suddenly be converted to more aggressive tumors specially grade 3 tumors which will lead to sudden deterioration).

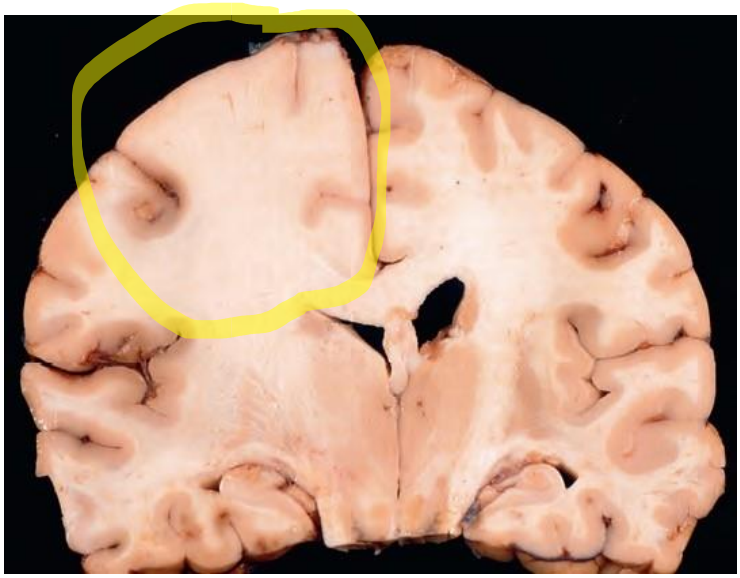
- Stratified into 3 groups based on histologic features:
  1. **Diffuse astrocytoma** (grade 2), mean survival is > 5 years.
  2. **Anaplastic astrocytoma** (grade 3), mean survival is 2-3 years.
  3. **Glioblastoma** (grade 4), mean survival is 15 months.

  - The prognosis gets poorer as the grade increases.
  - **NO grade 1 diffuse astrocytoma.**

❖ Now we can start today's lecture; **MORPHOLOGY of Diffuse (infiltrating) Astrocytoma**

Macroscopic features of grade 2 & 3 Diffuse astrocytoma:

- **Poorly defined, infiltrative tumors.**
- **Expand and distort the invaded brain.**
- **NO discrete mass.**
- **Infiltration beyond the grossly evident margins.**
- **+/- cystic degeneration**. (most of the Gliomas exhibit this cystic degeneration, which have a bubble-like appearance).



This is a coronal section form an autopsy of a patient with **expansion on the frontal left side**.

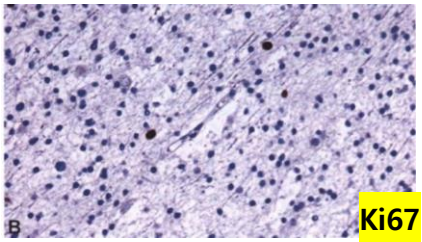
It may be hard to recognize the normal margins of the brain, but if you compare the right & left sides then the expansion will be apparent; as the left side is more "full" compared to the right side.

Still, you can't tell the start & end of the tumor (it's infiltrative). it doesn't form a discrete mass

the other interesting thing is the **blurring between the grey-white matter junction**. they are not well-define

Microscopic Features of grade 2 Diffuse Astrocytoma

❖ Here, **the transition between neoplastic and normal tissue is indistinct**. So, even if you could tell the start & end of the main tumor lesion, **tumor cells infiltrate normal tissue many centimeters from the main lesion** , that's why we called them infiltrative and we start grading them from grade 2 up to grade 4 , there is no grade 1 infiltrative or diffuse astrocytoma because these tumors have risk of recurrence and the complete surgical excision is hard.

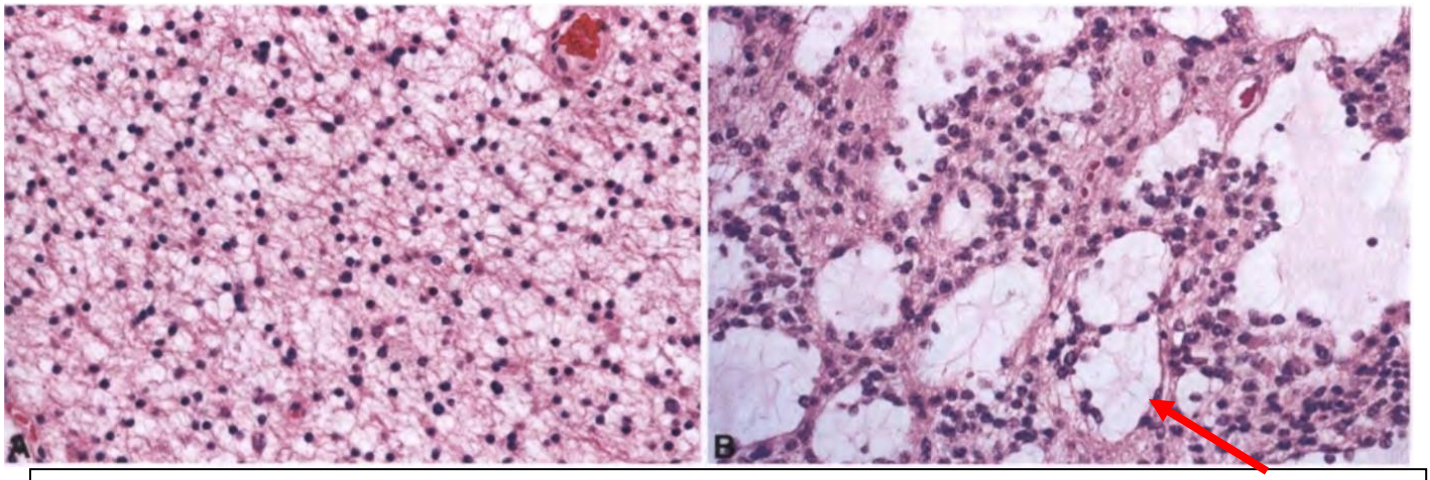


Ki67

❖ The Histological features are:

1. **<u>Hypercellular</u>** (compared to normal white matter): **mild to moderate** increase in the number of glial cells. [measured with the proliferative index, **Ki67**, which is minimal/mild here; less than 4%]
2. **<u>Cytologic atypia:</u>**
   Mild, allows for differentiating between tumor cells & the background.
   ✓ enlarged, elongated or irregular **hyperchromatic nuclei.** (they appear as dark dots)
   ✓ No prominent atypia.
3. **+ <u>Fibrillary background</u>** made of **<u>fine astrocytic cell processes</u>** (it has a pinkish color)

- **There will be <u>NO or rare Mitotic activity</u>, <u>NO necrosis</u>, <u>NO microvascular proliferation</u>.**
  - Recall that cyst formation is common here in Grade 2 Astrocytoma, as well as in grade 3.

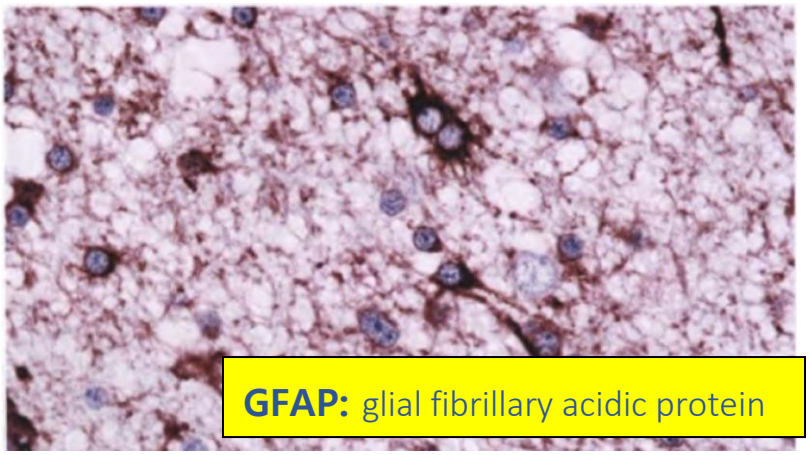


This is a histological section of Grade 2 astrocytoma, the pic on the left shows **microcyst formation.**

You can see **mild increase in cellularity**, **cytologic atypia** (dark dots representing **hyperchromatic nuclei**) and the fluffy, pinkish **fibrillary background.**

- Another thing about Grade 2 Astrocytoma is that it tests positive to **GFAP** stain. This stain tests positive in all glial cell tumors, it's not specific but it proves that we have a glial proliferation! in the morphology, the proliferating cells resemble astrocytes.

GFAP immune stain highlights the cytoplasm of the cells & their **processes**. this allows for the whole of the fibrillary background to be highlighted by this brown color.



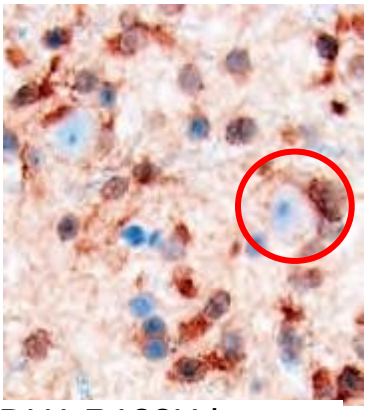
- The next step after testing GFAP would be an **IDH1 R132H immune stain**. If the result is negative, we test for the same mutation again (**IDH1**) but through **sequencing (PCR)** and for **IDH2 R172K.**

EXTRA: NOT FOR MEMORIZATION

what are these blue cells that are surrounded by the tumor brown cells? these are non-neoplastic neurons that are IDH negative. Tumor cells love to surround these neurons. This process is called perineuronal satellitosis.

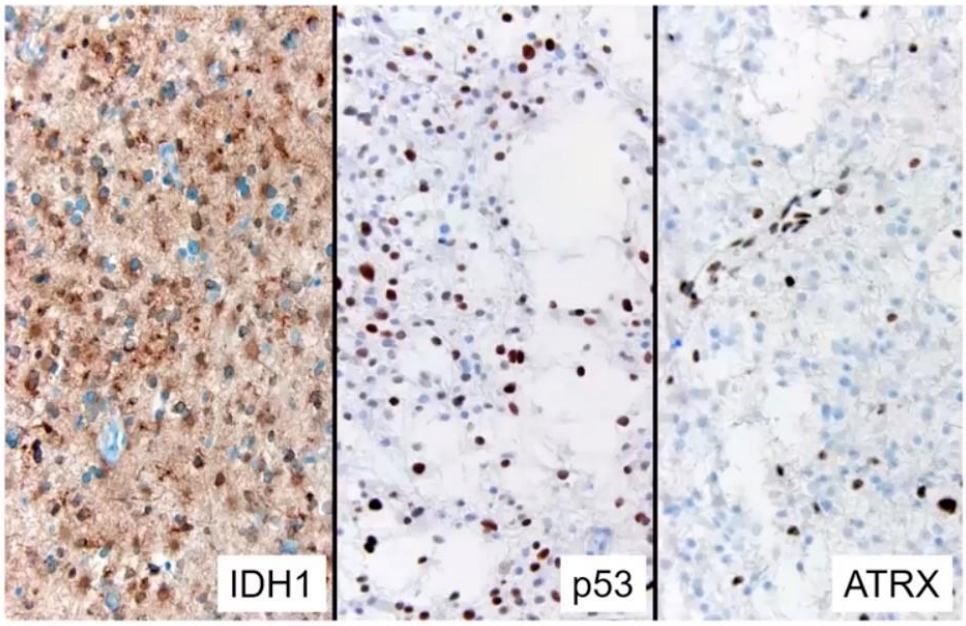
How do we know it's a neuron? from its shape & it would be positive for neuronal markers.

Again, NOT FOR MEMORIZATION



IDH1 R132H immune

Astrocytoma, IDH-mutant, CNS WHO grades 2-4



Mutations necessary for astrocytoma:

IDH +ve

p53: increase in the expression (it's a loss of function mutation)

Loss of expression of the ATRX.

Microscopic features of grade 3 Anaplastic astrocytoma:

1. **More cellular.** (than grade 2)
2. **nuclear pleomorphism.** (more atypia)
3. **MITOTIC FIGURES ARE PRESENT. (the most important factor for distinguishing the grade & increasing it from 2 to 3 is the mitotic activity.)**
4. **NO necrosis**
5. **NO microvascular proliferation**

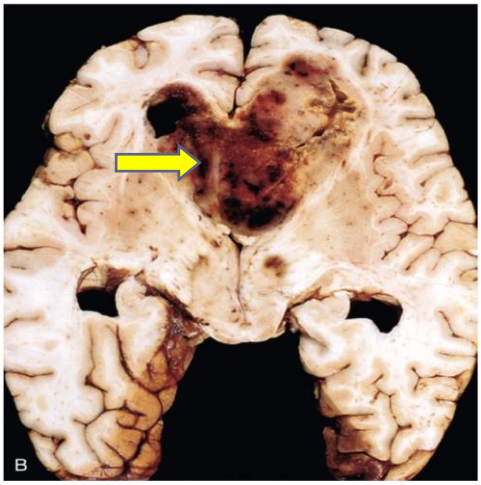
There is no clear-cut point for the no. of mitotic figures between grade 2 and 3. Previously, we used to consider 2 or less mitotic figures to be grade 2 and more than that is grade 3. But that depends on the size of biopsy; 2 mitotic figures in a 2 mm sample are more significant than in a 4cm biopsy.

Now, for us doctors we don't have to remember a specific cut point, but pathologists consider both the no. of mitosis and the size of biopsy and make an estimate.

Features of grade 4 Glioblastomas:

Macroscopic

- variation in the gross appearance of the tumor from region to region is characteristic (was called **glioblastoma multiforme**).
- Some areas are firm and white, others are soft and yellow and preserved (due to tissue necrosis), others show regions of cystic degeneration and hemorrhage and calcifications.



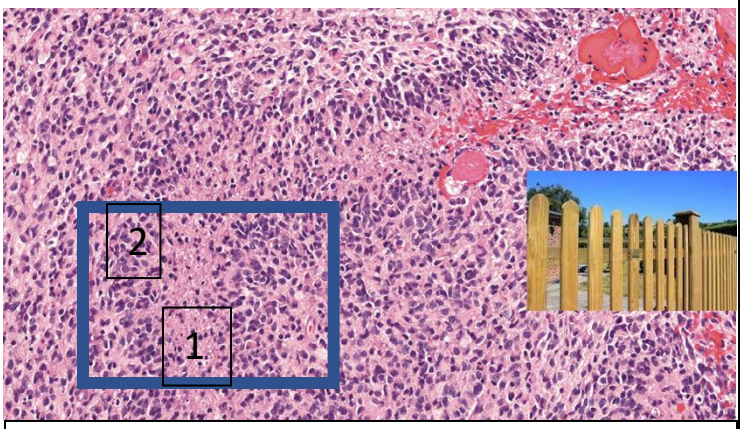
- Histologically you'll see areas that look like grade 2 & areas that are highly malignant. this is characteristic for grade 4. (hence the name glioblastoma *multi*forme)

➢ Microscopic

- **anaplastic astrocytoma features** (increased cellularity, atypia, mitotic activity) +either necrosis *or* microvascular proliferation:
- **Necrosis**: irregular zones of necrosis surrounded by dense accumulations of tumor cells (**palisading necrosis**) (fence-like structure).
  or
- **microvascular proliferation**:

  the presence of abnormal vessels with walls composed of 2 or more layers of vascular wall cells.

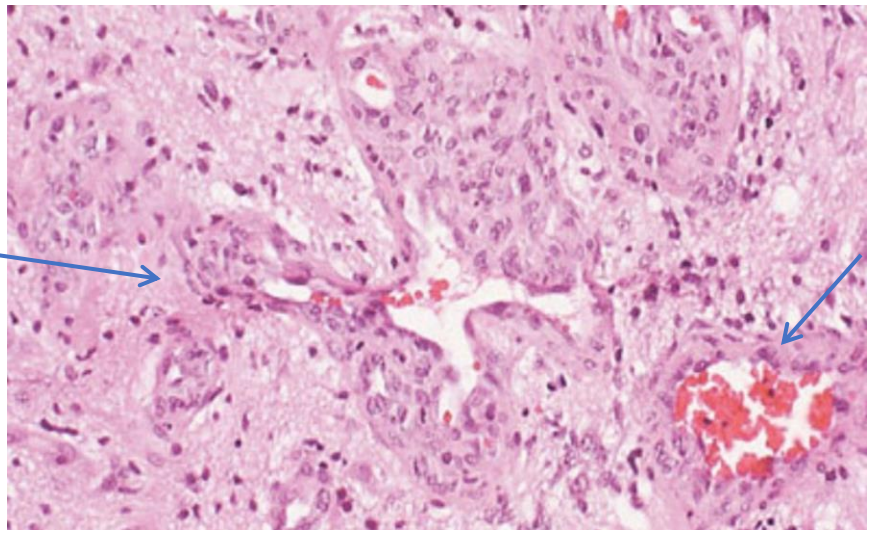
  sometimes there are so many layers that it starts to resemble the glomerulus of the kidney. That is called a glomeruloid structure.



This section shows palisading necrosis.

- Area 1 (the central pale area) is necrotic.
- Area 2 is viable tumor nuclei forming fence-like structure around the necrotic area. (hyperchromatic cells & nuclei)

You can see the increase in number of layers in these blood vessels.



❖ Now that we've finished discussing the macroscopic & microscopic features of **diffuse (infiltrating) astrocytoma** (grades 2, 3 & 4), we can finally discuss the **circumscribed astrocytic gliomas** (grade 1). We will only cover the Pilocytic Astrocytoma (PA).

Circumscribed astrocytic gliomas

Pilocytic Astrocytoma (grade 1):

- **Age at presentation**: children and young adults.
- **Location**:
  - cerebellum (especially in children) (most common).
  - Optic nerve.
  - Midline locations: Brainstem, optic chiasm/ hypothalamus, basal ganglia.
  - Spinal cord.
  - Cerebral hemispheres: Rare in children but happens in adults.

Recall:
2/3 of pediatric CNS tumors are infratentorial.
2/3 of adult CNS tumors are supratentorial.

- **Molecular profile:**
  - Activating mutations or translocations involving the gene encoding the **BRAF**, resulting in activation of the **MAPK** signaling pathway.
  - **Do not have mutations in IDH1 and IDH2**, supporting their distinction from the low-grade diffuse gliomas.

- **Macroscopic:**
  - well circumscribed (discrete) Cystic tumor +/- calcifications.
    There's no infiltration here & you can tell the start and end of the tumor. The Cyst can be: a cyst with some solid areas (called *cyst with a mural nodule*.), or *completely cystic*, or rarely *just solid* (mostly they're cystic). The calcification is variable.

- **Microscopic:**

• **Bipolar cells with long, thin GFAP positive "hair-like" processes:** hyperchromatic nucleus & processes on both sides. Characteristic for PA.

• **Rosenthal fibers:** brightly eosinophilic corkscrew shaped structures within the astrocytic processes & cytoplasm. they are basically intermediate filaments. Can be physiologic (gliosis) or pathologic (the PA tumor) and Alexander disease. Thus, they are not diagnostic/specific, but they help in the diagnosis.

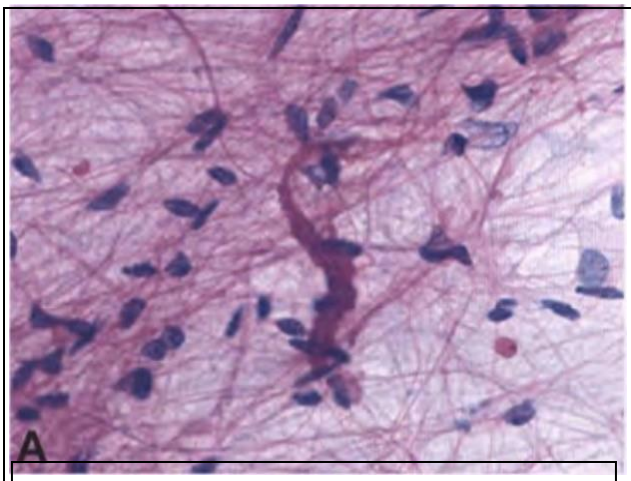
• **Eosinophilic granular bodies:** rounded hyaline droplets in cytoplasm of astrocytes seen in PA and ganglion-cell tumors (like ganglioglioma and gangliocytoma). Positive for PAS stain. not diagnostic.

• **Microcysts are often present.**

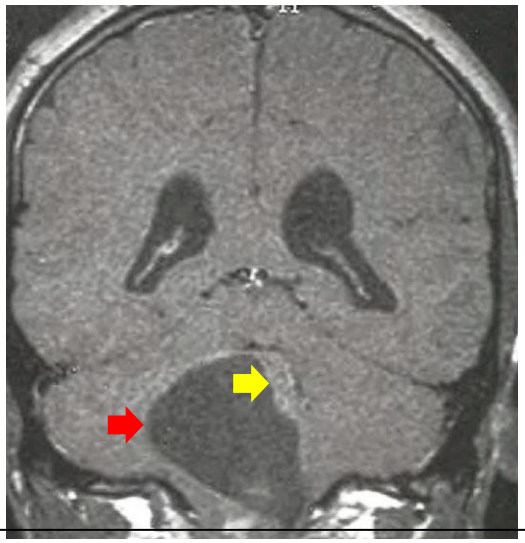
• **Necrosis and mitoses are rare.**

Extra Note from the doctor:

What about microvascular proliferation?
It does happen here, but it wasn't mentioned to avoid confusion.
Don't memorize it.

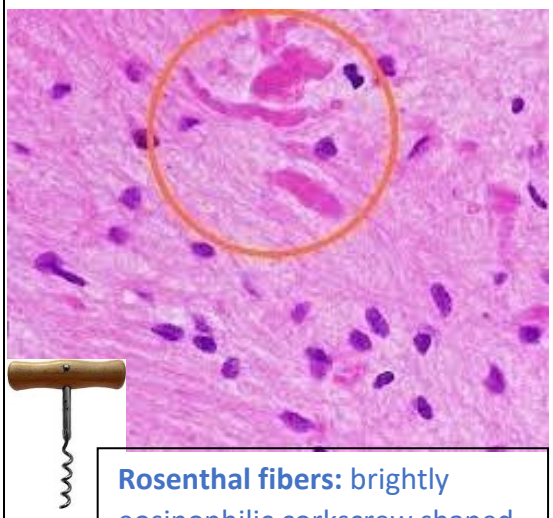


Bipolar cells with long, thin GFAP positive "hair-like" processes

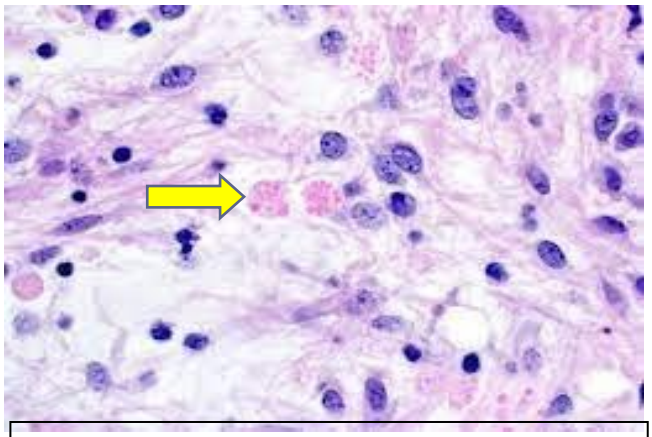


This image is of a child. The PA tumor here is in the cerebellum, which is the most common location.

well circumscribed, **cystic with a mural nodule** in the wall of the cyst or solid. The yellow arrow is the **mural nodule** (solid part) and the red arrow is the **cystic part**.



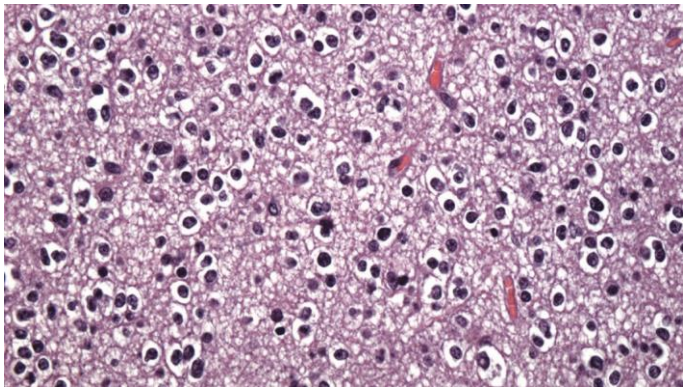
Rosenthal fibers: brightly eosinophilic corkscrew shaped



Eosinophilic granular bodies: hyaline droplets. PAS positive

- Now we've finished discussing one type of Gliomas; the Astrocytomas. Let's move on to the second type of Gliomas:

Oligodendroglioma



• **Definition**: **A diffusely infiltrating, slow-growing glioma** with **IDH1 or IDH2 mutation and codeletion of chromosomal arms 1p and 19q.** (Both must be present to make the diagnosis)

• Accounts for 5-15% of gliomas. (it's common relatively to other gliomas. Recall that Astrocytomas are the most common).

• **Age at diagnosis**: 40-50.

• **Location**: mostly in the white matter of cerebral hemispheres, mainly in the frontal lobe followed by the temporal, parietal and, less commonly in the occipital.

• The combination of surgery, chemotherapy, and radiotherapy yields an average survival of:

- 10-20 years for WHO grade 2.
- 5-10 years for WHO grade 3.

Recall: mean survival in Astrocytoma: -

grade 2 is > 5 years.
grade 3 is 2-3 years.
and grade 4 is 15 months.

• Grade 3 is more aggressive than grade 2 oligodendroglioma.

• Better prognosis than astrocytoma of the same grade!

• NO grade 1 OR 4 oligodendroglioma.

Grade 2 Oligodendroglioma:

Macroscopic:

• infiltrative tumors with blurring of grey matter-white matter boundary and no discrete mass.

• +/- cystic degeneration, focal hemorrhage, and calcification.

Microscopic:

• **Sheets of regular uniform cells resembling oligodendrocytes**: the increased cellularity helps differentiate it from the white matter background.

• **Round nuclei containing finely granular/stippled chromatin (salt and pepper)**.

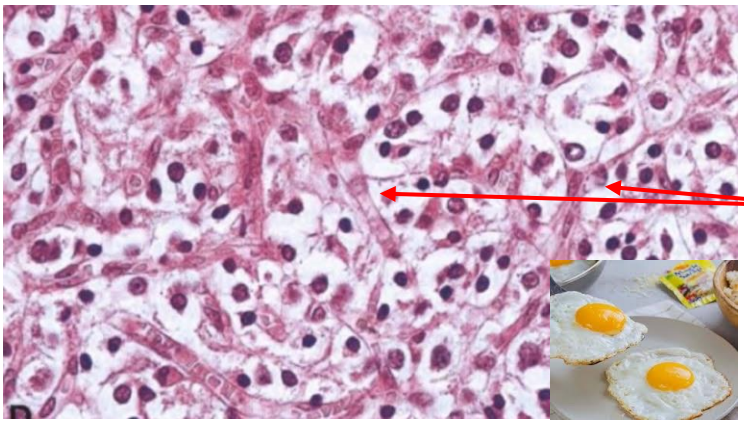
• **The nuclei are surrounded by a clear halo (transparent) of cytoplasm (fried-egg appearance**): this feature is an artifact that appears during histological preparation of the section.

• **Delicate network of anastomosing capillaries (chicken-wire).**

• **Calcification in 90% of tumors.**

• **Mitotic activity usually is absent or low (Ki67<5%).**

• **No spontaneous necrosis & No microvascular proliferation.**

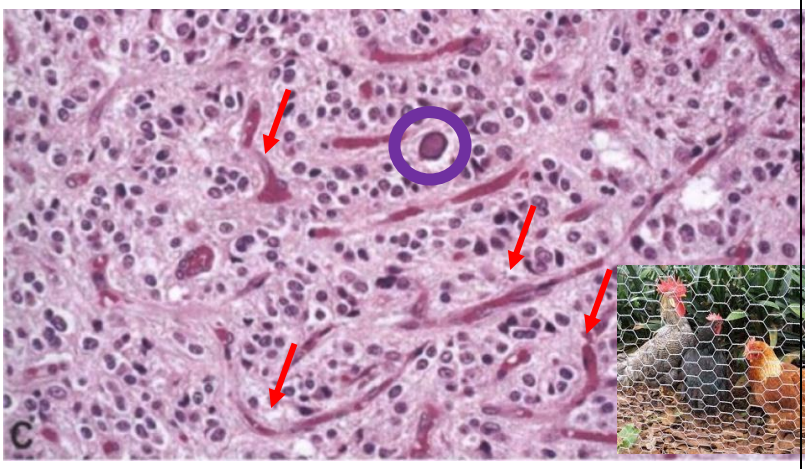


Notice the fried egg appearance of the cytoplasm. clear areas (clear halo) surrounding the nucleus.

So, in this section we have: cellular proliferation, uniform cells, salt & Pepper nucleus, network of capillaries in the background. This is diagnostic for Oligodendroglioma in the presence of IDH mutation & 1p19q codeletion.

Notice the chicken-wire appearance, which is a delicate network of anastomosing capillaries.

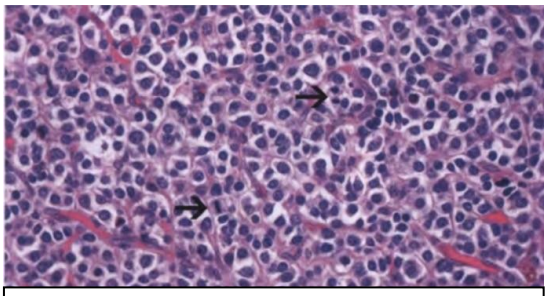
We can also see some **calcifications** in this section which are purple in color.



Grade 3, Anaplastic Oligodendroglioma:

- Defined as: An IDH-mutant and 1p/19q-codeleted oligodendroglioma with focal or diffuse histological features of anaplasia (in particular, **pathological microvascular proliferation and/or brisk mitotic activity with or without necrosis**). And/or **homozygous CDKN2A deletion.**

- We depend on the presence of either microvascular proliferation or brisk mitotic activity or both for deciding that the grade of Oligodendroglioma is 3 or 2. The presence or absence of necrosis doesn't influence the grade that much.
- is there a clear cut in the mitotic activity between grade 2 & 3 Oligodendroglioma?
  Yes. There has to be 2.5 mitosis/mm^2 in the field, or 6 mitosis per 10 in high power field, or more than 10% Ki67 to be able to say that it has brisk mitotic activity.



In this section, you can see that there's much more cellularity.

The black arrows point to mitotic figures (Brisk/significant mitotic activity).

May show microvascular proliferation.

CDKN2A: cyclin dependent kinase Inhibitor (2Aor 2B)

- The immune stains used in Oligodendroglioma: Codeletion of 1p19q, positive expression of IDH, negative expression of p53 (not mutated), ATRX is reserved (not mutant), homozygous CDKN2A deletion.

IDHm 1p/19q-codel Oligodendrogliomas, grades 2-3

Essential diagnostic criteria for oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade 2	Essential diagnostic criteria for oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade 3
A diffuse glioma	A diffuse glioma
WITH	WITH
an IDH1 codon 132 or IDH2 codon 172 missense mutation*	an IDH1 codon 132 or IDH2 codon 172 missense mutation*
AND	AND
combined whole arm deletions of 1p and 19q	combined whole arm deletions of 1p and 19q
AND	AND
absence of histological features of anaplasia	histological features of anaplasia, including brisk mitotic activity and/or pathological microvascular proliferation with or without necrosis AND/OR homozygous *CDKN2A* deletion**

these features change the grade of oligodendroglioma from 2 to 3

This table shows the similarities & differences between grade 2 Oligodendroglioma & grade 3 Anaplastic Oligodendroglioma.

In yellow box are the similarities, in red and green boxes are features of grade 2 and grade 3 Oligodendrogliomas, respectively.

A 59-year-old gentleman presented at the ER following a generalized tonic-clonic Seizure. He has experienced headaches for 2 months. On physical examination, he has weakness on the left side. A brain MRI shows large, irregular, 4.5-cm mass in the centrum semiovale of the right cerebral hemisphere that extends across the corpus callosum. A stereotaxic biopsy shows cellular proliferation of pleomorphic cells positive for GFAP, and IDH-1, and negative expression of ATRX. Three Mitotic figures were identified. No MVO or necrosis are identified. Based on the previous history your best diagnosis is?

A. Anaplastic Astrocytoma, WHO grade 3.
B. Glioblastoma.
C. Oligodendroglioma, WHO grade 2.
D. Diffuse Astrocytoma, WHO grade 2.
E. Anaplastic Oligodendroglioma, WHO grade 3.

Main clues:

GFAP positive: then it's a Glioma.

IDH-1 positive: then it's either Astrocytoma or Oligodendroglioma.

negative expression of ATRX: means that ATRX **is mutated.**

ATRX mutation rules out Oligodendroglioma & confirms Astrocytoma.

3 Mitotic figures: that is main factor that decides Astrocytoma is grade 3 & not grade 2 Astrocytoma.

Answer: A