

DIFFERENTIATION BETWEEN REACTIVE GLIOSIS AND DIFFUSE ASTROCYTOMA USING IMMUNOHISTOCHEMISTRY WITH AN ANTIBODY SPECIFIC FOR COMMON MUTANT FORM OF IDH1

Thesis submitted in partial fulfillment of MD. Degree in Pathology

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List Of Abbreviations

AA: Anaplastic astrocytoma.

AAF: Atypical or anaplastic features.

AD: Alzheimer's Disease.

AIDS: Acquired immunodeficiency syndrome.

AFP: alpha feto protein.

ALS: Amyotrophic Lateral Sclerosis

ARF: Alternative reading frame.

CDKs: Cyclin dependent kinases.

CEA: carcinoembryonic antigen

CNS: Central nervous system.

CNTF: ciliary neurotrophic factor

CSF: cerebrospinal fluid

DA: Diffuse astrocytoma.

EMA: Epithelial Membrane Antigen.

Fbx4: F box protein 4.

FGF2: fibroblast growth factor 2 .

G: Grade

GFAP: Glial fibrillary acidic protein.

GDNF: glial cell-derived neurotrophic factor .

GM: Glioblastoma multiforme.

H&E: Hematoxylin and eosin.

HCG: human chorionic gonadotrophin.

INK4a: Inhibitor of kinase 4a.

IDH: Isocitrate Dehydrogenase enzymes.

IL-6: cytokinesinterleukin 6 .

IFN- γ :interferon- γ .

LI: Labeling index.

LIF: leukemia inhibitory factor.

MIP: macrophage inflammatory protein-1.

NADP: Nicotinamide Adenine Dinucleotide Phosphate .

NAD : Nicotinamide Adenine Dinucleotide.

NF1: Neurofibromatosis 1.

NF2: Neurofibromatosis 2.

PA: Pilocytic astrocytoma.

PAS: Periodic acid Schiff.

PLAP: placental alkaline phosphates.

PNET: primitive neuroectodermal tumour.

PCNSL: Primary central nervous system lymphoma

PTAH: phospho-tungestic acid hematoxylin.

RB: Retinoblastoma.

TGF B: Transforming growth factor B.

TSC1: Tuberous sclerosis1.

TGF: Transforming Growth Factor.

TNF: Tumor Necrotizing Factor.

VEGF: Vascular endothelial growth factor.

WHO: World Health Organization.

Yrs: Years.

Introduction

Gliosis (Astrocytosis) is a process leading to scars in the central nervous system that involves the production of a dense fibrous network of neuroglia (supporting cells) in areas of damage.

Gliosis is a prominent feature of many diseases of the central nervous system including multiple sclerosis and stroke. After a stroke, neurons die and disappear with replacement by gliosis.

Astrocytoma (Glioma) is the general term applied to a diversity of glial tumors spanning from WHO grade I to grade IV , according to cellular features in each grade.

Astrocytic nuclear atypia is difficult to define and requires experience and a keen awareness of the clinical circumstances for which the tissue is being examined.

Nuclear atypia may be difficult to be recognized when compared with reactive gliosis. Astrocytic nuclear atypia is characterized by a comparatively larger nuclear size and increased nucleocytoplasmic ratio, angular nuclear profile while in gliosis smoother round uniform contour of the nuclei are observed.

Reactive gliosis may show mitotic activity of reactive astrocytes, a characteristic feature of demyelinating disease.

Perhaps one of the most striking example of abnormal cellular morphologies among reactive astrocytes may be seen by surgical neuropathologists in progressive leukoencephalopathy which show large cells with bizarre shaped nuclei.

Another form of reactive gliosis seen in Alzheimer disease showing metabolic astrocytes.

This is not usually a primary diagnostic issue in surgical neuropathology, however shouldn't be confused with other types of infiltrating neoplastic cells.

In small biopsies, one of the most important points in diagnostic neuropathology relates the differentiation between reactive gliosis and diffuse glioma.

This challenging differential diagnosis arises from two situations:

1-low cellularity edges of infiltrating astrocytoma and mild from a nearby reactive conditions.

2-florid astrocytosis, near a vascular malformation, and cellular astrocytoma.

Recently, IDH1 mutation utilizing PCR-based assay are found in astrocytoma but not in reactive gliosis (Horbinski C, Kofler J, Kelly LM et al 2009).

To date, detection of IDH1 mutations in low grade astrocytoma (WHO grade II) ranges from 59 – 88 % (Yan H, Parson DW, Jin G et al 2009).

Therefore, the use of immunohistochemistry with an antibody specific for the common mutant forms of IDH1 is a powerful and easy to practical neuropathological diagnosis.

Aim of the work:

The aim of this work is to evaluate the role of immunohistochemistry with an antibody specific for the common mutant form of IDH1 in differentiation between reactive gliosis and diffuse astrocytoma.

Anatomy and histology of Central Nervous System

Confined within the cranium and vertebral canal, the CNS is sheathed by connective tissue membranes that include a densely collagenous outer covering termed the pachymeninx or, dura matter and delicate inner investments known as leptomeninges or pia-arachnoid. Under normal circumstances, these are closely opposed and loosely joined by a layer of dural border cells that are easily disrupted to yield a sub-dural space that is, in fact, only a potential space compartment.

A sagittal dural fold known to as the falx cerebri lies between the cerebral hemispheres, a second such fold – the tentorium cerebelli- separating the superior cerebellar surfaces from the overlying temporal lobes of the cerebrum. Enclosed within the cranial dura, in addition to meningeal artery branches, are venous sinuses that serve both to drain the cerebral veins and to carry away the cerebrospinal fluid (CSF) transported from the subarachnoid space by arachnoid villi that projects into these conduits. Termed pacchionian granulations as they achieve grossly visible proportions with normal aging, these villi are draped by specialized arachnoidal cells. **(Fuller and Burger, 1997)**

Whereas the dura adhere tightly to the endosteal surfaces of the skull, at spinal levels it is attached only anteriorly to the vertebral bodies and is surrounded on its lateral and posterior aspects by a true compartment-the epidural space- which contains segments of the spinal nerve roots, blood vessels, and a very modest amount of adipose tissue. **(Fuller and Burger, 1997)**

The brain parceled into supratentorial versus infratentorial components, the former situated above and the later below the tentorium cerebelli. The cerebellum and most of the brain stem, including pons and medulla in their entirety, are infratentorial structures that may be collectively designated the

posterior fossa contents. The supratentorial CNS formed of cerebrum (subdivided into frontal, parietal, temporal and occipital lobes) and deep nuclei of the basal ganglia, thalamus and hypothalamus.

Within the CNS, connective tissue is few and essentially restricted to the adventitia of blood vessels. There are no resident lymphoid elements. The parenchyma of the brain and spinal cord is composed principally of the bodies and cytoplasmic processes of neuroepithelial cell types, including neurons and various classes of glia. Subsumed under the latter designation are supporting astrocytes, myelinating oligodendrocytes, and ependymal cells that line the ventricular surfaces. **(Fuller and Burger, 1997)**

Epidemiology of neurogenic tumours

Malignant primary brain tumours represent 2% of all cancers and 2.5% of cancer death annually in the United State. The male to female ratio is 3:2. The incidence peaks at 5 to 10 years of age and again at 50 to 55 years of age **(De Angiles et al., 2002)**

In Egypt neurogenic tumours accounted for 3.1% of all cancers (1999 – 2001). A large majority 85.2% of cancers were located in the brain in Egypt. **(Sadetzki et al., 2005)**

Recorded brain cancer incidence rates increased over the past several decades in most developed countries, particularly in the elderly, but this generally thought to be due to improved diagnosis more than to a real increase in incidence **(Legler et al., 1999)**. Incidence of glioma is positively related socioeconomic status **(Inskip et al., 2003)**. Cancer of the brain and other central nervous system is more common in males than females **(Parkin et al., 2002)**. A possible role of steroid hormones has been hypothesized, and a report noted reduced risk of glioma associated with early age of menarche and early age at first live birth **(Hatch et al., 2005)**. Several studies have indicated a reduced risk of astrocytoma among persons with history of allergies or certain infections, possibly indicating a role for immune factors **(Wiemels et al., 2004)**. Another report noted an inverse association between use of nonsteroidal anti-inflammatory drugs and glioblastoma **(Sivak-Sears et al., 2004)**.

World health organization classification for the tumours of the central nervous system 2007 (Louis, 2007)

• Index

- Tumors of Neuroepithelial tissue
- Tumors of cranial and paraspinal nerves
- Tumors of the Meninges
- Germ Cell Tumors
- Tumors of sellar region
- Metastatic Tumors

- Lymphomas and

Haemopoietic_Neoplasms

Tumors of Neuroepithelial Tissue

Astrocytic Tumors

1	9421/1(*)	Pilocytic Astrocytoma
	9425/3(**)	Pilomyxoid Astrocytoma
2	9384/1	Subependymal Giant Cell Astrocytoma
3	9424/3	Pleomorphic Xanthoastrocytoma
4	9400/3	Diffuse Astrocytoma
	9420/3	Fibrillary Astrocytoma
	9411/3	Gemistocytic Astrocytoma
	9410/3	Protoplasmatic Astrocytoma
5	9401/3	Anaplastic Astrocytoma
6	9440/3	Glioblastoma
	9441/3	Giant Cell Glioblastoma
	9442/3	Gliosarcoma

7 9381/3 Gliomatosis Cerebri

Oligodendroglial Tumors

8 9450/3 Oligodendroglioma

9 9451/3 Anaplastic Oligodendroglioma

Oligoastrocytic Tumors

10 9382/3 Oligoastrocytoma

11 9382/3 Anaplastic Oligoastrocytoma

Ependymal Tumors

12 9383/1 Subependymoma

13 9394/1 Myxopapillary Ependymoma

14 9391/3 Ependymoma

 9391/3 Cellular

 9393/3 Papillary

 9391/3 Clear Cell

 9391/3 Tanycytic

15 9392/3 Anaplastic Ependymoma

Choroid Plexus Tumors

- | | | |
|----|--------|------------------------------------|
| 16 | 9390/0 | Choroid Plexus Papilloma |
| 17 | 9390/1 | Atypical choroids Plexus Papilloma |
| 18 | 9390/3 | Choroid Plexus Carcinoma |

Other Neuroepithelial Tumors

- | | | |
|----|--------|---|
| 19 | 9430/3 | Astroblastoma |
| 20 | 9444/1 | Chordoid Glioma of the Third
Ventricle |
| 21 | 9431/1 | Angiocentric Glioma |

Neuronal and Mixed Neuronal-Glial Tumors

- | | | |
|----|--------|--|
| 22 | 9493/0 | Dysplastic Gangliocytoma of
Cerebellum (Lhermitte-Duclos)
Desmoplastic Infantile |
| 23 | 9412/1 | Astrocytoma / Ganglioglioma |
| 24 | 9413/0 | DysembryoplasticNeuroepithelial
Tumor |
| 25 | 9492/0 | Gangliocytoma |
| 26 | 9505/1 | Ganglioglioma |
| 27 | 9505/3 | Anaplastic Ganglioglioma |

28	9506/1	Central Neurocytoma
29	9506/1	Extraventricular Neurocytoma
30	9506/1	Cerebellar Liponeurocytoma
31	9509/1	Papillary Glioneuronal Tumor
32	9509/1	Rosette-forming Glioneuronal Tumor of the Fourth Ventricle
33	8680/1	Paranglioma

Tumors of the Pineal Region

34	9361/1	Pineocytoma
35	9362/3	Pineal Parenchymal Tumor of Intermediate Differentiation
36	9362/3	Pineoblastoma
37	9395/3	Papillary Tumor of the Pineal Region

Embryonal Tumors

38	9470/3	Medulloblastoma
	9471/3	Desmoplastic/Nodular Medulloblastoma
	9471/3	Medulloblastoma with Extensive