IMMUNOHISTOCHEMISTRY OF BRAIN TUMOURS

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Role of a pathologist remains a time honored one, namely, Accurate Diagnosis and assessment of prognosis.

IHC has made diagnosis more accurate
IHC and Molecular Markers have revolutionized a Pathologist role in Patient care by providing the following pivotal information.

1. Predicting the response to treatment
2. Selection of treatment based on molecular targets
Applications of IHC

- Diagnostic Immunohistochemistry
- Understanding of Molecular biology of Brain tumours
- Assessment of Proliferation of Brain tumours and their role in therapy
- Targeted therapies and biological modifiers for Brain tumours
Overview

• Role of IHC in diagnosis.
• Gliosis versus Glioma
• Proliferation studies in Glioma
• Targeted therapies in Brain tumour
Diagnostic Immunohistochemistry

• Intermediate Filaments - GFAP, Neurofilaments, Pancytokeratin, Vimentin
• S100
• Neurone specific enolase
• Synaptophysin
• Epithelial Membrane antigen,
• Beta Tubulin, Collagen, Factor VIII related antigen, desmin, Chromogranin
Neuroepithelial tumours

- Astrocytic Tumours
- Oligodendroglial tumours
- Ependymal tumours
- Mixed Neuronal/Glial
- Pinealoblastoma
- Pinealicytoma
- Embryonal group

GFAP

S100, Vimentin, Anti leu7, GFAP

GFAP, S100, EMA, Vimentin, NF

GFAP, Collagen, Desmin, Tubulin,

NSE, Synaptophysin, Chromogranin

Neurofilament, Nestin, Synaptophysin, GFAP, Vimentin
Tumours of Peripheral Nervous System and other tumours

- S100 in Peripheral nerve tumours
- Vimentin, Desmin, FacVIII R an, EMA, in Meningiomas
- Factor VIII related antigens for vascular tumours
- Lymphoma / Leukaemias - essential CD panel
- Melanoma - HMB45, S100
49 year old female with a large right parietal lobe tumour extending to the opposite left parietal lobe.

CT diagnosis - GBM
A diagnosis of metastatic deposits from a carcinoma was not accepted by the clinician as the CT picture was classical of GBM.
GFAP and Pan cytokeratin stain of the same case
52 Year old patient with Werthaim’s Hysterectomy done 2 years back for Endometrial Carcinoma came with a large Cerebellar tumour adjacent to the fourth ventricle.
GFAP positivity
S100 positive in tumour cells, GFAP was positive and Pancytokeratin was negative. Diagnosis - Ependymoma
34-year-old lady presenting with altered behavior and severe frontal headache of 3 months duration. CT scan showed a frontal partly solid, partly necrotic tumor. ST biopsy sent for quick diagnosis.
The sections revealed a lymphoma. Quick IHC carried out showed CD20, CD45 (LCA) positivity with increased Ki67 activity.
75 year old man with multiple small lesions scattered in frontal and Parietal area. Steriotactic biopsy.
LCA and CD20 stain in the same case
PNET tumour with NSE stain
Small cell variant of GBM
Gliosis versus Glioma
Fibrillary Astrocytoma
Protoplasmic Astrocytoma
Protoplasmic Astrocytoma
Steriotactic Biopsy from R-parietal SOL, 58 yrs old, Male with hemiplegia of 2 months duration
Is It a low grade glioma?

Is It the infiltrating edge of a glioma?

Or

Is the diagnosis “reactive gliosis”
Criteria's used to differentiate a Gliosis from a Glioma

- Histology
- IHC studies - GFAP, Proliferation markers, P53, EGFR
- Genetic studies - LOH of at least one allele on Chromosome 10, PTEN, P53, EGFR amplification gene, other Tumour suppressor gene
Biopsy from 72 year old male, Hemiplegic, SOL parieto-temporal region
38 year old male with GBM diagnosed at the age of 36 and followed up with Radiotherapy, came for a follow up. CT/ MRI diagnosis - Recurrent Neoplasia
Radiation induced gliosis
GFAP - IHC
GFAP positive and Proliferation marker negative
P53 IHC in GBM
EGFR in GBM - IHC
DEFINITION

Reactive Gliosis is the proliferation of Astrocytes as a hypertrophic and hyperplastic response to injury within the CNS resulting in the formation of scars.
Conditions associated with Gliosis

• Around Tumours
• Hypoxia / Traumatic Injury
• Post-Radiation effect
• Infections like Abscess, Tuberculoma, fungal infections
• Viral infections involving CNS
• Epilepsy
• Alzheimer’s disease
• Parkinsonism
• Stroke
• Multiple Sclerosis
Pathogenesis of Gliosis

- How do these agents induce gliosis? NOT KNOWN
- What type of Astrocyte reacts to injury? NOT KNOWN
  - ? Pre existing Astrocyte
  - ? Neuro-Glial precursors
• Histological criteria's are insufficient in most cases, to distinguish gliosis from low grade glioma.
• Use of IHC with proliferation marker has about 95 to 98% accuracy. P53 has 65% accuracy and EGFR about 30%.
• Genetic study with Loss of hetrozygosity of at least one allele is considered a definite indicator of Glioma. Unfortunately it is not available as a routine diagnostic method.
• Proliferation marker studies in Glioma and their implications in treatment of Gliomas
Cell Cycle

- G1 Phase
- S Phase
- G2 Phase
- Mitosis
Genetic and Molecular markers of prognosis in Gliomas

- MDM2 gene over expression induces growth by escaping TP53 regulation.
- The tumor suppressor genes - TP53, PTEN, MMAC etc - mutation induces tumor upgrading.
- P16, P15, P21, P27, RB genes which control at the G1 phase of cell cycle.
- Growth Factor Receptors like, EGFR, PDGF-control growth. If mutant, then tumor resistance to chemotherapy increases.
Properties of proliferation antibodies

- **Cyclin** - positive in G1, G2, S and M phase
- **Ki67** - Positive in G2, M and S phase only on frozen sections
- **MiB-1** - Variant of Ki67 - Positive in G2, M and S Phase on paraffin embedded Sections
<table>
<thead>
<tr>
<th>WHO Grade</th>
<th>WHO Designation</th>
<th>No of cases</th>
<th>Age Range</th>
<th>Sex Male</th>
<th>Sex Female</th>
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<tbody>
<tr>
<td>I</td>
<td>Pilocytic Astrocytoma</td>
<td>5</td>
<td>4 – 22</td>
<td>2</td>
<td>3</td>
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<tr>
<td>II</td>
<td>Diffuse Astrocytoma</td>
<td>36</td>
<td>14 – 50</td>
<td>25</td>
<td>11</td>
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<tr>
<td></td>
<td>a. Fibrillary</td>
<td>10</td>
<td></td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>b. Protoplasmic</td>
<td>17</td>
<td></td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>c. Gemistocytic</td>
<td>2</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>d. Oligodendroglioma</td>
<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>e. Oligo Asrocytoma</td>
<td>6</td>
<td></td>
<td>6</td>
<td>0</td>
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<tr>
<td>III</td>
<td>Anaplastic Astrocytoma</td>
<td>16</td>
<td>28 – 65</td>
<td>14</td>
<td>5</td>
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<tr>
<td></td>
<td>Anaplastic</td>
<td>3</td>
<td></td>
<td>3</td>
<td>0</td>
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<tr>
<td></td>
<td>Oligodendroglioma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Glioblastoma Multiforme</td>
<td>15</td>
<td>10 – 61</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>75</td>
<td></td>
<td></td>
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Table 2

<table>
<thead>
<tr>
<th>Tumor Grade (WHO)</th>
<th>No.</th>
<th>Mitosis / 10 HPF</th>
<th>Mib-1 ≤10/10 HPF</th>
<th>Mib-1 &gt;10/10 HPF</th>
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<tr>
<td></td>
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<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>0.5 – 1</td>
<td>1 – 9</td>
<td>5</td>
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<tr>
<td>II</td>
<td>36</td>
<td>0.5 – 5</td>
<td>1 – 9</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>19</td>
<td>5 – 10</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>2 – 20</td>
<td>--</td>
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</tbody>
</table>
Protoplasmic Astrocytoma Grade 2 progressing to Grade 3
MiB-1 Counts in each case of Grade 2 Diffuse Gliomas
### Table 3

Comparison of outcome of disease in cases with low and high MiB-1 counts / 10 HPF.

<table>
<thead>
<tr>
<th>Cases</th>
<th>No.</th>
<th>Recurrence of disease</th>
<th>Follow up</th>
<th>Lost to follow up</th>
<th>Expired</th>
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<tbody>
<tr>
<td>Low MiB-1 (&lt;10/10 HPF)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>5</td>
<td>Nil</td>
<td>(2 – 7 yrs)</td>
<td>--</td>
<td>Nil</td>
</tr>
<tr>
<td>Grade II</td>
<td>20</td>
<td>3 (all grade II)</td>
<td>(2 – 8 yrs)</td>
<td>3</td>
<td>Nil</td>
</tr>
<tr>
<td>High MiB-1 (&gt; 10/10 HPF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>16</td>
<td>3 (all grade III)</td>
<td>(1 – 5 yrs)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Grade III</td>
<td>19</td>
<td>--</td>
<td>--</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Grade IV</td>
<td>15</td>
<td>--</td>
<td>2 cases (1, 1 ½ yrs)</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>
MiB-1 Study is simple, cost effective and does not need sophisticated Molecular biology labs.

The G1 Phase of cell cycle does not stain for MiB-1, hence low false positive counts.

Counting of stained cells in 10 consecutive fields is simple and counts give reliable information on the biological behavior of the Gliomas.

MiB-1 counts do not throw any further light on the prognosis of grade 3 and grade 4(GBM) Gliomas.
• MiB-1 are most useful in Grade 2 Diffuse Gliomas.
• MiB-1 counts below 10/10HPF especially in Grade 2 Diffuse Gliomas carry better prognosis.
• The sub group of grade 2 Gliomas which are strongly positive for MiB-1 (Counts >10/10hpf) appear to be potentially aggressive.
• The clinical outcome of the patients in this subgroup of aggressive Grade 2 Gliomas resembles the grade 3 tumors.
• Post surgical evaluation, aggressive therapy and close follow up may be essential in these cases for better care of these patients
• Targeted therapies and biological modifiers for Brain tumours
• Growth receptors:
  1. EGFR family ie EGFR 1; EGFR 2 or her2, c erb 2; EGFR 3;EGFR4.
  2. Platelet derived growth factor
  3. Vascular endothelial growth factor 1, 2, and 3 (VEGFR)
  4. Insulin like growth factor
  5. Fibroblast growth factor receptor
  6. Nerve growth factor
• Hormone receptor studies like ER receptors.
• Adhesion receptors like CD99, E-Cadherin, Neural cell adhesion molecule N-CAM
• Apoptosis assessment with Bcl-2 gene studies, CD95
• Retinal S antigen studies
• P53 mutation, RAS gene, MDM2, RB gene, other gene mutation studies
<table>
<thead>
<tr>
<th>Receptors</th>
<th>Name of Monoclonal antibody</th>
<th>Market name</th>
<th>Route</th>
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<tbody>
<tr>
<td>VEGFR</td>
<td>Bevacizu mab</td>
<td>Avastin</td>
<td>IV</td>
</tr>
<tr>
<td>EGFR</td>
<td>Gefitinib</td>
<td>Iressa</td>
<td>oral</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Tarseva</td>
<td>oral</td>
<td></td>
</tr>
<tr>
<td>C-kit</td>
<td>Imatinib</td>
<td>Gleevec</td>
<td>oral</td>
</tr>
<tr>
<td>PDGFR</td>
<td>Imatinib</td>
<td>Gleevec</td>
<td>oral</td>
</tr>
<tr>
<td>EGFR 2</td>
<td>Trastuzumab</td>
<td>Herceptin</td>
<td>IV</td>
</tr>
</tbody>
</table>
Steriotactic biopsy in a 56 year old man from Tempero-parietal lobe - GBM
P53
VEGFR stain
Glioma grade 3 progressing to grade 4
EGFR in GBM - IHC
Glioma grade 2 progressing to grade 3
Same case Estrogen Receptors
Same case VEGFR
Medulloblastoma in a 43 year old women
P53
Conclusion

• IHC is useful in diagnosis, understanding the biological properties, predicting the response to treatment and selection of targeted therapies of brain tumours.

• Pathologists play a role in patient care and management.
Thank you