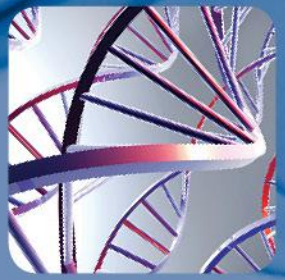
Exceptional People. Exceptional Care.





Neuropathology 101

Dr David Wong

Anatomical pathology

Mater Pathology

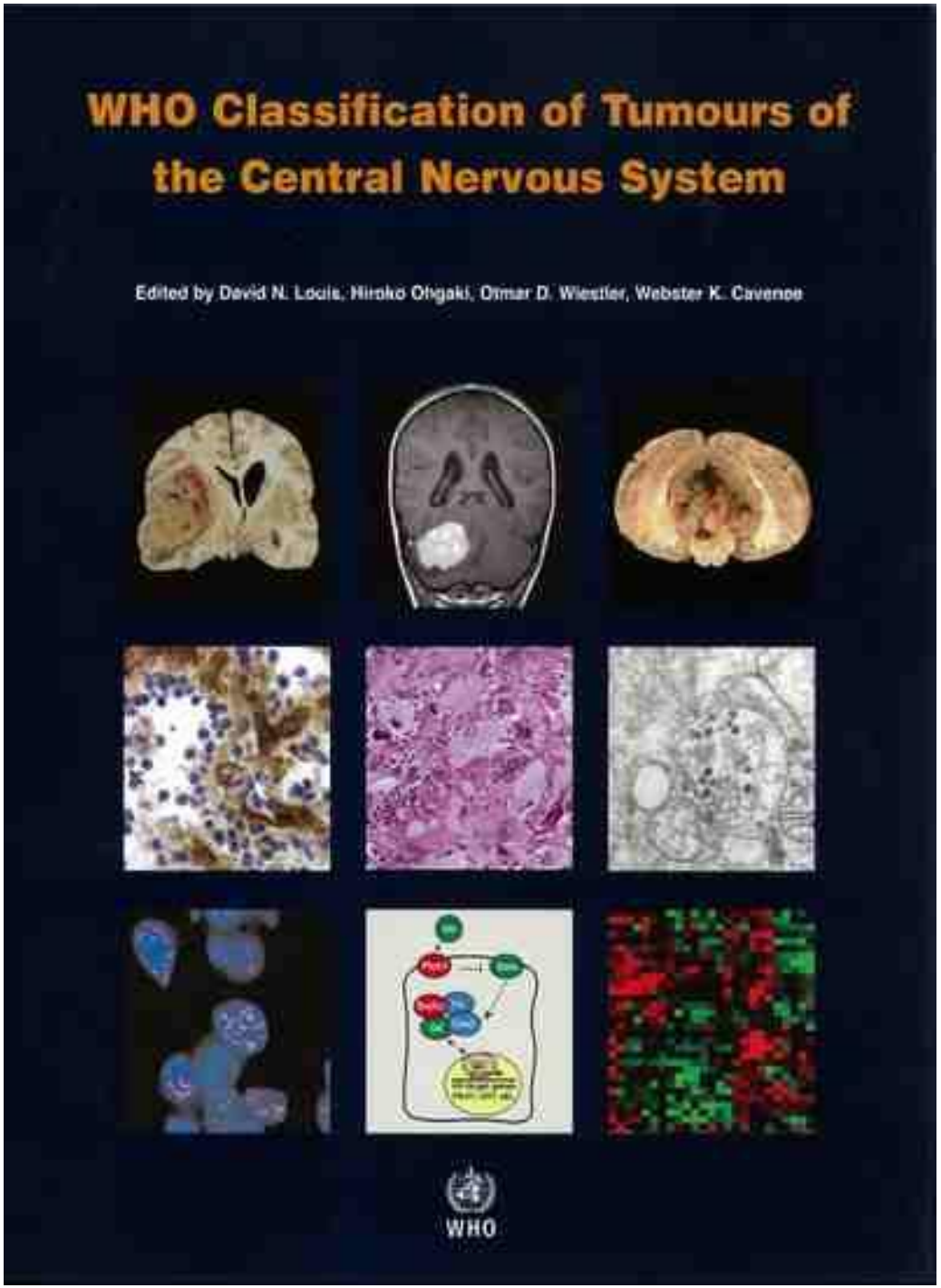
Goals

- CNS tumour classification
- Pre/Intraoperative differential diagnosis
- Gliomas
- Some practical molecular classification of diffuse gliomas.

Neuropathology at Mater

- Diagnostic neurosurgical biopsies

- Perinatal autopsies
- Adult autopsies


WHO Classification of Tumours of the Central Nervous System
Edited by David N. Louis, Hiroko Ohgaki, Otmar D. Wiestler, Webster K. Cavenee
WHO

WHO 2007

CNS tumour classification schemes

1. Cell of origin
2. Tumour grading
3. Molecular markers

WHO classification of CNS tumours 1/2

Astrocytic tumours

Pilocytic astrocytoma	WHO gr I
Pilomyxoid astrocytoma	WHO gr II
Subependymal giant cell astrocytoma (SEGA)	WHO gr I
Pleomorphic xanthoastrocytoma (PXA)	WHO gr II
Diffuse astrocytoma	WHO gr II
Anaplastic astrocytoma	WHO gr III
Glioblastoma	WHO gr IV
Giant cell glioblastoma	
Gliosarcoma	
Gliomatosis cerebri	

Oligodendroglial tumours

Oligodendroglioma	WHO gr II
Anaplastic oligodendroglioma	WHO gr III

Mixed glial tumours

Oligoastrocytoma	WHO gr II
Anaplastic oligoastrocytoma	WHO gr III

Ependymal tumours

Subependymoma	WHO gr I
Myxopapillary ependymoma	WHO gr I
Ependymoma	WHO gr II
Anaplastic ependymoma	WHO gr III

Neuronal and mixed neuronal-glial tumours

Gangliglioma and gangliocytoma	WHO gr I
Anaplastic ganglioglioma	WHO gr III
Desmoplastic infantile astrocytoma and ganglioglioma	WHO gr I
Dysembryoplastic neuroepithelial tumour (DNET)	WHO gr I
Central neurocytoma and extraventricular neurocytoma	WHO gr II
Cerebellar liponeurocytoma	WHO gr II
Papillary glioneuronal tumour	WHO gr I
Paraganglioma (spinal)	WHO gr I

Choroid plexus tumours

Tumours of the pineal region

Pineocytoma	WHO gr I
Pineal parenchymal tumour of intermediate differentiation	WHO gr II-III
Pineoblastoma	WHO gr IV
Papillary tumour of the pineal region	WHO gr II-III

WHO classification of CNS tumours 2/2

Embryonal tumours

Medulloblastoma	WHO gr IV
CNS PNET	WHO gr IV
Atypical teratoid/rhabdoid tumour	WHO gr IV

Tumours of the cranial and paraspinal nerves

Schwannoma	WHO gr I
Neurofibroma	WHO gr I
Perineurioma	WHO gr I-III
Malignant peripheral nerve sheath tumour (MPNST)	WHO gr II-IV

Meningeal tumours

Meningioma	WHO gr I
Atypical meningioma	WHO gr II
Anaplastic meningioma	WHO gr III
Haemangiopericytoma	WHO gr II
Anaplastic haemangiopericytoma	WHO gr III
Melanocytic lesions	
Haemangioblastoma	WHO gr I

Tumours of the sellar region

Craniopharyngioma	WHO gr I
Granular cell tumour of the Neurohypophysis	WHO gr I
Pituicytoma	WHO gr I
Spindle cell oncocytoma of the Adenohypophysis	WHO gr I

Tumours of the haematopoietic system

Malignant lymphoma

Histiocytic tumours

Germ cell tumours

Familial tumour syndromes

Metastatic tumours

WHO grading

- Histological grading is a means of predicting biological behaviour and influences choice of therapies.
- **Grade I lesions:** generally low proliferative potential; possibility of cure following surgical resection alone.
- **Grade II lesions:** generally infiltrative, have low level proliferative activity but often recur; some progress to higher grade malignancy.
- **Grade III lesions:** generally have histologic evidence of malignancy (atypia, mitoses); most receive adjuvant radiation and/or chemo. **Grade IV lesions:** are cytologically malignant, mitotically active, necrosis-prone; ass w rapid disease evolution and fatal outcome.

  Eg GBM, most embryonal tumours and many sarcomas. Some tend to have widespread infiltration and propensity for craniospinal dissemination.

Prognosis and survival

- WHO grade, age, performance status, tumour location; radiology (eg contrast enhancement), extent of surgical resection; proliferation indices; genetic alterations

- WHO Gr II: typically survive >5yrs
- WHO Gr III: typically survive 2-3yrs
- WHO Gr IV: GBM typically succumb within a year. Others such as medulloblastoma and germ cell tumours are rapidly fatal if untreated, but radiation and chemotherapy result in 5-yr survival 60% & 80% respectively.

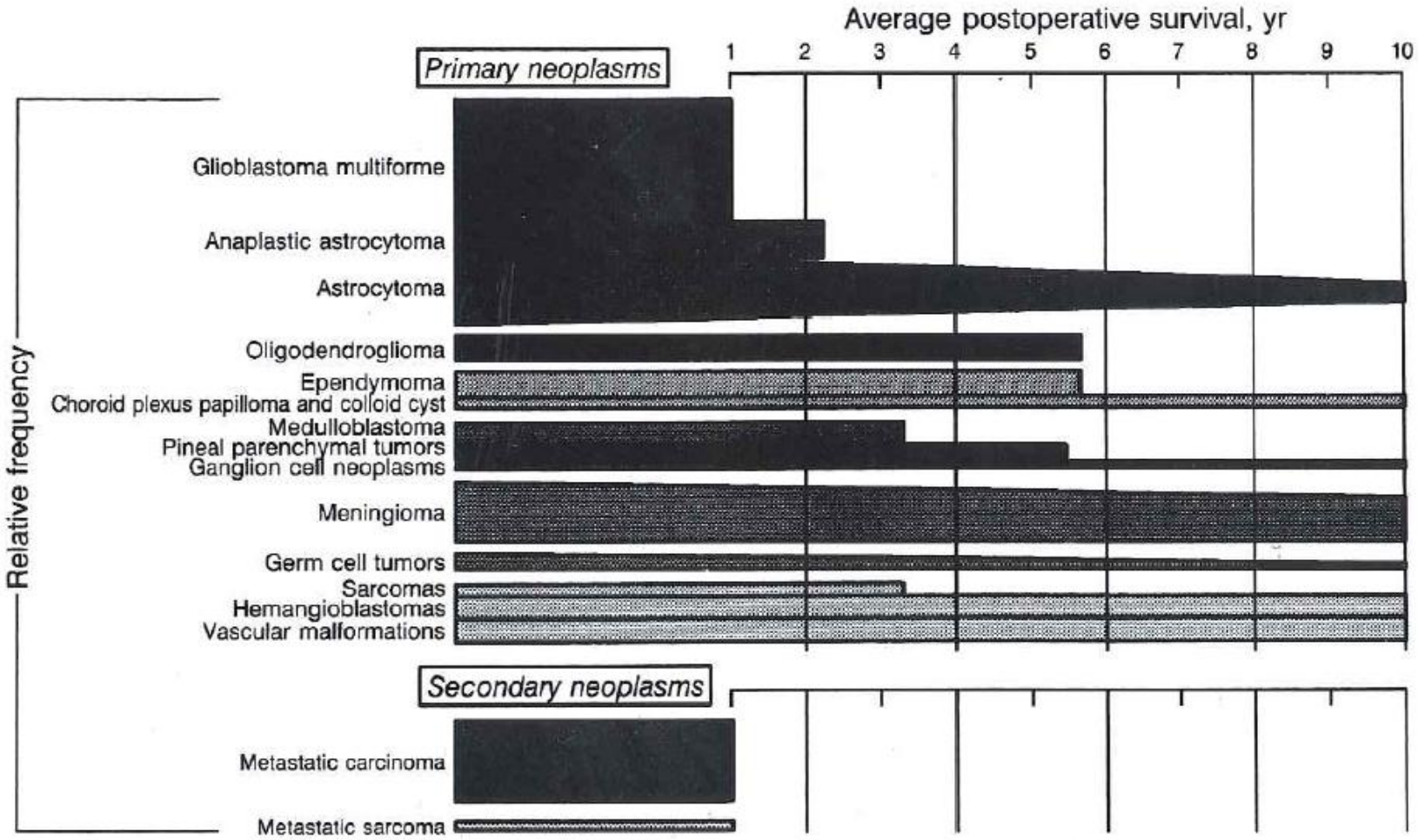


FIGURE 4–1 PRIMARY AND METASTATIC TUMORS OF THE BRAIN: INCIDENCE AND SURVIVAL RATES

Relative incidence and mean postoperative survival rates are indicated by the width and length of the individual arms, respectively.

Preoperative differential diagnosis

1) **Patient details**: **Age** (adult or child), gender (eg met breast CA), known tumours, previous therapy (eg irradiation), rapid onset, immuno-compromised (eg tumour, abscess, CNS lymphoma, PML), genetic disorders (eg NF, TS, Turcot's)

2) **Radiology: Site**, intra or extra axial, supratentorial or infratentorial, **pattern of enhancement**, solid or cystic, necrosis, haemorrhage, margins, calcification.

3) **Surgeon's observations**

Common CNS tumours by location, age and imaging characteristics 1/4

Location	Child / Young adult	Older adult
Cerebral / Supratentorial	Ganglioglioma (TL, cyst-MEN, E) DNET (TL, intracortical nodules) PNET (solid, E) AT/RT (infant, E)	Diffuse glioma Gr II-III (NE, focal E) GBM (E or rim E, "butterfly") Metastases (grey-white junc^{n}, E or rim E) Lymphoma (periventricular, E)
Cerebellar / 4th ventricle	PA (cyst-MEN) Medulloblastoma (vermis, E) Ependymoma (4th v, E) Choroid plexus papilloma (4thv, E) AT/RT (infant, E)	Metastases (multiple, E or rim E) Hemangioblastoma (cyst-MEN) Choroid plexus papilloma (4th v, E)
Brainstem	"Brainstem glioma" (pons, +/-E) PA (dorsal, exophytic, cyst-MEN)	Gliomatosis cerebri (multifocal, +/-E)

AT/RT, Atypical teratoid/rhabdoid tumour; **DNET,** Dysembyoplastic neuroepithelial tumour; **E,** Enhancing; **GBM,** Glioblastoma multiforme; **MEN,** Mural enhancing nodule; **PA,** Pilocytic astrocytoma; **PNET;** Primitive neuroectodermal tumour; **TL,** Temporal lobe

Common CNS tumours by location, age and imaging characteristics 2/4

Location	Child / Young adult	Older adult
Spinal cord (intramedullary)	Ependymoma (E, +/- syrinx) PA (cystic, E) Drop metastases (cauda equina, E) MPE (filum terminale, E)	Ependymoma (E, +/- syrinx) Diffuse astrocytoma (ill defined, +/-E MPE (filum terminale, E) Paraganglioma (filum terminale, E)
Spinal cord (intradural, extramedullary)	Clear cell meningioma (+/- dural tail, E) Schwannoma (NF2, nerve origin, dumbbell shape, E) Drop metastases (leptomeningeal, E)	Schwannoma (nerve origin, dumbbell shape, E) Meningioma (+/- dural tail, E)
Spinal cord (extradural)	Bone tumour spread (EWS/PNET, usually E) Meningioma (+/- dural tail, E) Abscess (E) Vascular malformations (dilated BV, +/- E)	Herniated disc (T1-spin echo, NE) Postoperative scar (E) Secondary lymphoma (E) Metastases (E) Abscess (E)

BV, blood vessels; **E,** Enhancing; **EWS:** Ewing's sarcoma; **MPE,** Myxopaillary ependymoma; **NE,** non enhancing **PNET;** Primitive neuroectodermal tumour;

Common CNS tumours by location, age and imaging characteristics 3/4

Location	Child / Young adult	Older adult
Intrasellar	Pituitary adenoma (solid, E) Craniopharyngioma (cystic, E) Rathke's cleft cyst (cystic, +/- E)	Pituitary adenoma (solid, E) Craniopharyngioma (cystic, E) Rathke's cleft cyst (cystic, +/- E)
Suprasellar / hypothalamic/ optic pathway/ 3rd ventricle	Germinoma (solid, E) Craniopharyngioma (cystic, E) PA (cyst-MEN) Pilomyxoid astrocytoma (infant, solid, E)	Colloid cyst (3rd v, +/- E) Craniopharyngioma (cystic, E)
Pineal	Germinoma (solid, E) Pineocytoma (solid, E) Pineoblastoma (solid, E) Pineal cyst (cystic, NE)	Pineocytoma (solid, E) Pineal cyst (cystic, NE)
Thalamus	PA (cyst-MEN) AA/GBM (E or rim E)	AA/GBM (E or rim E) Lymphoma (E, +/- multifocal)

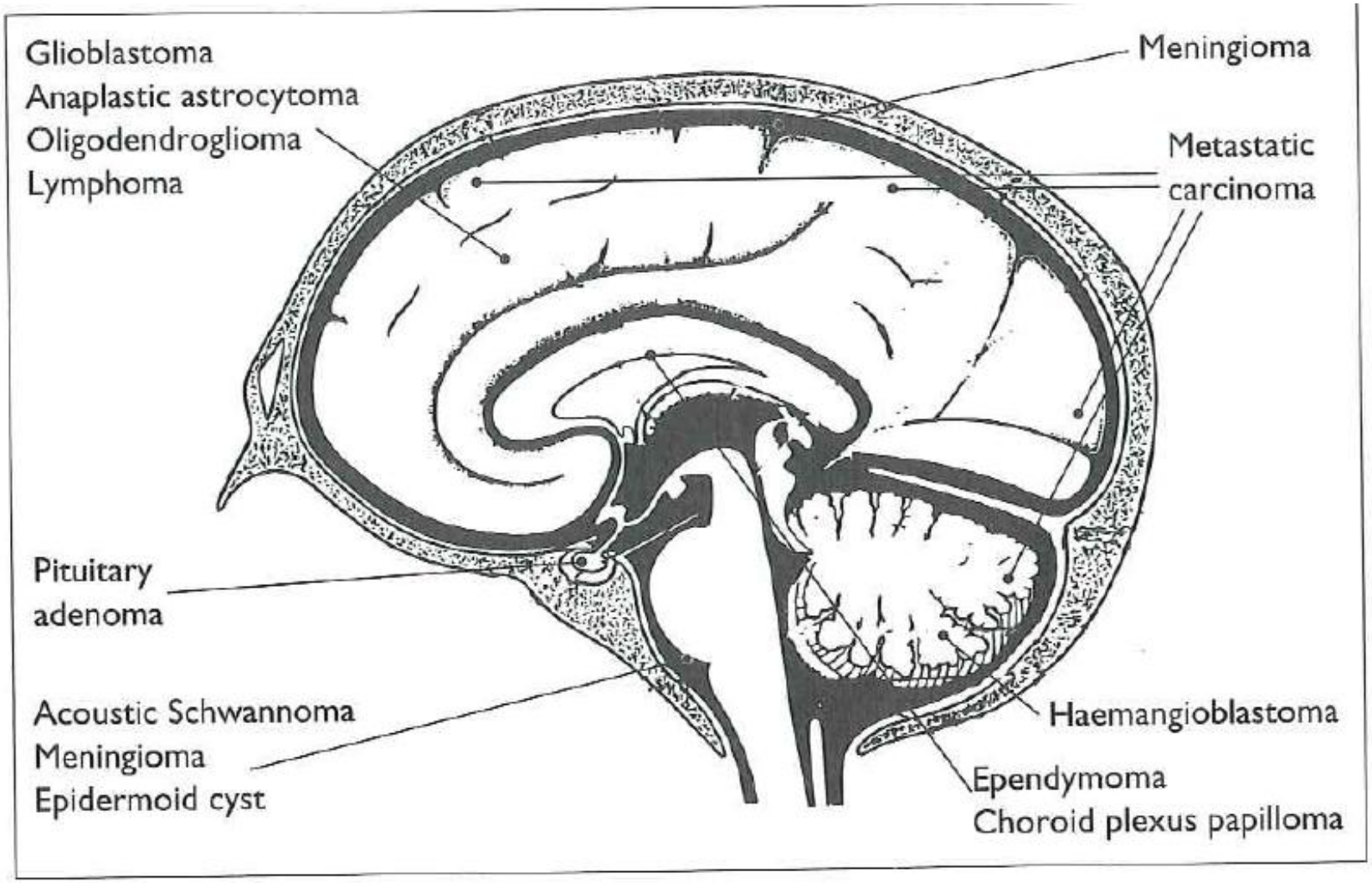
AA, Anaplastic astrocytoma; **E,** Enhancing; **GBM,** Glioblastoma; **MEN,** mural enhancing nodule; **NE,** non enhancing; **PA,** Pilocytic astrocytoma;

Common CNS tumours by location, age and imaging characteristics 4/4

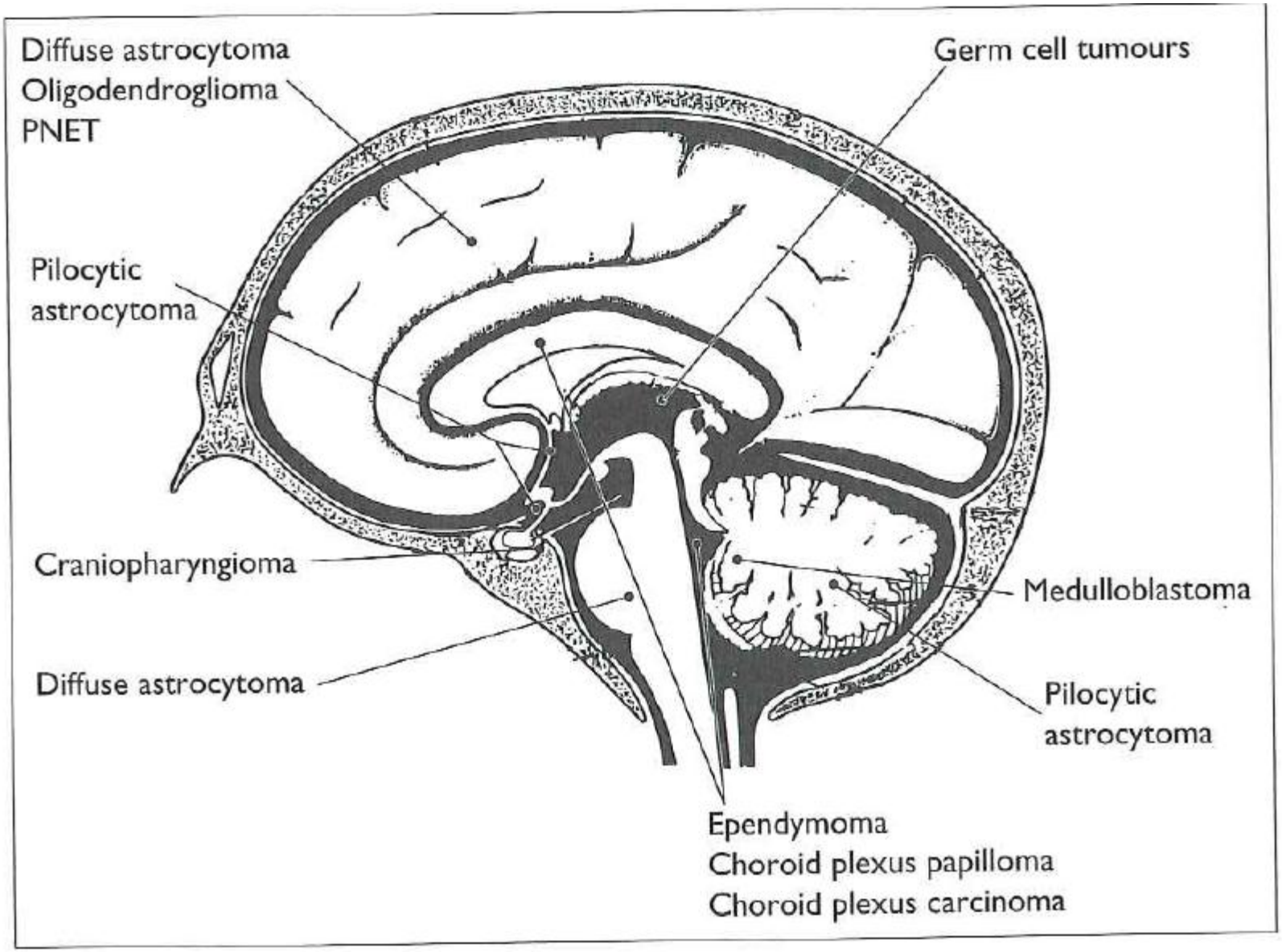
Location	Child / Young adult	Older adult
Cerebellopontine angle	Vestibular schwannoma (NF2, E, involves internal auditory meatus) Choroid plexus tumour (E, component in 4th v)	Vestibular schwannoma (NF2, E, involves internal auditory meatus) Meningioma (+/- dural tail, E)
Lateral ventricle	Central neurocytoma (E) SEGA (tuberous sclerosis, E) Choroid plexus papilloma (E) Choroid plexus carcinoma (infant, E, large, invasive)	Central neurocytoma (E) SEGA (tuberous sclerosis, E) Choroid plexus papilloma (E) Subependymoma (+/- E) Meningioma (+/- dural tail, E)
Nerve root / paraspinal	Neurofibroma (NF1, E) MPNST (NF1, E, necrotic)	Schwannoma (dumbbell shape, E) Meningioma (+/- dural tail, E) Secondary lymphoma (E) Neurofibroma (NF1, E) MPNST (E, necrotic)

E, Enhancing; **MPNST,** malignant peripheral nerve sheath tumour; **NF1**, neurofibromatosis type 1
SEGA, subependymal giant cell astrocytoma

Anatomical distribution of commonest brain tumours in adults.



Anatomical distribution of commonest brain tumours in children.

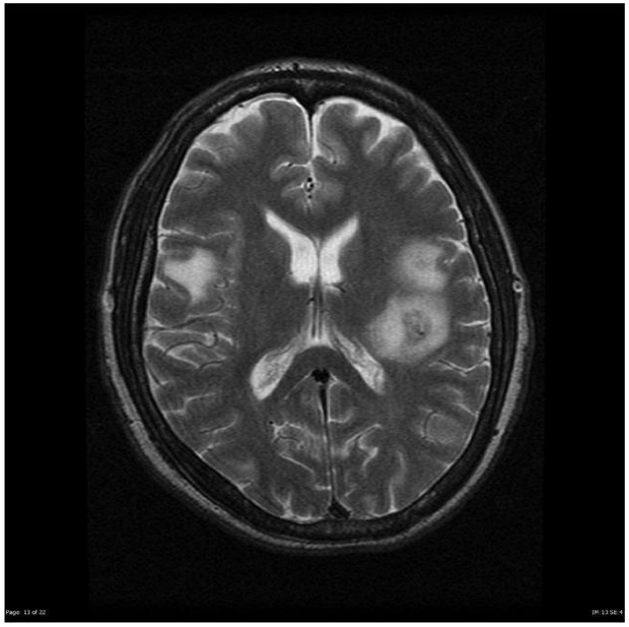


Interpreting the MRI

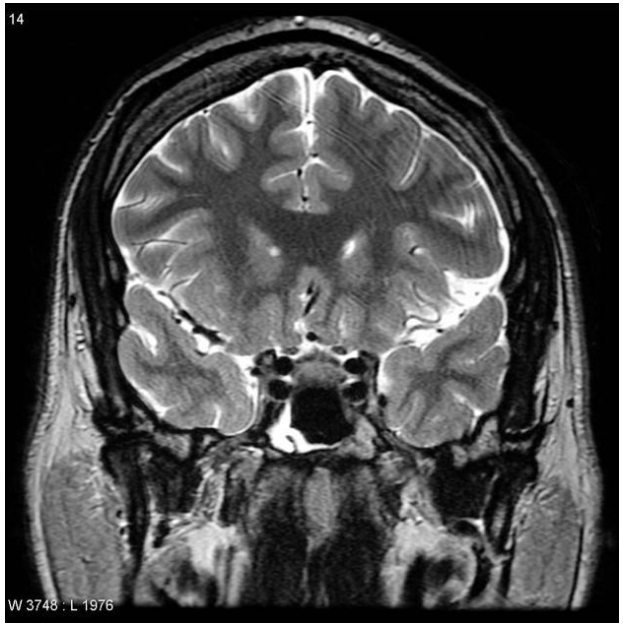
- **3 planes of section**

  Axial (this has all the T1, T2, etc thus go to that plane),

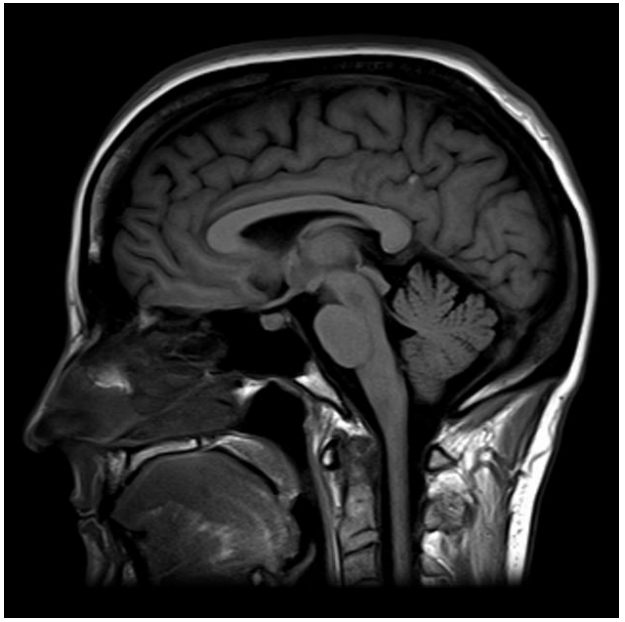
  Coronal, Sagittal (latter 2 would usually only have T1 post contrast)



Axial



Coronal



Sagittal

Interpreting the MRI

- **4 main sequences**

  T1 pre, T1 post contrast (gadolinium – look for bright BV & choroid plexus compared with pre contrast), T2, T2-flair (CSF is suppressed)

- **3 simple principles**

I. On T2 = H2O (CSF, oedema)

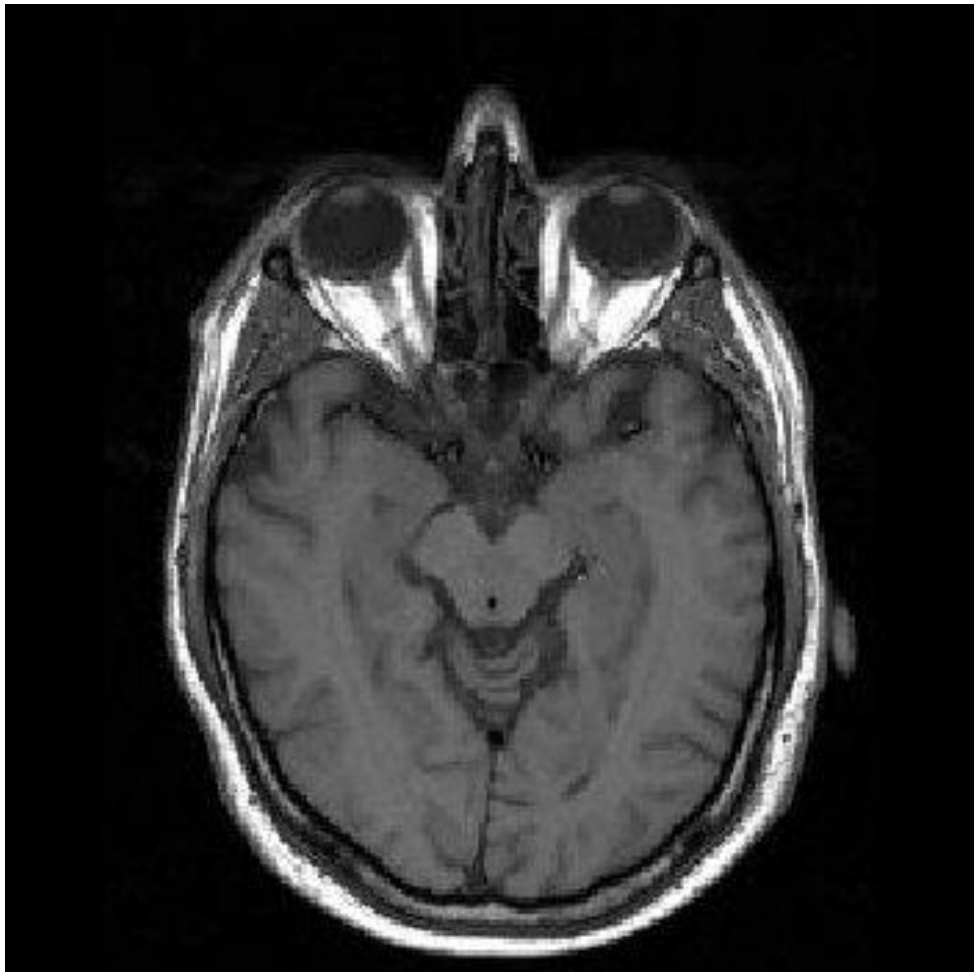
II. White matter is rich in lipid myelin, thus contains less water than grey matter, thus white matter is darker (hypointense) than grey matter on T2

Thus if cortex is brighter (hyperintense) than white matter = T2 (vice versa in T1 where CSF is also black)

III. Contrast enhancement is assessed on T1 (not on T2; thus do not confuse T2 brightness for contrast enhancement) NB: Blood is bright on the T1 pre contrast, thus need to compare T1 pre and post)

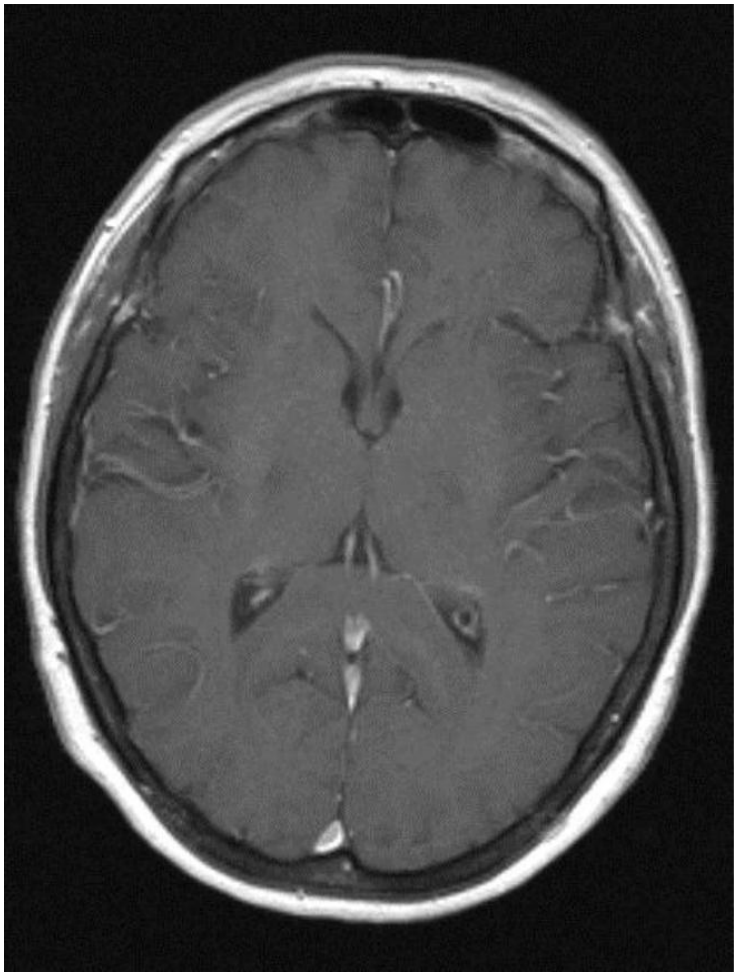
- For quick intraoperative look, pick T1 post (for enhancement) and T2-flair (for extent of lesion including oedema) sequences on the axial planes.

T1 pre



Cortex is darker than white matter

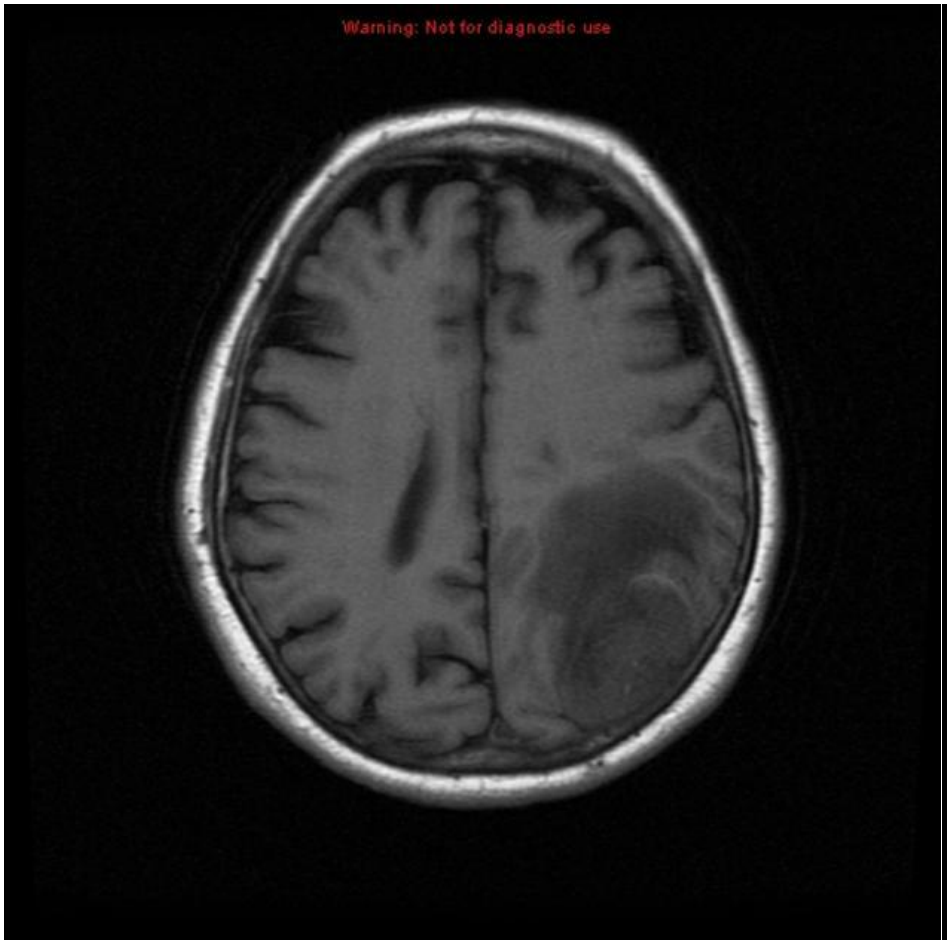
T1 post

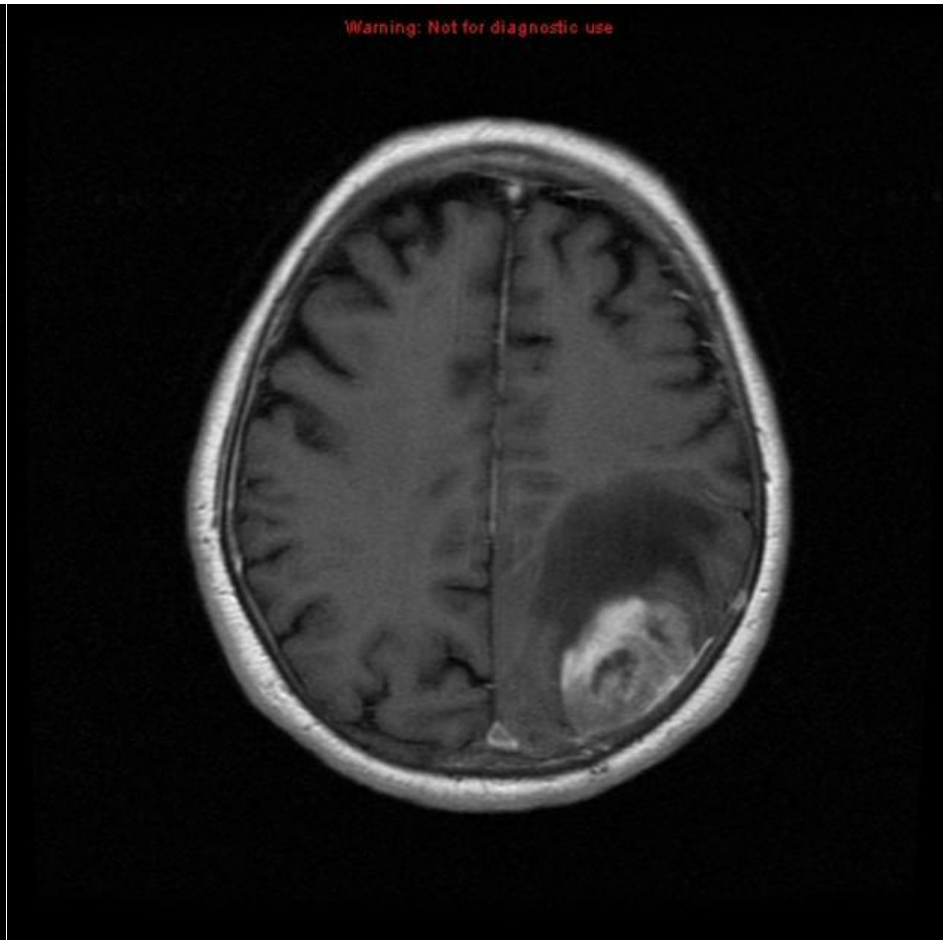


Contrast indicated by bright blood
Vessels and choroid plexus

T1 pre

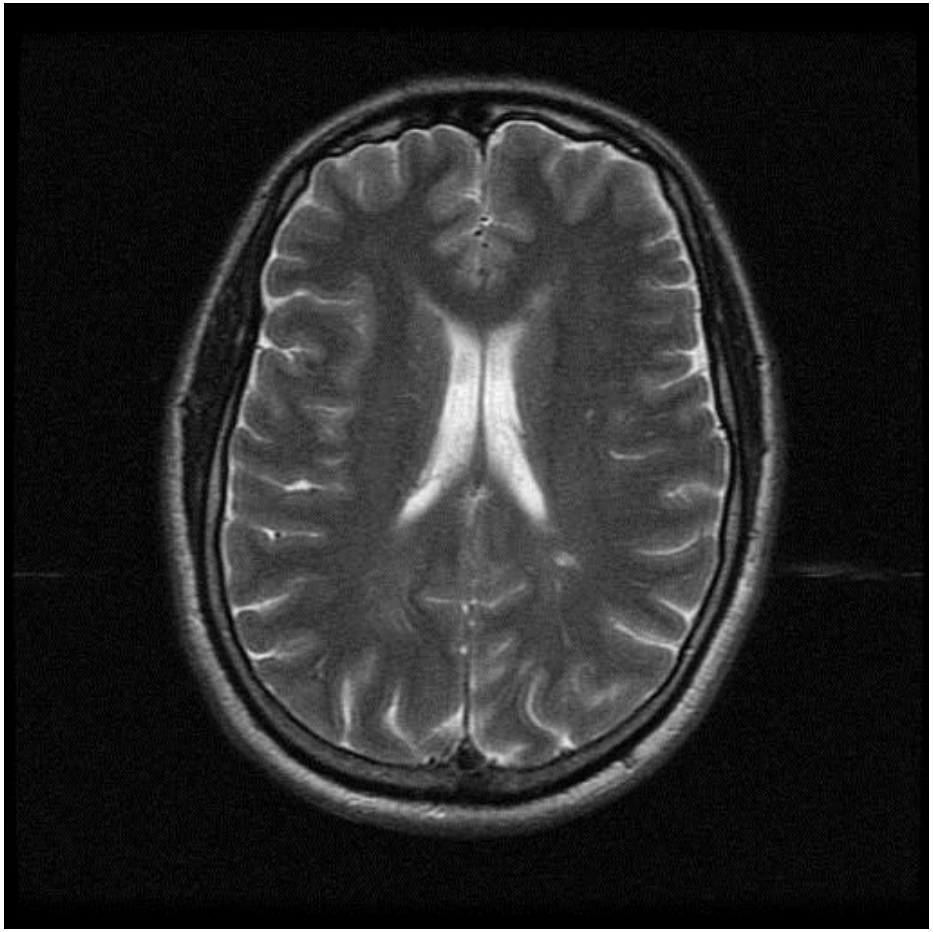
T1 post





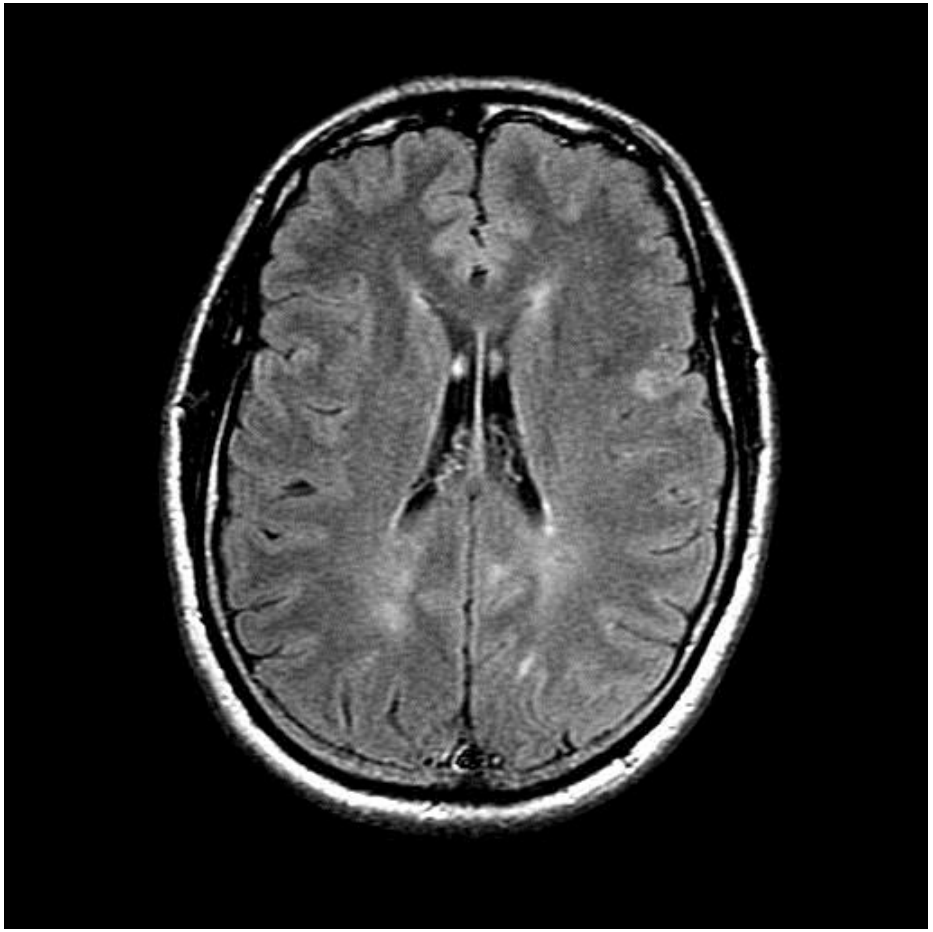
Enhancing lesion (GBM)

T2



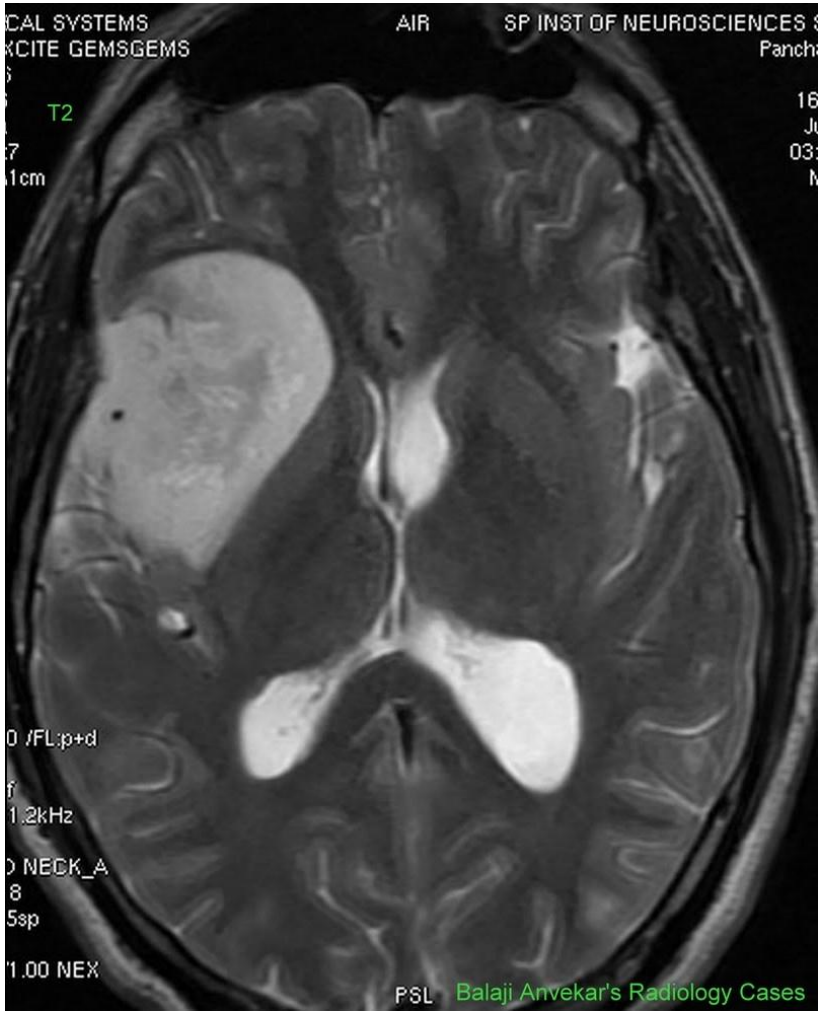
Cortex brighter than white matter
CSF is bright

T2 flair



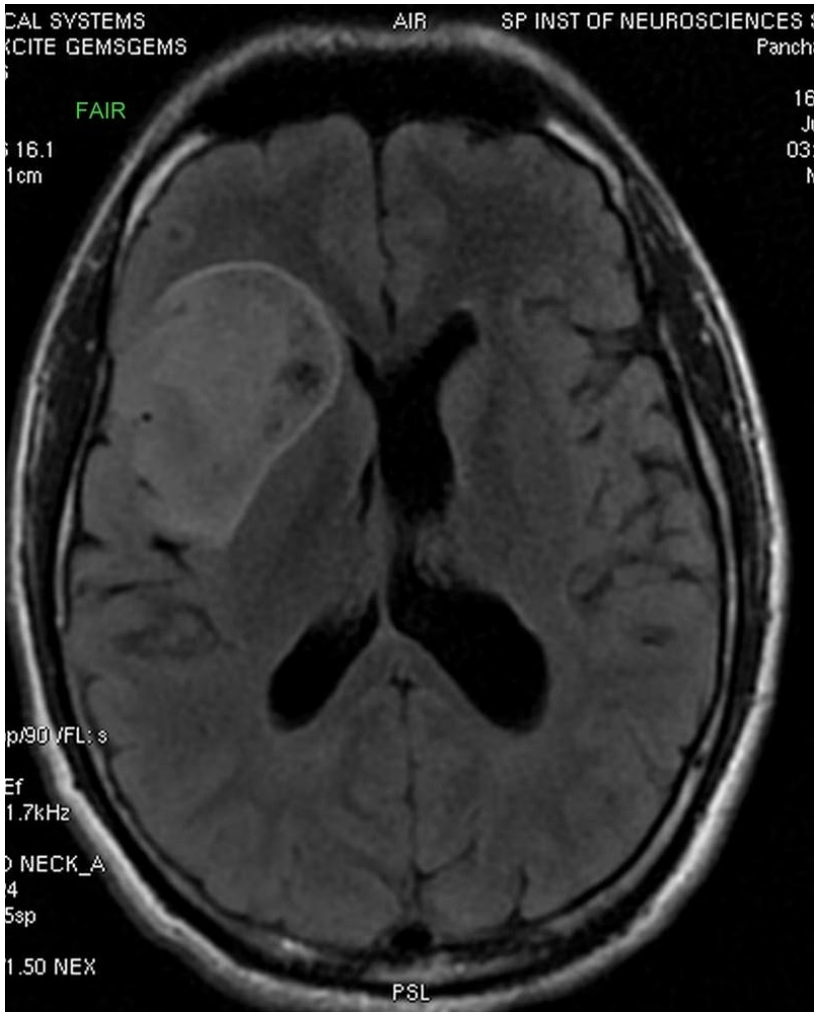
Fluid attenuated: ie brightness of CSF is attenuated and is now dark.

T2



Cortex brighter than white matter
CSF is bright
(Anaplastic astrocytoma)

T2 flair



With CSF suppressed, water such as Oedema can be assessed better
NB: Oedema (& CSF) are dark on T1

Other T2 based MRI sequences

1. **DWI:** Diffusion – Weighted Imaging

- "Restricted diffusion" - Bright on DWI Movement of water is "restricted" in
  - In acute infarct (6hr-7d),
  - Abscess (eg helpful in a ring enhancing lesion with ddx GBM, met),
  - Epidermoid cyst,
  - Hypercellular tumours (PCNSL, PNET, etc)

2. **T2-GRE (T2*):** Gradient Echo

- Useful for detecting (appears hypointense/dark)
  - Blood products (eg cavernoma)
  - Iron
  - Calcium

3. **SWI:** Susceptibility-Weighted Imaging

- Useful for detecting (appears hypointense/dark)
  - Blood products
  - Iron
  - Calcium
  - Small veins

Information to be gained on MRI

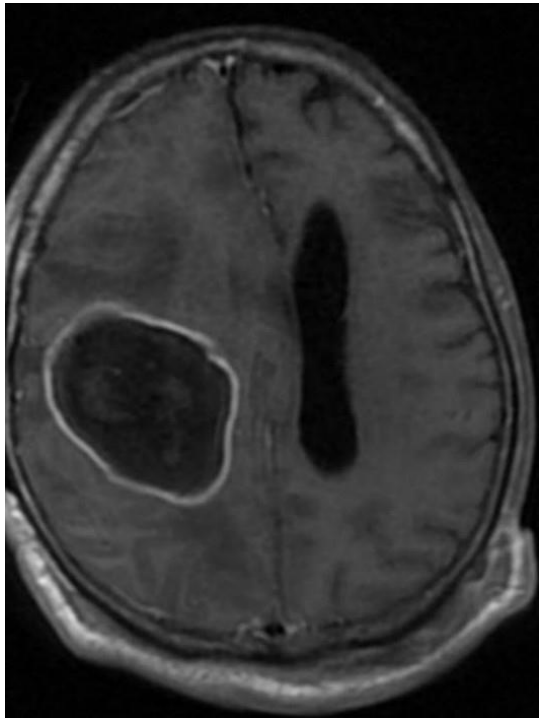
1. **Anatomic location**
2. **Interface of lesion border with brain**
   - Sharply circumscribed: eg Met, PXA, PA, ganglioglioma, hemangioblastoma
   - Diffuse: 2 things - Diffuse gliomas & lymphomas

NB: Half of diffuse gliomas invade cortex & blurs gray/white on T2-flair (cf vasogenic edema is limited by gray/white)

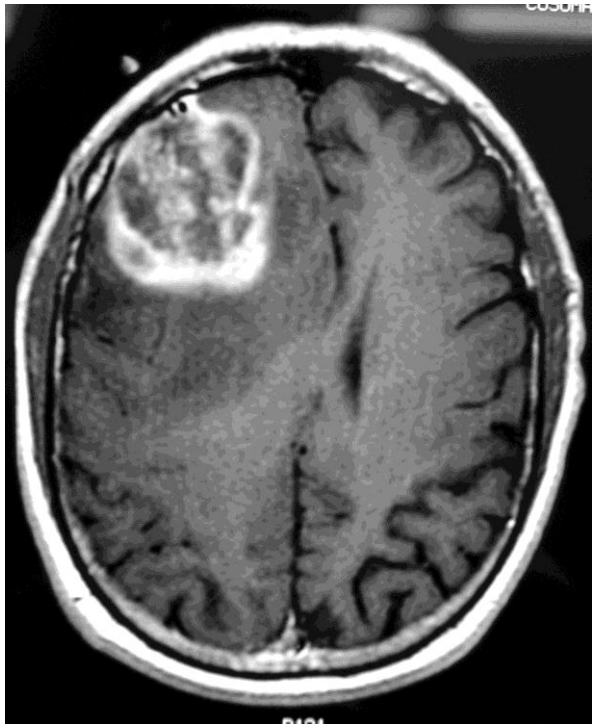
3. **Presence of contrast enhancement**
   - Eg GBM (strong), JPA (uniform), etc
4. **Pattern of contrast enhancement**
   - Smooth ring: Abscess (confirm with DWI)
   - Ragged ring: GBM, Met
   - C-shaped ring: Demyelinating
   - Solid, uniform: Meningioma (look for dural tail), PCNSL (NB HIV can have necrosis), Schwannoma (ie no central necrosis, slow growing, LG, except lymphoma)
   - Cyst w nodule: PA, PXA, Ganglioglioma
   - Dark ring*: Cavernoma, Abscess

* T2, T2-flair, T2-GRE, T2-SWI

Patterns of contrast enhancement

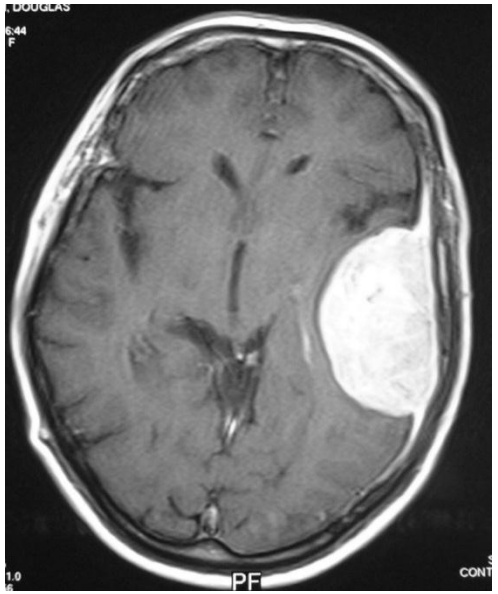


Abscess: Uniformly thick & smooth contoured ring enhancement. (NB: Fluid diffusion restricted On DWI)

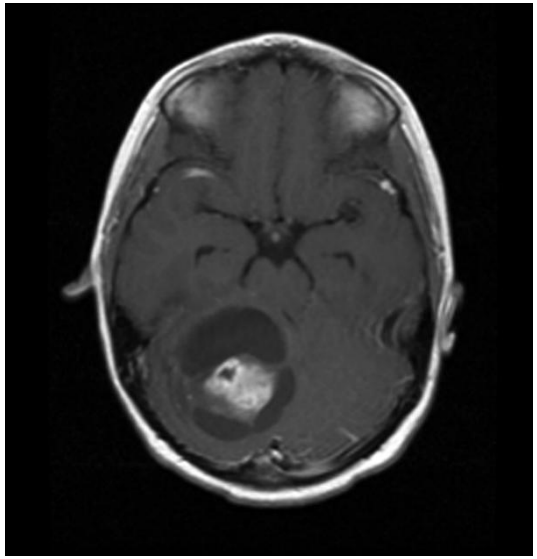


↑ **GBM:** Ragged ring

Pilocytic astrocytoma: → Cystic with enhancing nodule



↑ **Meningioma:** Solid uniform enhancement; dural tail

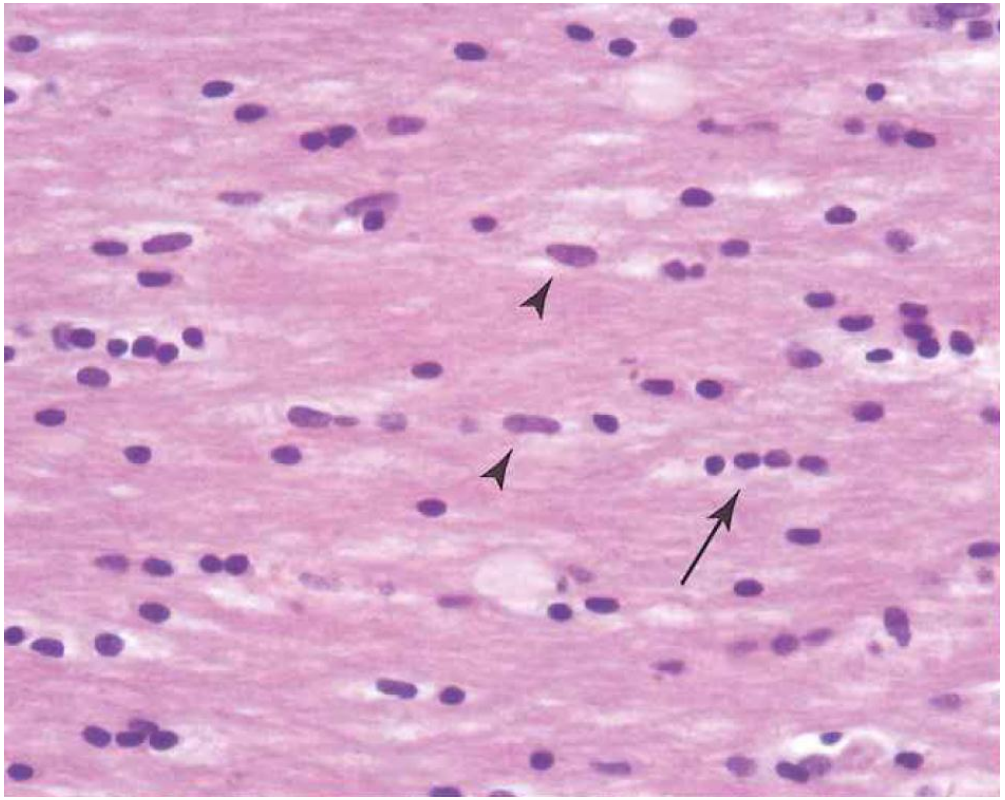


HISTOPATHOLOGIC ASSESSMENT

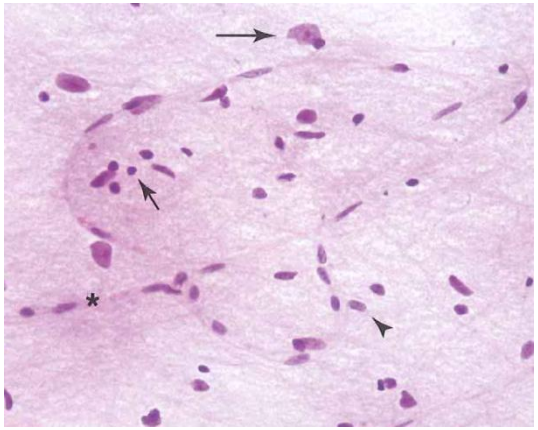
Combination of

- **Histopathology**
  - Histology patterns
- **Immunohistochemistry**
  - Glial markers: GFAP, S100
  - Neuronal markers: Synaptophysin, chromogranin, NeuN (mature neuronal diff), NFP (axons)
  - Epithelial markers: CK
  - Proliferation markers: Ki67
  - Prognostic: IDH1
- **Molecular studies**
  - FISH: 1p19q deletion (oligo), N-myc or c-myc (in large cell/anaplastic medulloblastoma)

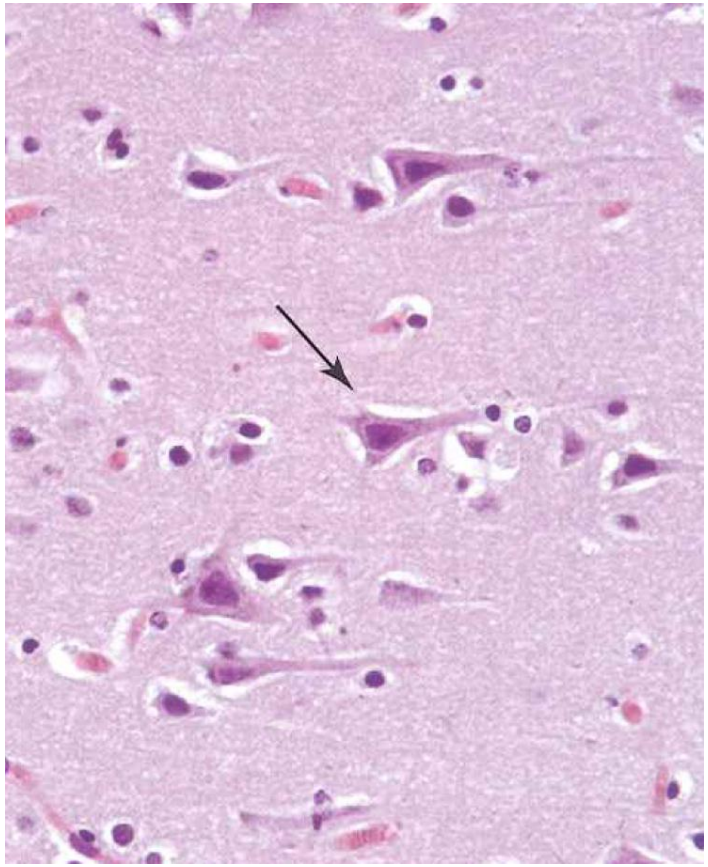
Normal brain



Astrocytes (arrowhead)
Oligodendrocytes (arrow)

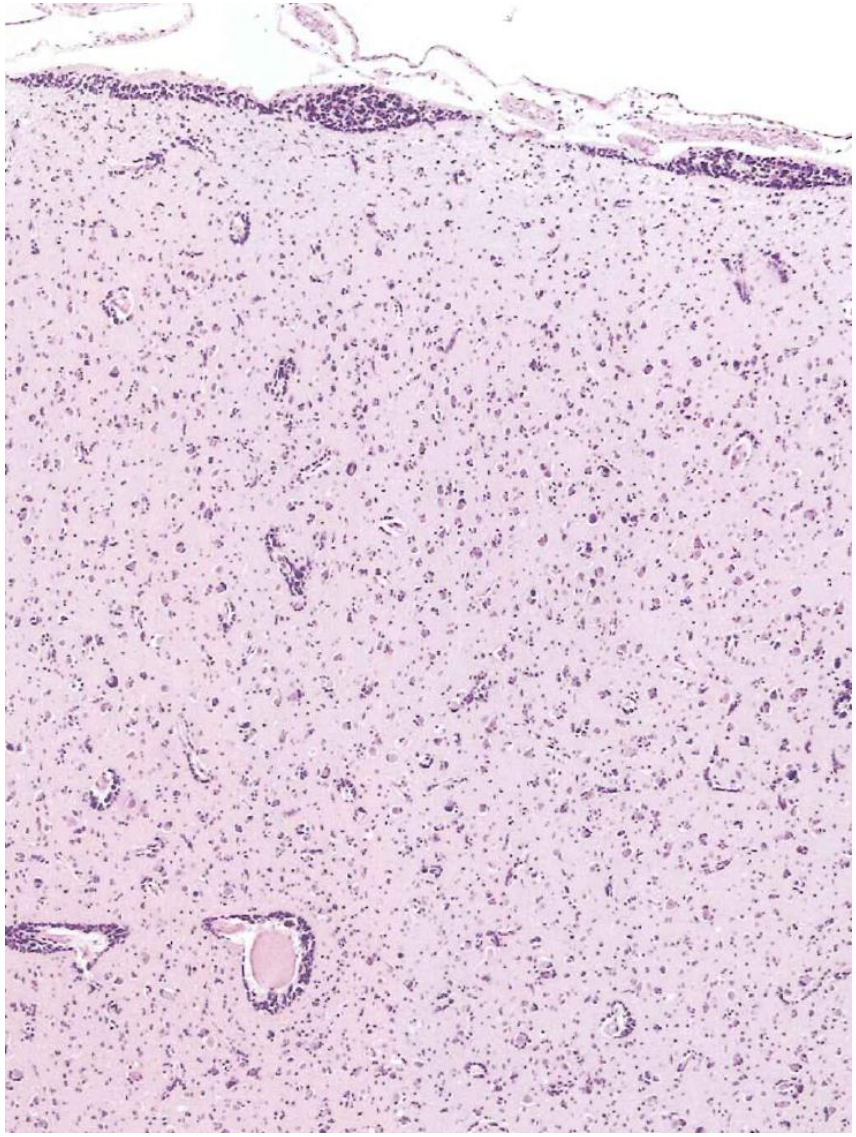


Smear cytologic
Preparation →

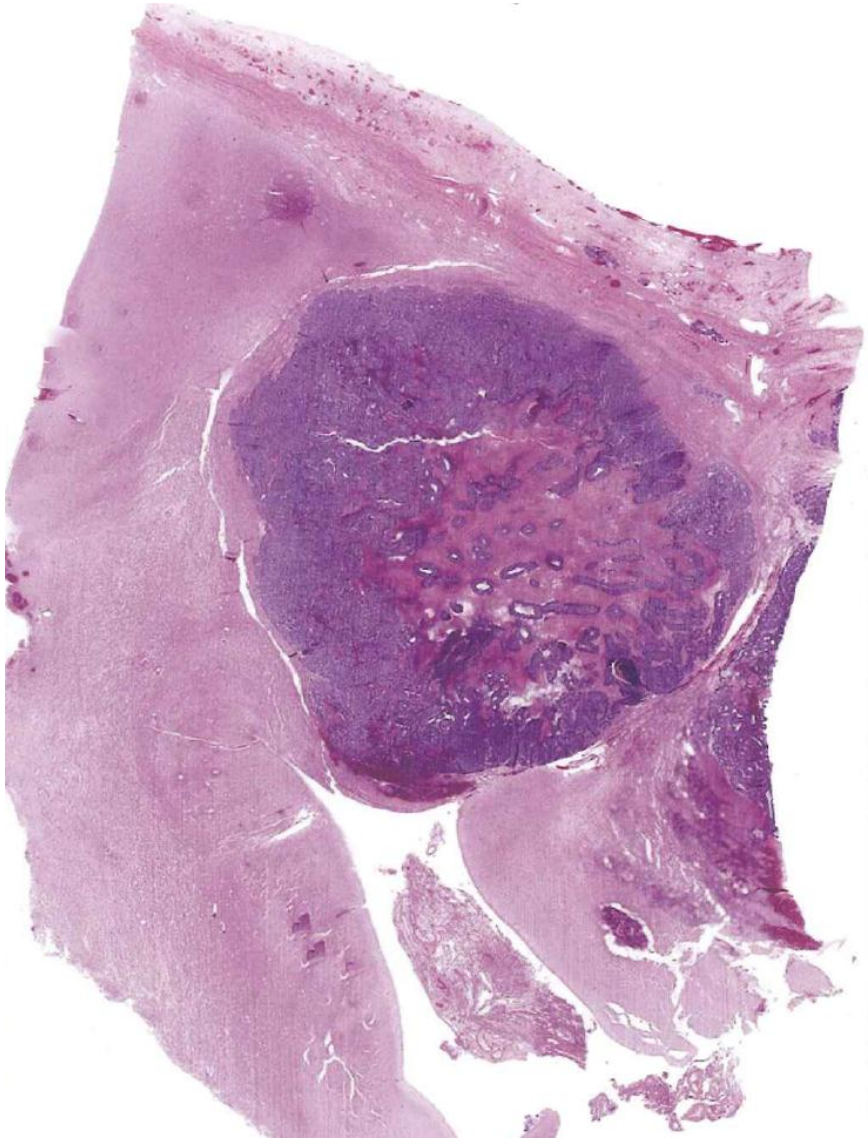


↑(Pyramidal) neurones

Some neoplastic patterns

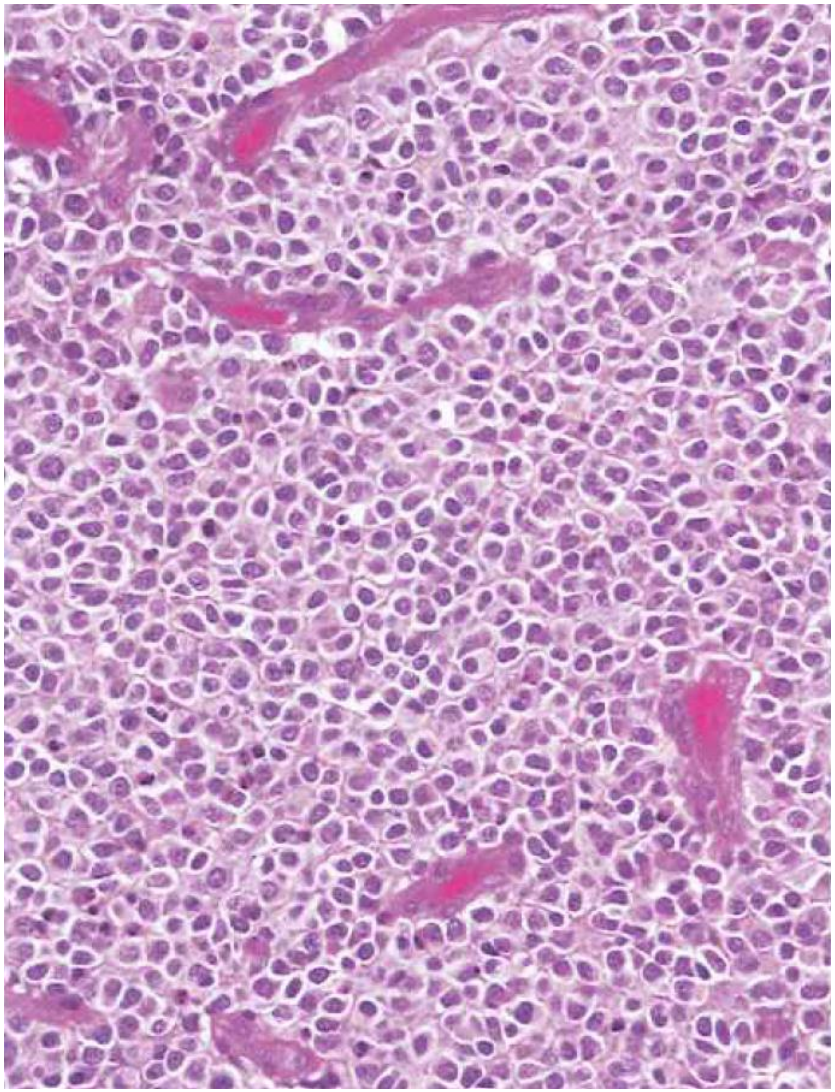


Diffuse hypercellular parenchymal infiltrate (glioma)

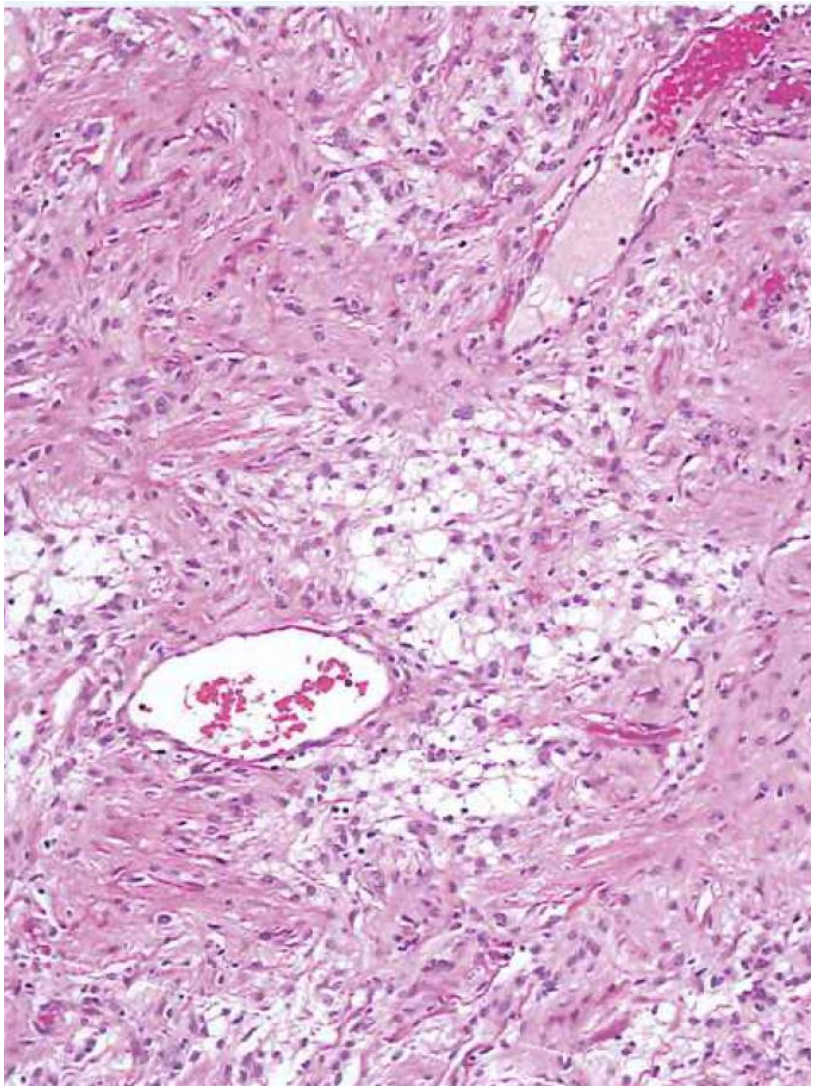


Discrete circumscribed mass (eg metastatic adenocarcinoma)

Some neoplastic patterns

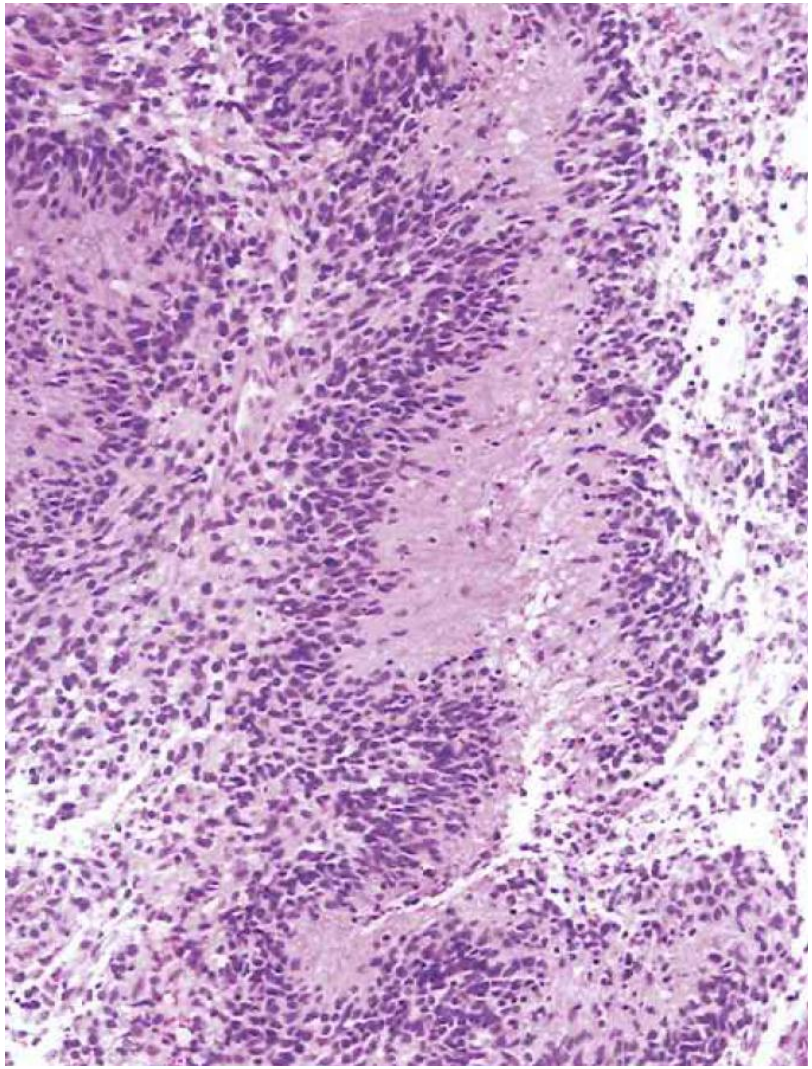


"Fried egg" or Clear cells: Eg Oligodendro-glioma, small cell GBM, clear cell ependymoma, central neurocytoma

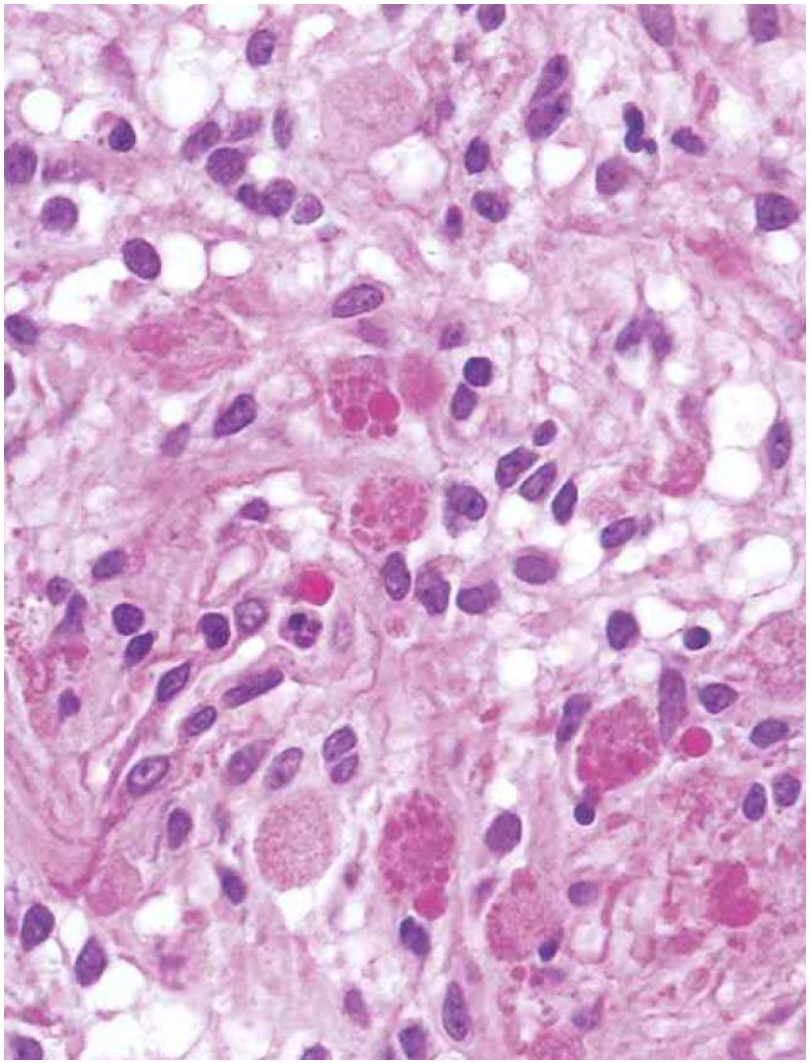


Biphasic (loose and compact): Eg PA, PXA, Ganglioglioma, Schwannoma

Some neoplastic patterns

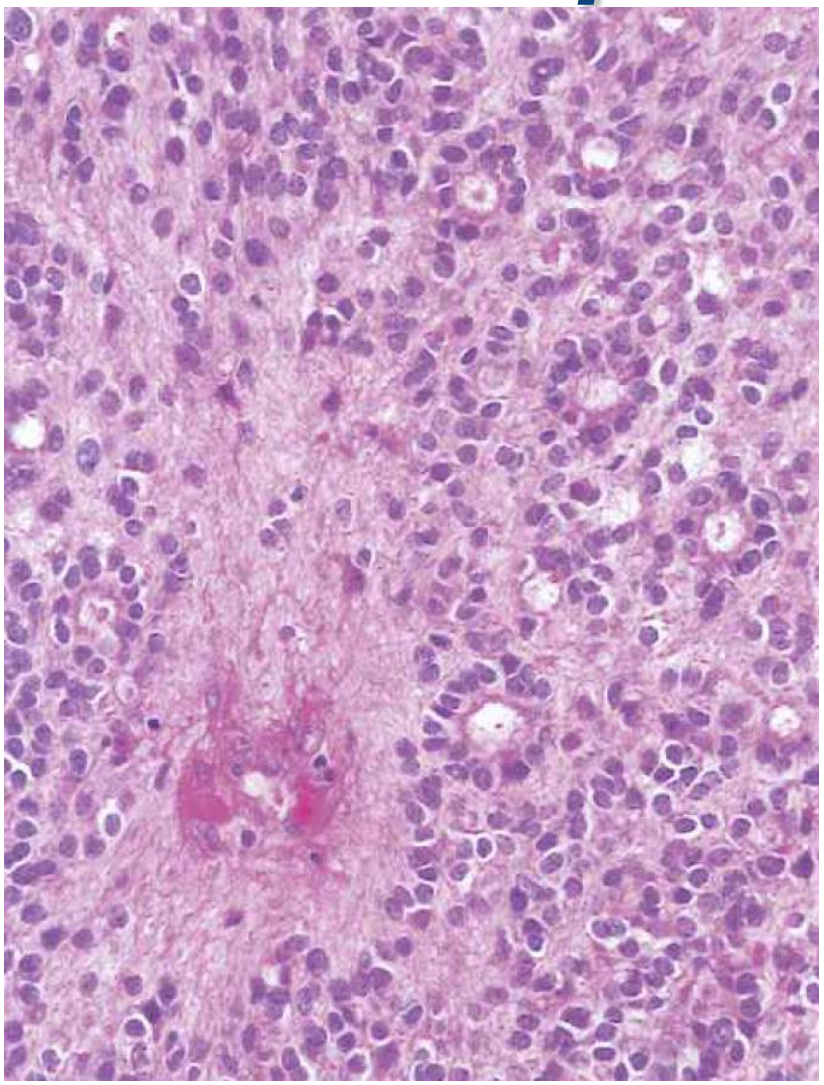


Pseudo/Palisading cells: GBM (pseudopalisading necrosis), Schwannoma (Verocay bodies)

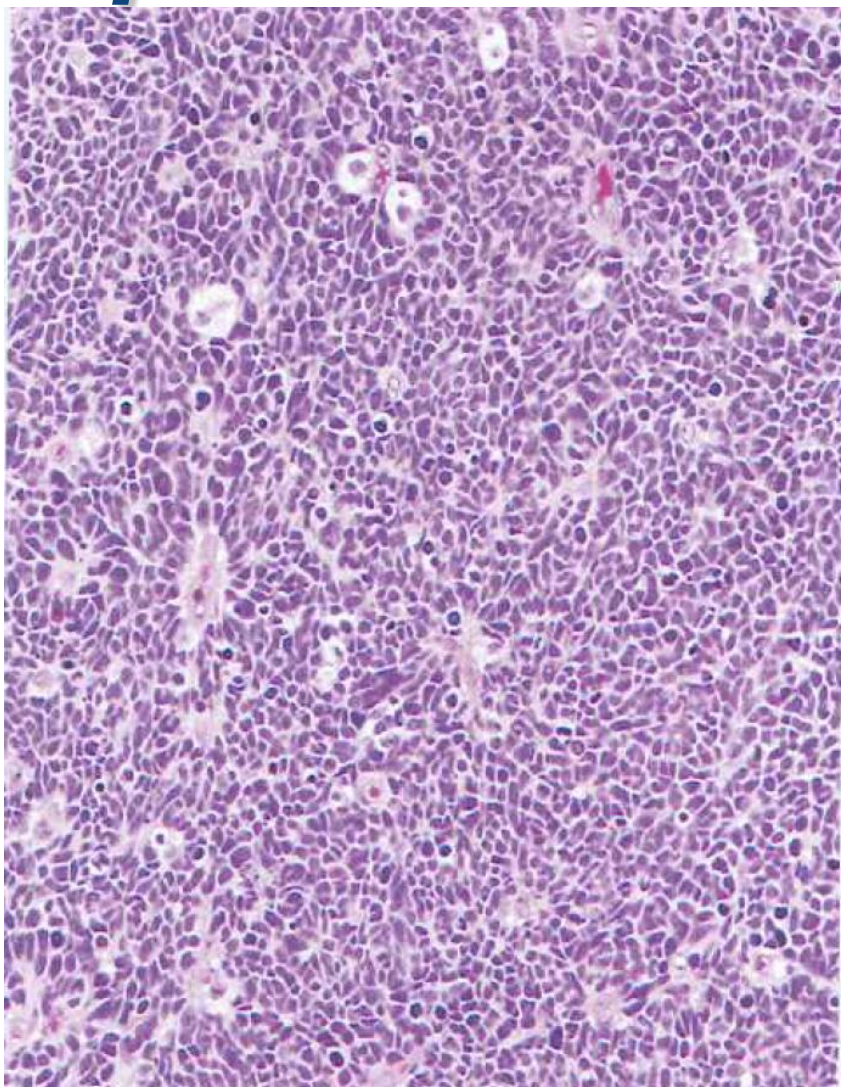


Rosenthal fibres / Eosinophilic granular Bodies: PA, ganglioglioma, PXA, piloid gliosis adjacent slow growing neoplasms

Some neoplastic patterns



Rosette forming: Eg Ependymoma (true rosette), Medulloblastoma (Homer Wright), CNS PNET



“Small blue cells” (Primitive): Medulloblastoma, CNS PNET, AT/RT, GBM, lymphoma, melanoma

ASTROCYTOMAS

- **Diffusely infiltrating** astrocytomas: Low gr diffuse astrocytoma, anaplastic astrocytoma, GBM
- **Circumscribed** astrocytomas: Pilocytic astrocytoma, Pleomorphic xanthoastrocytoma (PXA)
- Most frequent intracranial neoplasms (>60% of all 1^{o} brain tumours)
- **Diffuse astrocytomas** share the following features:

  1) arise at any CNS site, preferentially in cerebral hemispheres,

  2) usu manifest clinically in adults,

  3) diffuse infiltration of adjacent brain and structures irrespective of grade,

  4) tendency for progression to anaplasia (anaplastic or GBM)
- Recurrent lesions tend to be of a higher grade than the previous resection.

Grading is based on areas showing highest anaplasia.

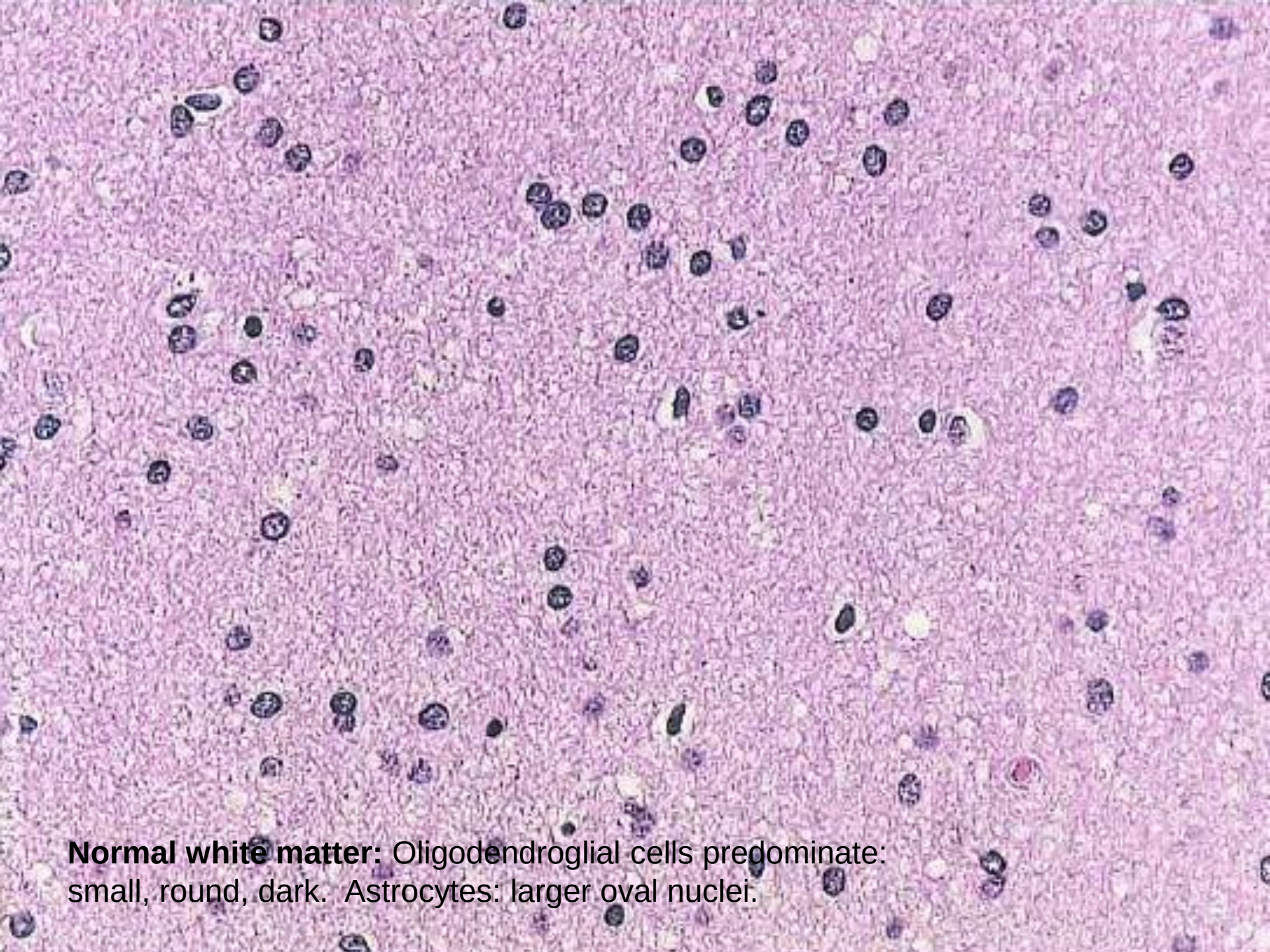
Diffuse Grade II: Cytologic atypia alone

Anaplastic Grade III: Anaplasia & mitoses

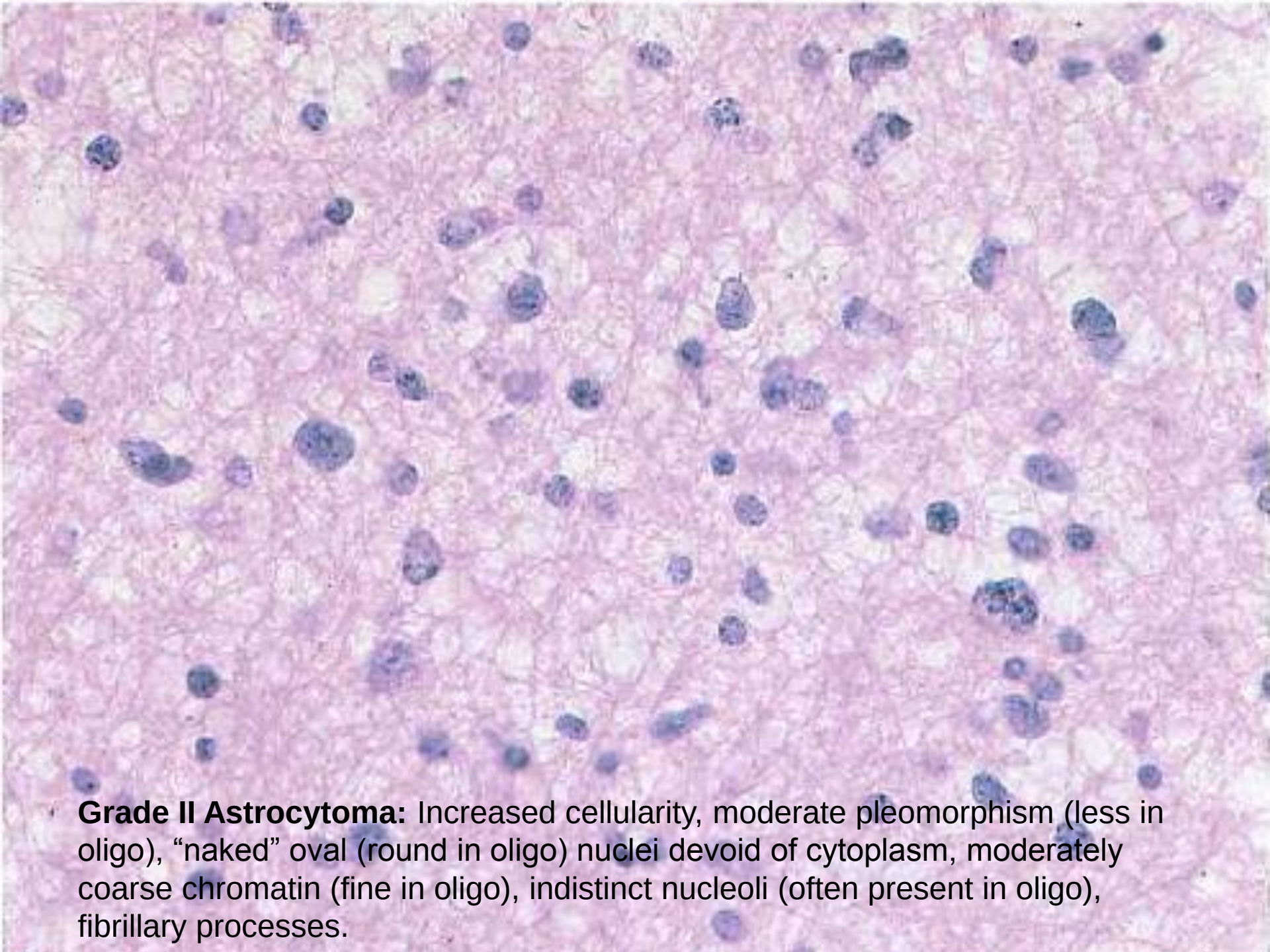
Glioblastoma Grade IV: Vascular endothelial proliferation and/or necrosis

Definitions:

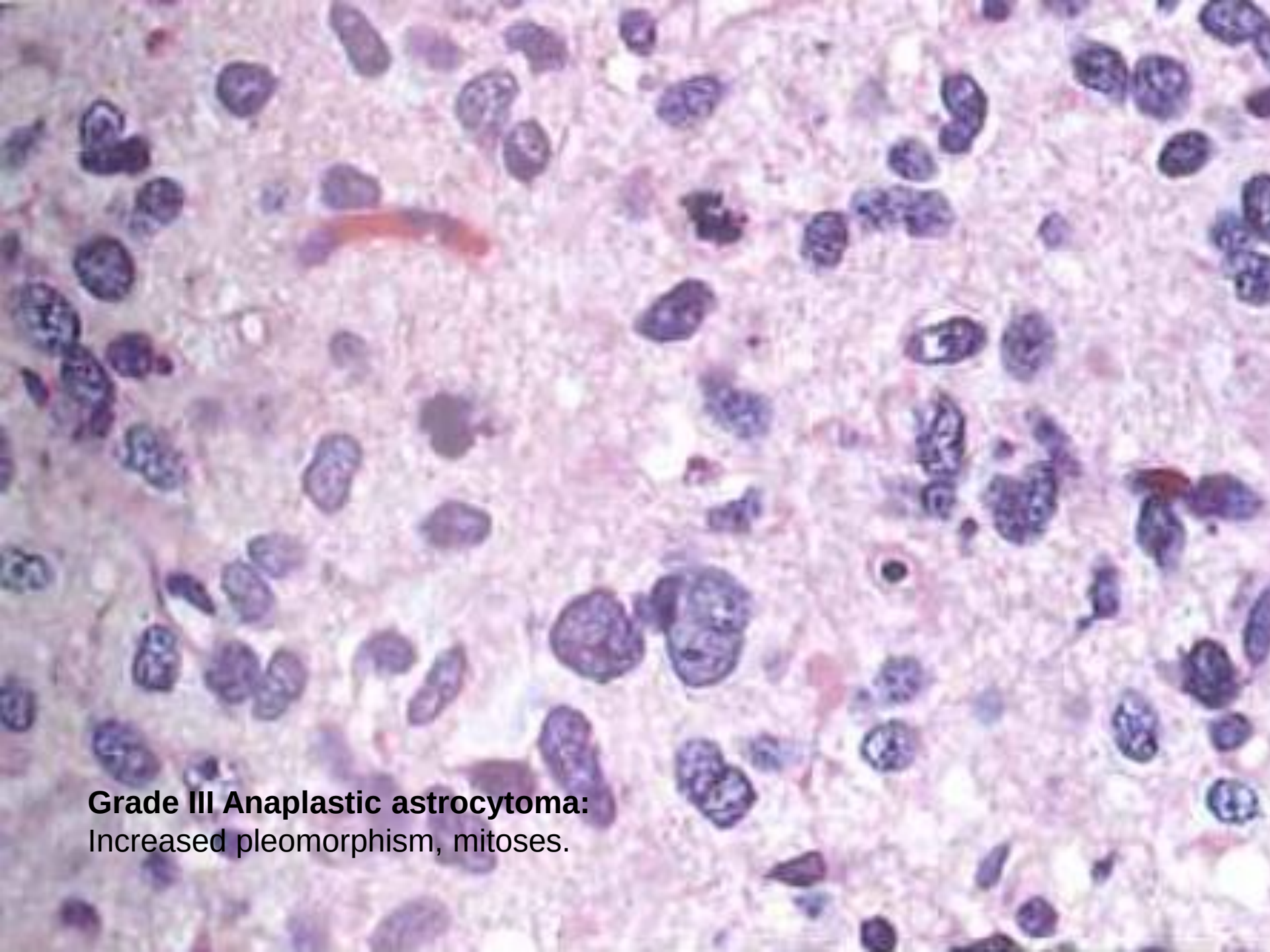
- **Atypia:** variation in nuclear shape or size with hyperchromasia
- **Mitoses:** must be unequivocal (but no number criteria). A single mitosis does not confer gr III behaviour (may do Ki67 immunostain: a proliferative marker)
- **Microvascular (vascular endothelial) proliferation:** Apparent multilayering of endothelium or glomeruloid vasculature
- **Necrosis:** May be of any type; perinecrotic palisading need not be present.



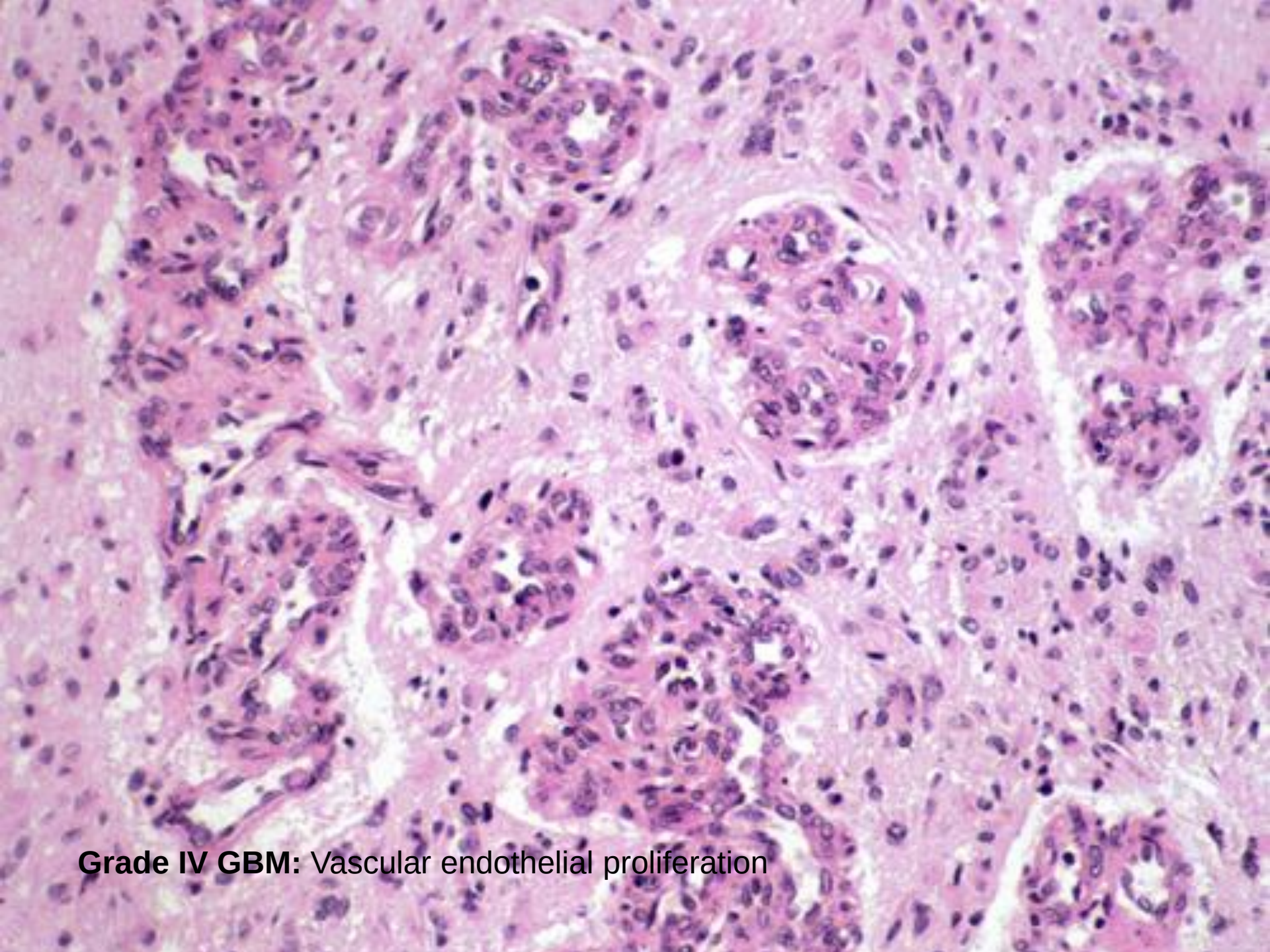
Normal white matter: Oligodendroglial cells predominate: small, round, dark. Astrocytes: larger oval nuclei.



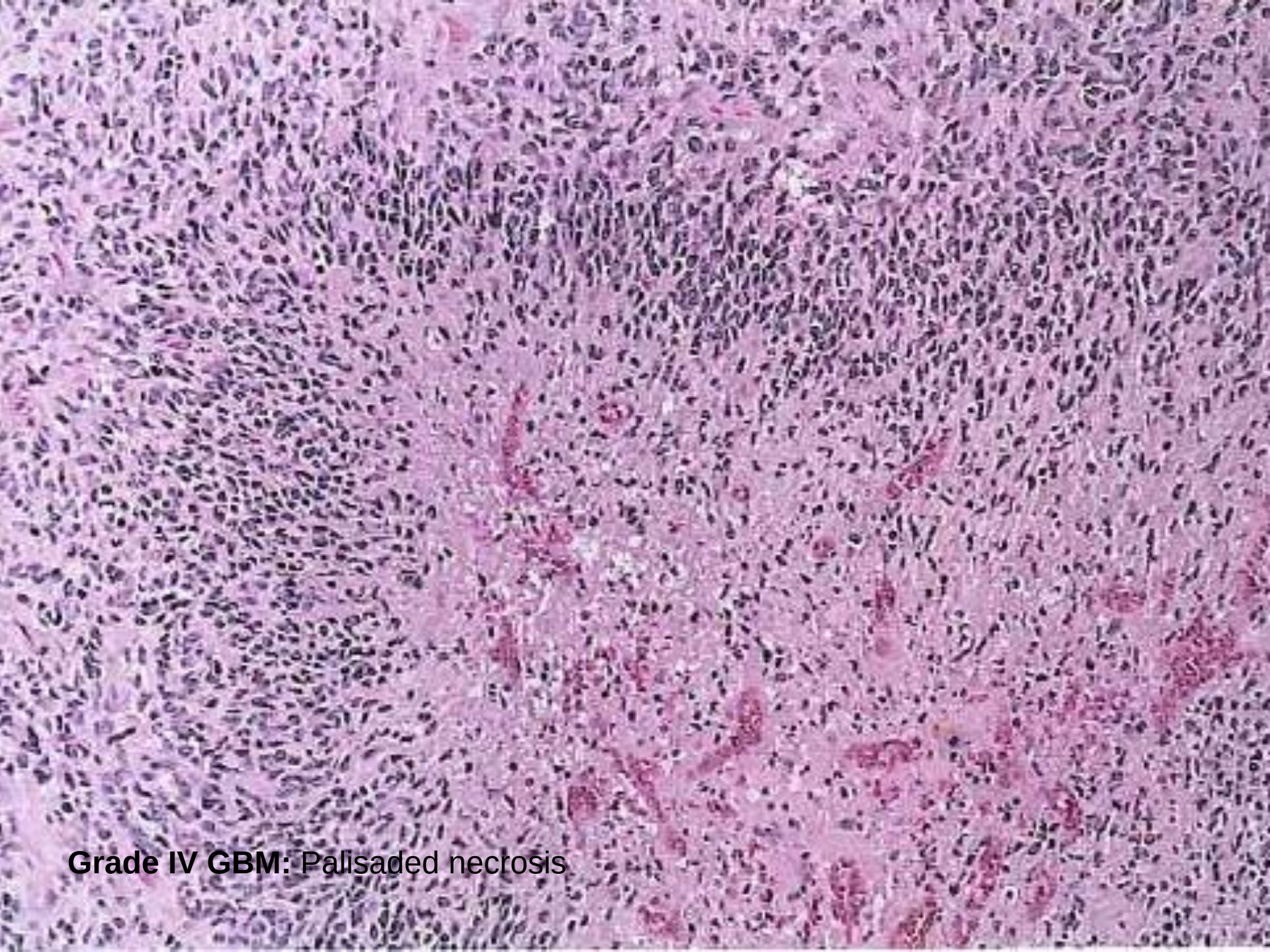
Grade II Astrocytoma: Increased cellularity, moderate pleomorphism (less in oligo), “naked” oval (round in oligo) nuclei devoid of cytoplasm, moderately coarse chromatin (fine in oligo), indistinct nucleoli (often present in oligo), fibrillary processes.



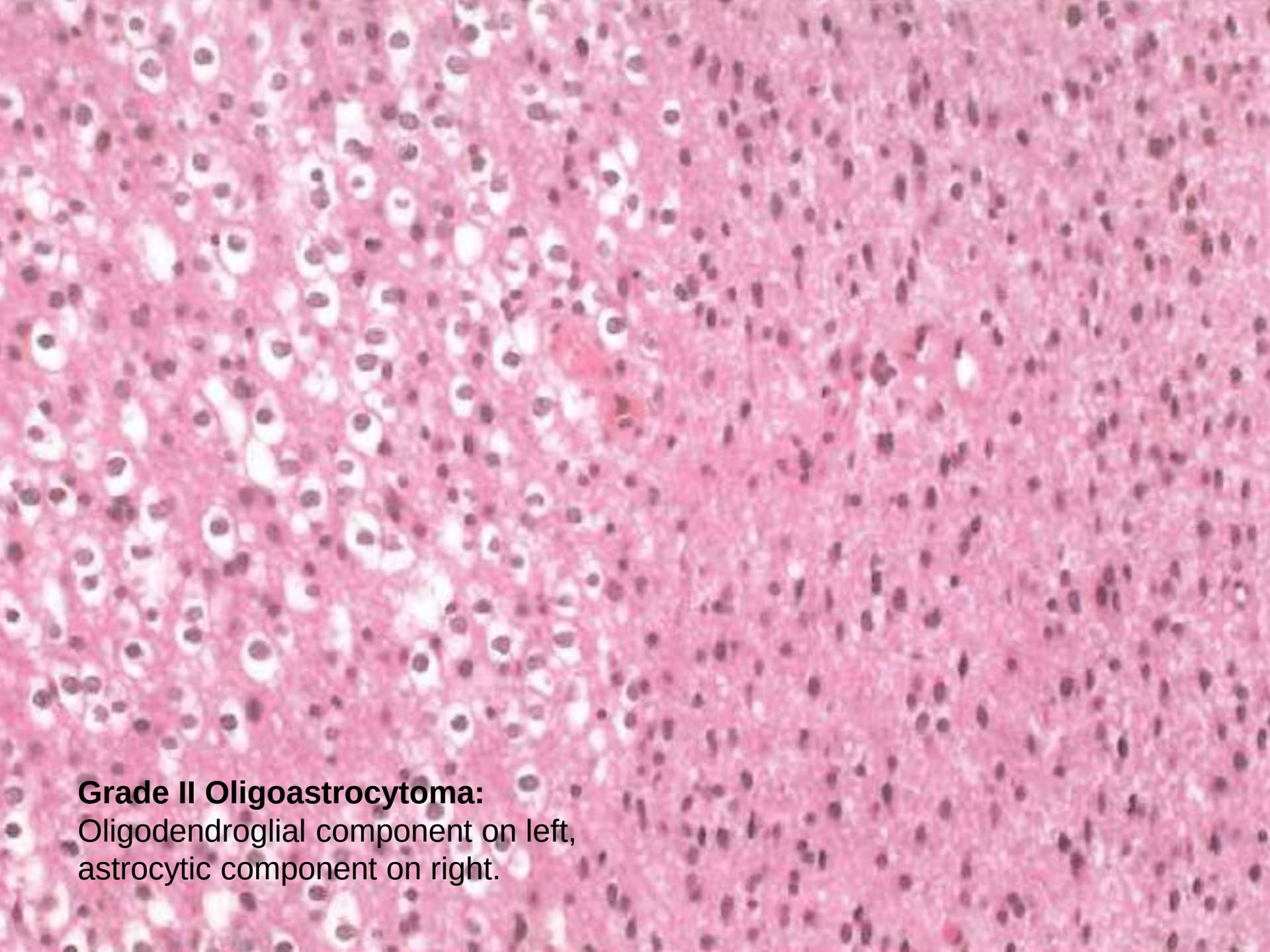
Grade III Anaplastic astrocytoma:
Increased pleomorphism, mitoses.



Grade IV GBM: Vascular endothelial proliferation



Grade IV GBM: Palisaded necrosis



Grade II Oligoastrocytoma:
Oligodendroglial component on left, astrocytic component on right.

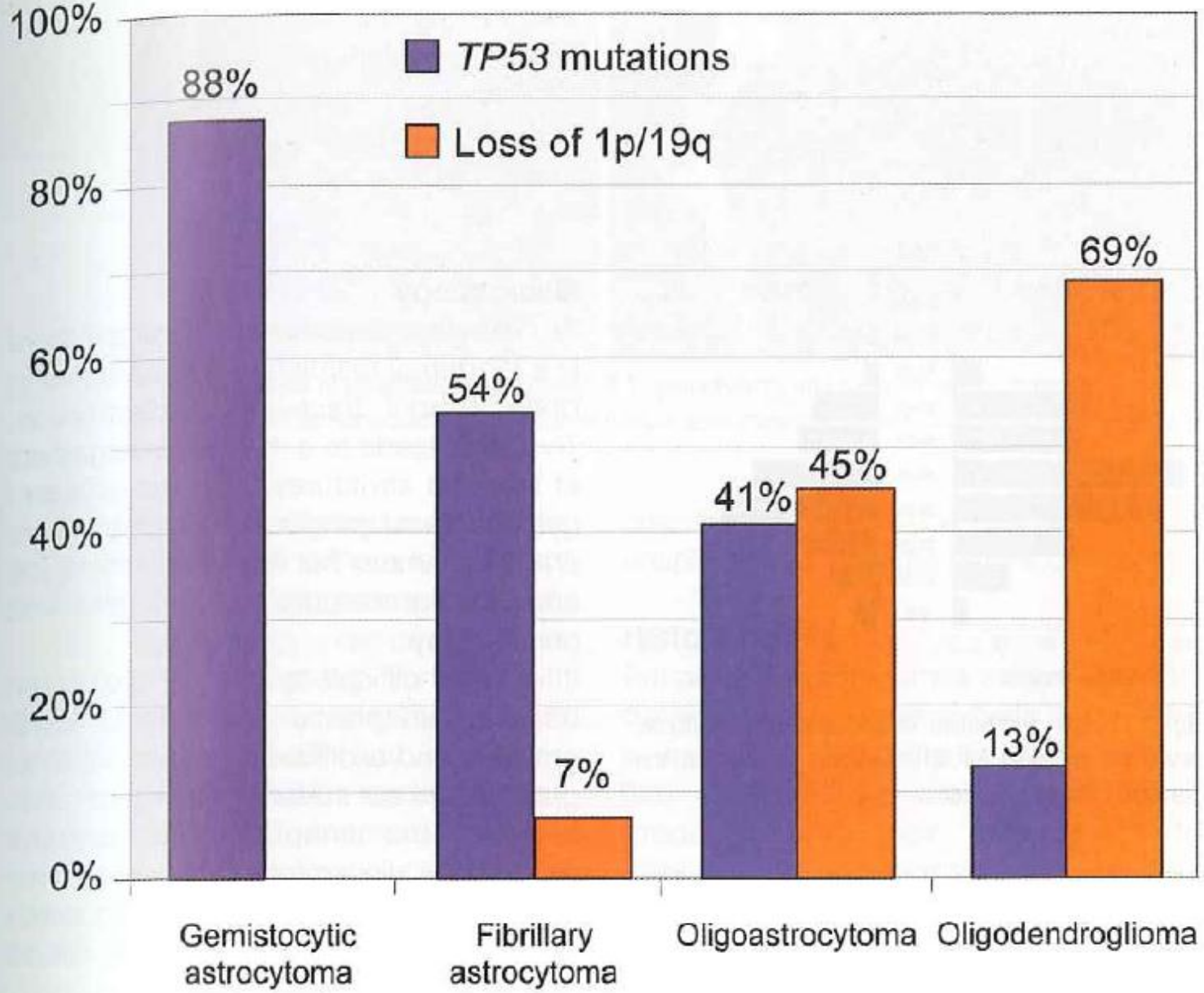


Fig. 1.21 *TP53* mutations are the genetic hallmark of low-grade astrocytomas in particular gemistocytic astrocytomas, whereas loss of 1p and 19q are frequent in oligodendrogliomas. Oligoastrocytomas commonly show either *TP53* mutations or loss of 1p/19q, with these changes being largely mutually exclusive. Modified from Okamoto *et al.* {1634}.

Timing and frequency of genetic alterations in the evolution of glioblastoma

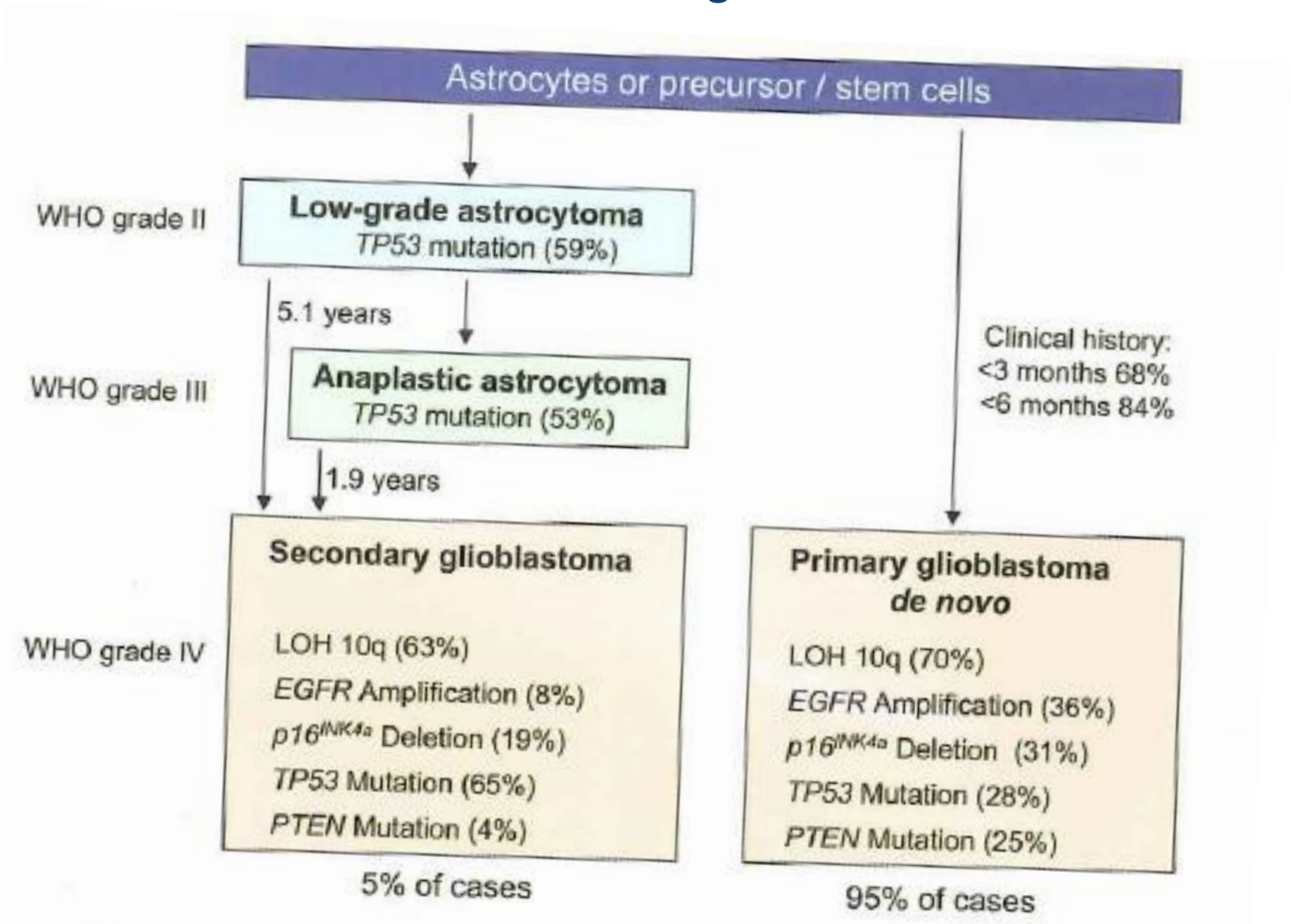


Fig. 1.36 Timing and frequency of genetic alterations in the evolution of glioblastoma. Note that LOH 10q is frequent in both primary and secondary glioblastomas, and *TP53* mutations are early and frequent genetic alterations in the pathway leading to secondary glioblastoma. Modified from Ohgaki *et al.* {1620}.

Practical molecular classification of diffuse gliomas

- There are currently only 2 clinically relevant (diagnostic, prognostic and predictive) diffuse glioma molecular markers
  - 1p/19q codeletion
  - Isocitrate Dehydrogenase (IDH) mutation

1p19q co-deletion

- 5-10% of all primary brain tumours are oligodendrogliomas
- FISH studies
- 1p19q found in
  - LG oligodendrogliomas: Nearly 85%
  - Anaplastic oligodendrogliomas: 65%
  - GBM with oligo component: 5-15%
- Probably an early event in tumorigenesis.
- High inter-observer variation in diagnosing an oligodendroglioma component.

- Presence of 1p19q co-deletion:

a.Diagnostic role: To refine classification of gliomas and support an oligodendroglioma component.

b.Prognostic (longer survival) in anaplastic and probably also for LG gliomas. This is inconsistent for GBM with oligo.

NB: Prognostic significance of 1p del without 19q del and vice versa is not definitely understood.

c.Predictive: Chemosensitive

- Thus co-deletion is a favourable genetic signature, correlates with oligodendroglioma morphology (it is the molecular definition of oligo) and impacts treatment regimen.

IDH1/2 mutation

- Originally discovered in 2008
- IDH1 mutation is most common (95%). IDH1(R132H) immunohistochemistry available
- IDH1/2 mutation found in
  - LG (WHO gr II) gliomas: 70-80%
  - Anaplastic (gr III) glioma: 65-70%
  - Including all 1p19q codeleted oligos
  - Secondary GBM (gr IV): >80%

  BUT only
  - Primary GBM (gr IV): <10%
- Probably an early tumour event (like 1p19q co-del and p53).

- IDH1Often occurs with p53 mutation in astrocytoma
- P53 and 1p19q del are generally mutually exclusive

- Presence of IDH1/2 mutation:

a.Diagnostic role: Nil.

NB: Not in pilocytic astrocytoma Gr I

b.Prognostic: Better survival than gliomas with wild-type IDH.

c.Predictive: Not currently predictive for outcome to a specific therapy

- Thus mutation is a favourable genetic signature which is used to stratify grade II-III gliomas

MGMT promoter methylation

- A methylated (thus silenced) MGMT promoter, encoding a DNA-repair enzyme, is present in many cancers including
  - LG (WHO gr II) gliomas: up to 93%
  - Anaplastic (gr III) glioma: 50-80%
  - Secondary GBM (gr IV): 70%
  - Primary GBM (gr IV): 40%
- Not routinely tested. Antibodies are available for detection of MGMT protein, but interpretation is challenging.

- MGMT promoter methylation:

a.Diagnostic role: Nil.

b.Prognostic:

c.Predictive: Partially predictive of enhanced response to DNA alkylating chemotherapeutics (temozolomide) and benefit from radiotherapy. However standard of care of GBM is radiotherapy + temozolomide regardless of MGMT status.

EGFR mutation

- Typically in GBM (45%, especially small cell GBM 70%) and anaplastic astrocytoma (10%), rather than oligodendroglioma.

- MGMT promoter methylation:

a.Diagnostic role: In GBM and AA, but not oligo (eg anaplastic oligo).

b.Prognostic: Inconclusive

c.Predictive: EGFR tyrosine kinase inhibitors (TKI) in clinical trial settings.

CONCLUSION

- **CNS tumour classification**
  - Cell of origin
  - Tumour grading
  - Molecular
- **Pre/Intraoperative differential diagnosis**
  - Age (adult vs paediatric),
  - Tumour site
  - Radiologic characteristics (circumscribed vs diffuse, enhancing vs non-enhancing, pattern of enhancement)
- **Gliomas**
  - GBM, astrocytomas and oligodendrogliomas are the commonest primary brain neoplasms
- **Molecular classification of diffuse gliomas** (gr II-III) with 1p19q codeletion and IDH1 mutation status have become routine practice