Primary and secondary glioblastoma and tumor boundaries of glioblastoma: a morphometrical and immunohistochemical study.

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Dr. Giovani Vandewalle

Promotoren:
Prof. Dr. J.M. Brucher
Prof. Dr. J. Creemers
Prof. Dr. E. Beuls

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Hans Joachim Scherer (14.05.1906-16.04.1945)

"A tumor is not a heap of cells independent from the tissue in which it grows. It can only be understood when studied in all its parts, especially in its growth zone and in its natural relationship with the tissue from which it originates" [198].
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ABBREVIATIONS

A  astrocytoma
AA anaplastic astrocytoma
AFIP Armed Forces Institute of Pathology
CD cell density
CNS central nervous system
CT computed tomography
cm centimeter
df degrees of freedom
EC endothelial cell
FG fast growing
GFAP glial fibrillary acidic protein
GB glioblastoma
GMVP glomeruloid multilayered vascular proliferation
HPF high power field
ITC isolated tumor cells
LG lower-grade
LPF low power field
MIB-1 Institute of molecular immunology Borstel
µm micrometer
mm millimeter
mmc millimeter corrected for tissue shrinkage
MRI magnetic resonance imaging
MT Masson trichrome stain
MVP multilayered vascular proliferation
N number of nuclei
n number of HPF
PCI cell proliferation index
PGB primary glioblastoma
PGBP periglioblastomatous tissue
PM photomicrograph
PN MIB-1 immunopositive nuclei
PS pial surface
rs Spearman’s rank correlation coefficient
SD standard deviation
SG slow growing
SGB secondary glioblastoma
TTP tumor tissue proper
VFG very fast growing
WHO World Health Organisation
ZI zone of infiltration
ZDI zone of dense infiltration
ZITC zone of isolated tumor cells
INTRODUCTION and AIMS OF THIS STUDY
Recently, molecular genetics have caused a renewed interest in Scherer's concept (1940) of primary and secondary glioblastoma (GB) [197]. It has become clear that at least two distinct genetic pathways lead to the glioblastoma phenotype. The first, de novo, pathway involves amplification of the epidermal growth factor receptor gene (primary GB), whereas the other, the progression pathway, is characterised by the sequential accumulation of a number of genetic alterations initiated by mutation in the p53 tumor suppressor gene (secondary GB) [20, 93, 129, 135, 233]. In the 50 years interval, the issue of primary and secondary GB was highly controversial, most authors regarding the GB as derived from a lower-grade astrocytic tumor by anaplasia [43, 124, 186]. At present, it is of general belief that there is no morphological distinction between primary and secondary GB [19, 130, 233, 239].

Another highly debated issue is that of the tumor boundaries in GBs. Whereas some authors consider the GB a localised disease largely inferred from its high tendency for local recurrence [1, 16, 50, 99, 142], others think of the GB as a more diffuse lesion [39, 90, 120]. This divergence of opinions can be related to the difficulty of identifying isolated infiltrating tumor cells in histological sections and to the indiscriminative use of the term GB in both primary and secondary GBs.

The aims of this study were therefore to investigate whether primary GBs can be distinguished morphologically from secondary GBs and to assess the extent of infiltration in GBs. To meet these goals, large surgical specimens of untreated GB were studied using immunohistochemical detection of the MIB-1/Ki-67 defined nuclear antigen and morphometry.
This study has been outlined as follows. Chapter I reviews the historical development of the glioblastoma terminology and the present knowledge of GB. Chapter II deals with the materials and methods employed. In chapter III the clinical, histopathological and morphometrical data of the clinical cases are presented and analysed. In chapter IV the obtained results will be discussed and related to literature findings.
CHAPTER I

SALIENT FEATURES OF THE GLIOBLASTOMA
I. Historical notes on glioblastoma terminology and definition.

"On ne connaît bien une science que si l'on connaît son passé"
(Auguste Comte)

Historically, at least three periods can be distinguished, each lasting approximately half a century.
The first was a pure descriptive period with close parallelism between discoveries in normal neuro-anatomy and tumor pathology. Although some knowledge of the macroscopical features of intrinsic neoplasms of the CNS had already been gained at the beginning of the 19th century [198, 256], Virchow (1846) was the first investigator to combine macro- and microscopical studies. His discovery of an interstitial substance in the CNS [230] which he coined "Nervenkitt" [231], was soon followed by the introduction of the name "glioma" [232] designating a group of often large, ill-defined tumors with a brain-like appearance. These tumors with slow clinical evolution, consist microscopically of glial cells and fibers. They were distinguished from the other main group of tumors, the "cerebral sarcomas", more globuliform and circumscribed tumors with a rapid, often apoplectic evolution. Microscopically, they are rich in cells and blood vessels, often showing fatty degeneration.
The identification by Deiters [63] and Jastrowitz [110] of the "fiberforming" or "spider" cells, which had led to the publication of the "spider cell glioma" by Simon [211] in 1874, inspired Golgi [85] in 1875 to state that for the histological diagnosis of glioma the presence of fiberproducing spider cells was essential, as was the presence of spindle or round cells for sarcomas. Glioma research at the end of the century was largely devoted to the demonstration of the glial nature of sarcomas [23,
95, 217, 219] and resulted in names as “gliosarcoma” [23] or “glioma
sarcomatodes” [219] meaning a tumor of glial origin morphologically
resembling a sarcoma.

The second “cytogenetic” period was greatly influenced by the
embryonic cell theory of Cohnheim [53], which postulated that tumors
originate from scattered embryonic cell nests, arrested at different stages of
the neurocytogenesis. Early exponents were Pick and Bielschowsky [175],
Ribbert [181], Strauss and Globus [218]. The latter three almost
simultaneously suggested in 1918 the term spongioblastoma to denote a
highly cellular neoplasm with varying cytological composition, depending
on the degree of maturation of the spongioblast from which the tumor arose.
The spongioblast had been described by His in 1904 [98]. In analogy to
the name “gliome polymorphe” of Roussy, Cornil and Lhermitte [185],
Bailey and Cushing suggested at the 15th Annual Meeting of the American
Neurological Association, Philadelphia 1924, to add the adjective
multiforme to the name spongioblastoma hereby emphasizing its
polymorphous structure and symptomatology. This change in name first
appeared in print in 1925 in an article of Globus and Strauss [84] entitled:
“Spongioblastoma multiforme. A primary malignant form of brain
neoplasm; its clinical and anatomical features.”
The second period culminated in the works of Bailey and Cushing [7] and
del Rio-Hortega [101, 102]. As opposed to the latter who “had little doubt
as to the general embryonic character of gliomas”, Bailey and Cushing had
merely adopted the cytogenetic principle as a working hypothesis to
classify gliomas [9] rather than as an oncologic dogma, as is often
erroneously thought [192]. They spoke of GB as a diffuse, invasive,
rapidly growing tumor of anaplastic polymorphous cells, essentially neuroglial, located typically in the cerebral hemispheres of adults, prone to necrosis, softening and hemorrhage. In order to avoid confusion with their unipolar spongioblastoma, but mainly because the component cells did not seem to resemble any embryonic cell very closely, but were rather anaplastic cells presumably derived from protoplasmic astrocytes of the cortex by dedifferentiation, they already proposed in their monograph of 1926 [7] in the form of a footnote to replace the name spongioblastoma by glioblastoma which seems to have been used for the first time by Mallory [150] in 1914 but indiscriminately from the term glioma. Arguments in support of this concept of dedifferentiation from preexisting cells were the finding of occasional mitoses in protoplasmic astrocytes and the observation that tumors, essentially composed of protoplasmic astrocytes, were found dedifferentiated to GB at a subsequent surgical intervention [9, 11]. One year later, in 1927, the GB appeared in Bailey’s simplified classification [8] under its actual name, but it lasted until the early 1930’s before it became widely accepted.

Meanwhile some early opposition had arisen against these cytogenetic concepts from authors such as Tooth [224], Roussy, Lhermitte, Cornil [185] and Cox [56], but it was Scherer [197] with his “technique of complete glioma examination” who first proved the existence of dedifferentiation of astrocytoma into GB. He stressed the importance of distinguishing these dedifferentiated astrocytomas from primary GBs because they differed substantially in their clinical and biological features. The concept of primary and secondary GB was born.
The third period was dominated by the concept of anaplasia, enunciated by von Hansemann [234] in 1898 and reintroduced by Kernohan et al. [123] in 1949. Tumors were no longer considered to develop from embryonic cell nests, but from adult cells still capable of proliferation, by a process of dedifferentiation or anaplasia. The facts that tumor cells of GB more closely resemble an astrocyte than a spongioblast, that some astrocytomas recur as GB at subsequent operations and the frequent finding of glioblastomatous foci in an otherwise typical astrocytoma, led to the assumption that the GB was nothing else but an increased degree of malignancy of the more benign astrocytoma [123]. It was suggested to eliminate the GB from the nomenclature of gliomas and to replace it, in analogy with the four-tiered grading system of Broders for carcinomas [25, 26], by the designation astrocytoma grade 3 and 4. The same principle was later adopted in the first series of the Atlas of Tumor Pathology published by the Armed Forces Institute of Pathology (AFIP) [124] which probably accounted for its widespread acceptance. Almost simultaneously, Ringertz [182] proposed in 1950 a three step grading system for hemispheric gliomas with the GB representing the highest degree of anaplasia common to all three varieties of benign glioma ("the common final pathway").

Zülch [251, 252] on the other hand conceived of the GB as "a primary malignant brain tumor sui generis", totally different from the rare secondary GB accounting for only 5 to 10% of the astrocytomas.

The second series of the AFIP Atlas of Tumor Pathology, which appeared in 1972 by Rubinstein [187], differed only slightly from the first series with regard to the GB being "the extreme manifestation of anaplasia of mature glial tumor cells, mostly astrocytic."
In the first edition of the WHO Histological typing of Tumors of the Central Nervous System edited by Zülch [255] the GB was classified under the heading of poorly differentiated and embryonal tumors and regained its nosographic position of malignant glial tumor sui generis.

However in the second edition of the “Blue book” edited by Kleihues, Burger and Scheithauer [128] a somewhat predictable reverse action took place and the GB was reconsidered to be predominantly of astrocytic nature. Evolution from an oligodendroglioma was admitted to occur, whereas an anaplastic ependymoma rarely reached the stage of GB. Somewhat less conspicuous perhaps has been the omission of the adjective multiforme from the WHO nomenclature as it had not only become redundant but also incorrect in some cases of GB which are composed of uniform small cells.

In the third series of the AFIP Atlas of Tumor Pathology edited by Burger and Scheithauer [43] a similar view was adopted to a large extent, only differing in a narrowed histogenetic derivation of the GB. It was advocated to classify vaguely ependymal tumors as malignant ependymoma rather than GB, and it was suggested that “only under rare circumstances” GB evolved from an oligodendroglioma and even then only by the acquisition of a predominant astrocytic component.

Now, with the advent of molecular genetics in the field of neuro-oncology, we find ourselves at the eve of a new period of which we may hope that the detection of molecular milestones will contribute to a better nosologic understanding of the different oncotypes. With regard to the GB, the controversy has finally been settled and it is now widely accepted that at least two types of GB exist, of which the primary (“de novo”) type seems to prevail [129, 233].
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1836</td>
<td>Ependyma (Purkinje)</td>
</tr>
<tr>
<td>1846</td>
<td>Interstitial substance in the CNS (Virchow)</td>
</tr>
<tr>
<td>1863/65</td>
<td>Glioma - Sarcoma (Virchow)</td>
</tr>
<tr>
<td>1865</td>
<td>Fiber forming cells (Deiters)</td>
</tr>
<tr>
<td>1870</td>
<td>Spinal cells (Cajal)</td>
</tr>
<tr>
<td>1874</td>
<td>Spider cell glioma (Simon)</td>
</tr>
<tr>
<td>1875</td>
<td>Golgi dictum</td>
</tr>
<tr>
<td>1875</td>
<td>Anemone (von Lenhossek)</td>
</tr>
<tr>
<td>1902</td>
<td>Gliosarcoma (Boeck)</td>
</tr>
<tr>
<td>1902/3</td>
<td>Ependymoma - Choroid plexus papilloma (Mallory, Sauer, Sauerbeck, Mutran)</td>
</tr>
<tr>
<td>1904</td>
<td>Spongioblast (Hir)</td>
</tr>
<tr>
<td>1911</td>
<td>Glioma sarcocromodius (Stumpf)</td>
</tr>
<tr>
<td>1913</td>
<td>Three main types of neuroglia (Cajal)</td>
</tr>
<tr>
<td>1914</td>
<td>Cibulum (Mallory)</td>
</tr>
<tr>
<td>1918</td>
<td>Spongioblastoma (Strauss, Globus, Rubbert)</td>
</tr>
<tr>
<td>1921</td>
<td>Third element of Cajal: oligodendrocytes and microglial cells (del Rio Hortega)</td>
</tr>
<tr>
<td>1924</td>
<td>Oligodendroglia (Bailey)</td>
</tr>
<tr>
<td>1924</td>
<td>Gliome polymorphique (Roussy, Cornil, Lhermitte)</td>
</tr>
<tr>
<td>1925</td>
<td>Spongioblastoma multiforme (Globus, Strauss)</td>
</tr>
<tr>
<td>1926/27</td>
<td>Glioblastoma multiforme (Bailey, Cushing) Astrocytoma (Bailey, Cushing)</td>
</tr>
<tr>
<td>1940</td>
<td>Primary and secondary glioblastoma (Schuler)</td>
</tr>
<tr>
<td>1949</td>
<td>Astrocytoma grade 2-4 (Kernohan, Sivek, Adlon)</td>
</tr>
<tr>
<td>1950</td>
<td>Common final pathway (Ringertz)</td>
</tr>
<tr>
<td>1952</td>
<td>AFFP 1 (Kernohan, Sivek)</td>
</tr>
<tr>
<td>1956</td>
<td>Turner's aneurysm (Ziehl)</td>
</tr>
<tr>
<td>1976</td>
<td>AFFP 2 (Rabinstein)</td>
</tr>
<tr>
<td>1979</td>
<td>WHO 1 (Ziehl)</td>
</tr>
<tr>
<td>1993</td>
<td>Genetic subset of GB - (von Deimling)</td>
</tr>
<tr>
<td>1993</td>
<td>WHO 2 (Kleinsch, Berger, Scheithauer)</td>
</tr>
<tr>
<td>1994</td>
<td>AFFP 3 (Berger, Scheithauer)</td>
</tr>
</tbody>
</table>

Table I-1. Milestones in the historical development of neurocytology and glioma pathology.
2. Definition

In the preceding section, the historical development of the current GB terminology and definition has been described. In contradistinction to the common definition of GB as the most malignant astrocytic tumor [129], in the present study the GB will be thought of as a very fast growing glioma either present in all parts or only focally in a more quiescent glioma. In order to explain this aberrant point of view, we first need to discuss the concept of malignancy in the CNS.

In intracranial tumors, malignancy has both a biological and clinical connotation [256]. Merely by their growth in a closed rigid box, the skull, with only very limited volumetric compensations of the cerebrospinal fluid and cerebrovascular blood volume all brain tumors will eventually be malignant without intervention. This clinical malignancy is not only influenced by the type of growth, but also by the reaction of the surrounding tissue (oedema), the anatomical site, the accessibility of the tumor to resection, the extent of resection, the radio- and chemosensitivity of the tumor and by the age and general condition of the patient. Since most of these factors are in general beyond the reach of the pathologist, he necessarily has to confine himself to the evaluation of the former [109].

What then indicates biological malignancy? The classical clinicopathological concepts of benignity and malignancy inherited from general pathology have proven to be difficult to apply in the field of neurooncology. Not only are primary CNS neoplasms poorly metastatic outside the CNS in the absence of prior surgery [5, 100, 173], the other main criterion of malignancy, invasiveness, seems also to have lost most of its discriminative value since the vast majority of the intrinsic CNS neoplasms
display an infiltrative mode of growth [196, 187, 256]. On the other hand, the rapidity of tumor growth remains, in view of the closed chamber properties of the bony calvarium, one of the foremost characteristics of malignancy in the CNS [27, 104, 254]. Therefore one may attempt to subdivide brain tumors according to the quality (rapidity) and quantity (extent i.e. circumscribed or diffuse) of growth, resulting in three “grades”: slow growing activity in all parts (SG), focal fast growing activity (FG), and very fast growing activity (VFG) either focally or in all parts (table I-2, fig. I-1). The rapidity of tumor growth can be morphologically inferred from the evaluation of three histopathological variables: the degree of cyto-and histoarchitectural differentiation, cell density (CD) and proliferation activity.

<table>
<thead>
<tr>
<th>SG activity in all parts in a circumscribed* or diffuse growth.</th>
<th>Cell density</th>
<th>Proliferative activity</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG activity focally present in a circumscribed* or diffuse growth.</td>
<td>low to moderate</td>
<td>absent to low</td>
<td>well</td>
</tr>
<tr>
<td>VFG activity in all parts or focally in a circumscribed* or diffuse growth.</td>
<td>moderate to high</td>
<td>low to moderate</td>
<td>less</td>
</tr>
<tr>
<td>VFG activity in all parts or focally in a circumscribed* or diffuse growth.</td>
<td>high</td>
<td>high</td>
<td>anaplastic</td>
</tr>
</tbody>
</table>

Table I-2. Schematic representation of the subdivision of primary CNS neoplasms according to the rapidity and extent of the growth activity (* truly circumscribed or with a narrow zone of infiltration).
For each group of primary CNS neoplasms we can define a number of proper cyto- and histoarchitectural features similar to the "genuine architectures" of Zülch [256]. They not only typify the oncotype but also are at least partly the expression of good differentiation, being progressively lost in the process of malignant transformation. For instance for the diffusely growing astrocytic subgroup we have to mention the stellate cytoplasmic configuration, the fibrillary background, the so-called perivascular bushes [202] or feufrage pérvascuialaire [30] consisting of a multitude of perivascular processes reminiscent of the specialized end feet attachments to the vessels.

The oligodendroglial tumors are characterized by their uniform and round dark nuclei, fried egg artifact or honeycomb appearance, the so-called chickenwire pattern of the vasculature, the frequent microcalcifications and by their greater tendency towards cortical infiltration.

Typical for the ependymal tumor group are the perivascular pseudorosettes and ependymal rosettes or canals, mimicking their normal lining function.

Finally, the choroid plexus papillomas are typified by their papillary architecture consisting of a single layer of cuboidal or columnar cells.
resting on a basement membrane and a core of underlying fibrovascular stroma, closely resembling normal choroid plexus.

“Anaplasia” will not be used here as a synonym of fast growing activity but in the original definition of von Hansemann [234] as a low degree of differentiation of a tumor cell in comparison with the parent cell from which it originated. The loss of differentiation can be conceived as “the logical consequence of the fast growing activity of the neoplastic cells, which have no time for maturation between mitotic divisions” [27]. Besides the aforementioned proper cyto- and histoarchitectural features which are gradually lost - at least some of them - in the process of malignant progression, some universal changes are seen in the cells such as nuclear hyperchromasia and an increase of the nuclear/cytoplasmic ratio. To the contrary of what is often thought, cellular (nuclear and/or cytoplasmic) pleomorphism including tumor giant cells is not a universal indicator of anaplasia in astrocytic tumors as it is also encountered in the pilocytic and pleomorphic xanthoastrocytoma.

When used in a restrictive manner within one and the same oncotype, increased cell density [129] is without any doubt the most reliable sign of accelerated growth as it is largely unaffected by the employed histological techniques.

Concerning the last histopathological parameter, until very recently the assessment of the proliferative activity was equivalent with calculating the mitotic index. In daily practice this remains true to a large extent, although the application of proliferation markers such as PCNA, Ki-67/MIB-1 appears to find more and more acceptance since they are a more reliable measure of proliferative activity than mitotic figures representing only a relatively short phase of the cell cycle [104].
In this concept of malignancy based on the histopathological evaluation of the extent and rapidity of growth, necrosis and microvascular proliferation, hallmarks of GB, may be considered indirect signs of accelerated growth. However some caution is necessary since, hypoxia, the basic pathophysiological mechanism underlying both features [136], may be induced by a variety of means. Not only overcrowding, creating an imbalance between oxygen/ nutritional demands and supply, probably accounting for the small, serpiginous foci of necrosis in GBs or medulloblastomas and for the intervacular necrosis of metastatic tumors, but also thrombotic occlusion, responsible for the large, geographical, ischemic form of necrosis in GBs, and intramural or extramural vascular changes such as hyalinization and encirclement by cytoplasmic processes - both often encountered in pilocytic astrocytomas - may induce hypoxia in tumor cells. These hypoxic tumor cells may then react by secreting angiogenic factors [178, 215, 241] inducing angiogenesis and multilayered vascular proliferation in order to restore the imbalance or they may proceed to cell death when damaged irreversibly.

Applied to astrocytic tumors, the astrocytoma is then defined as a diffuse SG astrocytic neoplasm implying indistinct tumor margins, low to moderate hypercellularity, no sparse mitotic activity and good differentiation (i.e. stellate cytoplasmic configuration, slight nuclear pleomorphism and hyperchromasia, fibrillary background and feutrage périvasculaire). The anaplastic astrocytoma depicted as a focal FG astrocytoma, denotes an otherwise quiescent astrocytoma containing more or less extensive foci of increased cellularity, mitotic activity and less differentiated cells (i.e. more hyperchromatic and pleomorphic nuclei, increased nuclear cytoplasmic ratio, less well developed cytoplasmic
processes and feutrage). When these foci become indistinguishable from GB i.e. demonstrating high hypercellularity, high proliferative activity (including the “indirect signs” necrosis and microvascular proliferation) and anaplasia, we speak of highly anaplastic astrocytoma or secondary GB. The GB on the other hand denotes a VFG glial neoplasm present in all parts from the beginning, which may be termed primary in contradistinction to the former. The numerical predominance of the astrocytic variant of secondary GB may be accounted for by the relative higher frequency of astrocytic tumors among gliomas [187, 256].
3. Epidemiology

In sharp contrast to the relative high frequency of primary CNS neoplasms in the pediatric age group, where they rank second after leukemia's, accounting for approximately 20% of all cancer-related deaths under the age of 15 [171, 209], in adults they are responsible for only a minor 2% of all cancer burden [114, 209] amounting to 7-10 new cases per 100,000 inhabitants per year in most of the industrialized countries [172]. Recent epidemiological studies have indicated increasing brain tumor incidence and mortality rates over the past two decades, especially in the elderly, which cannot be explained only by improved diagnostic and coding procedures [59, 76, 87, 166, 209]. In general, men are more often affected than women [76, 166, 179, 209] and share a higher mortality/incidence ratio [208], which can be explained by a higher incidence of gliomas and a lower incidence of meningiomas in men [187, 256]. Among races, Caucasians are more often affected than Africans or Asians [172, 179].

Some 15-23% of primary CNS tumors are due to GB [225], constituting the most frequent primary brain tumor, with annually 2-3 new cases per 100,000 population for most of the European and North American countries [76, 94, 96]. The cerebral hemispheres are most often involved, in particular the frontal and temporal lobes, whereas the occipital lobes are relatively spared, this being grossly proportional to the amount of white matter present in each cerebral lobe [187]. Brainstem locations of GB are more frequently encountered in children [65, 79] whereas cerebellum [141] and spinal cord [256] are uncommonly affected at any age. Rare under the age of 30, GBs show a peak incidence between 45 and 65 years [256]. A slight male preponderance is noted with a male-to-female ratio of 3:2 in most of the series [187, 256].
4. **Clinical and neuroradiological features.**

The clinical symptoms and signs of GB are similar to those of other brain tumors, albeit often more abrupt in onset and rapid in evolution. They include headache, papilloedema, mental status changes, focal neurological deficits and seizures.

**Headache** is a major symptom in GB patients, being present in more than 75% [189] at some time during the clinical course. Although often intermittent and nonspecific, certain features of the headache may be suggestive of brain tumor e.g. pain that increases in severity, in frequency and duration, pain that awakes the patient or is more severe in the morning, or pain that increases while coughing or exercising.

**Papilloedema**, formerly a frequent (58%) sign of increased intracranial pressure [189] is since the advent of CT and MRI less often encountered as a result of earlier diagnosis. In a recent study, only 8% of patients had papilloedema at the time of diagnosis [72].

**Changes in mental status** are experienced in approximately 40% of patients by the time of diagnosis [189, 243] and may span a wide spectrum from subtle to severe alterations in personality (disinhibition, irritability, impaired judgement, emotional lability), in cognitive functions (memory loss, intellectual decay, problems with concentration and abstract thinking) and in consciousness culminating in stupor and coma.

**Site-specific neurologic symptoms and signs** are encountered in 40 to 60% of GB patients and include hemiparesis, hemianesthesia, visual field defects, and dysphasias [189, 243].

**Seizures** are the first symptom in approximately 37% of patients with GB [189], half of which are focal. This relative low incidence in comparison with that of other gliomas (70%) is explained by the rapid growth of the
GB [222]. Depending on the epileptogenicity of the involved brain area, seizures occur in 59% of the frontal, 42% of the parietal, 35% of the temporal and 33% of the occipital tumors [204]. A prolonged history of seizures in patients with malignant glioma appears to be associated with a better prognosis since it may correlate with the evolution from a prior low-grade lesion [156]. Status epilepticus, change in seizure pattern, postictal paralysis, refractory epilepsy and the presence of additional neurologic symptoms have been found to increase the likelihood of a neoplastic etiology [21].

In general, duration of the symptoms tends to be short unless the GB developed from a symptomatic less malignant precursor lesion [35, 129, 189]. In a study of 71 supratentorial GBs in adults [220] duration of 2-3 months was noted in 32%, of less than 6 months in 50% and of more than one year in approximately 28% of the cases. A similar mean duration of 5.4 months was reported by Burger [35].

The introduction of computed tomography (CT) in the early-to-mid 1970s and magnetic resonance imaging (MRI) in the early-to-mid 1980s have both greatly influenced the diagnosis and management of brain tumors. These "autopsy in vivo" techniques allow to locate accurately, to diagnose earlier and to determine more objectively gross residual tumor after surgical excision [1]. Moreover, they form the basis of modern stereotactic neurosurgical procedures.

Four to 10% of GBs do not enhance after administration of contrast agents [47, 205]. They appear on CT scans as hypo- or isodense lesions. On MRI these tumors characteristically present as low signal intensity areas on T1-weighted and high signal intensity on T2-weighted images [244]. However, the vast majority of GBs shows either a
homogeneous or, more typically, a ring-shaped pattern of varying thickness in contrast enhancement, on both contrast-enhanced CT scans and contrast-enhanced T1-weighted MR images [44, 148, 189]. The pathological basis of the rapid uptake of contrast agents in brain tumors has been ascribed to a local disruption of the blood-brain barrier as shown in several ultrastructural studies demonstrating defective tight junctions, increased numbers of pinocytic vesicles, presence of fenestrations, defects in the basement membrane and abnormalities of the glial investment [88, 145]. The structural vascular abnormalities result in increased permeability of the capillaries leading to the extravasation of a protein-rich filtrate of plasma into the extracellular space. Whereas the oedematous fluid is propelled, by bulk flow, often far beyond the boundaries of the macroscopic tumor and hereby showing a clear predilection for white matter [115, 222], the leakage of radiographic contrast agents remains confined to the region of blood-brain barrier disruption [44]. At this point it should be noted that glucocorticoids, trying to restore the blood-brain barrier, reduce the volume of contrast enhancement and peritumoral oedema, as well as the intensity of contrast enhancement [45].

Kelly et al. [120] distinguished four CT/MRI-defined volumetric zones in GBS, which correspond histopathologically to: a central area of necrosis (zone 1), a rim of solid tumor tissue with neovascularity and endothelial proliferation (zone 2), peritumoral vasogenic oedema usually containing isolated tumor cells (ITC) (zone 3 and 4). In a subsequent study [70], the same authors found ITCs outside the zone of T2-weighted abnormality in 2 of the 4 studied cases. The CT/MRI defined zones depicted in Fig. I-2 are: zone 1, an internal CT hypodense zone; zone 2, a zone of contrast enhancement; zone 3, the peripheral CT hypodense zone
and zone 4, the CT isodense zone lying outside the peripheral CT hypodense zone but within the region of increased T2 signal.

Calcification, although uncommon in GBs, has been clearly associated with progression from a preexisting low-grade tumor [148, 189, 200, 220, 244].

![CT/MRI-defined volumetric zones](image)

Fig. 1-2. CT/MRI-defined volumetric zones in GB.

The principal neoplastic and non-neoplastic conditions to be differentiated radiographically of untreated GB are metastastic brain tumor, primary CNS lymphoma, brain abscess and cerebral infarction [42]. They all can show a ring pattern of enhancement on CT or MRI scans. Some 10% of GBs have their epicenter at the gray-white matter junction [189], 2 to 3% are multicentric tumors [15, 136, 187], but the vast majority of GBs are single, deep in the white matter seated lesions. Moreover, the zone of peritumoral oedema, being approximately of the same size as the tumor tissue proper in GBs, is usually larger in metastastic carcinoma [189].

A ring pattern of enhancement may be seen in primary CNS lymphomas as a result of central necrosis. More typically however, lymphomas display a
solid, homogeneous pattern of contrast enhancement and are hyperdense on unenhanced CT scan. Additional features of primary CNS lymphomas are the often-observed periventricular and bilateral location [21, 42].

Following the stage of cerebritis, the capsule of a brain abscess surrounding an inner core of necrotic debris may enhance brightly on a post-contrast CT scan. The thickness of the enhanced rim is usually more uniform than that of a GB [189].

A cerebral infarction can also mimic radiographically a GB [42]. The clinical context and the sequence in time of initial low-density mass effect with the appearance of ring enhancement about one week into course usually permit distinction. A wedge-shaped configuration is generally only encountered in large infarcts [189].

We may therefore conclude that at present, modern imaging techniques are unable to depict precisely, in all cases, the outer tumor limits [70, 120, 148] as they depend on the presence of oedema, that can be absent e.g. in gray matter without significant mass effect [115] or be reduced as a response to antineoplastic and/or the administration of glucocorticoid therapy [140]. In consequence, the radiographic evaluation of the response to treatment should be carried out under constant or reduced corticoid doses [148].
5. **Neuropathological features**

There are many excellent descriptions of the neuropathological features of GB [42, 43, 129, 136, 187, 202, 256], therefore only some aspects will be briefly discussed.

**Histopathological criteria**

Most of the currently employed histopathological grading systems require three criteria for the diagnosis of GB, which can all be related to the tumor's VFG activity.

In the Duke scheme by Burger [35], the triad consists of moderate to marked hypercellularity, moderate to marked pleomorphism and necrosis, the key diagnostic criterion. Vascular proliferation is optional.

The UCSF scheme by Davis [61], used at the University of California, San Francisco, differs from the foregoing by the requirement of vascular proliferation with necrosis as an optional feature.

The St.Anne/Mayo system [58] and the revised World Health Organization system (WHO-2) [128] require one of either together with nuclear atypia and mitoses in the St.Anne/Mayo system, and with poorly differentiated neoplastic astrocytes and hypercellularity in the WHO-2 system.

The Kernohan grading system [122, 123], still in use in some laboratories [69, 126, 165], although outstripped in clarity and prognostic usefulness by the more recent grading schemes, requires 7 criteria for the diagnosis of grade 3 and 4 astrocytomas. Included are the percentage of normal appearing astrocytes (50-75% in grade 3, few in grade 4), degree of anaplastic changes in the remaining cells i.e. nuclear and cellular pleomorphism and nuclear hyperchromatism (moderate in grade 3, marked
in grade 4), increased cellularity (1.5 times that of normal brain in grade 3, 3 times that of normal brain in grade 4, mitotic figures (at least 1 in every other HPF in grade 3, 4-5 in every HPF in grade 4), vascular changes (increased vascularity and frequent and often pronounced endothelial proliferation in grade 3, high vascularity and marked proliferation in almost every instance in grade 4), necrosis (frequent throughout the tumor in grade 3, frequent and extensive in grade 4), narrow zone of infiltration (grades 3 and 4).

**Proliferative activity**

GBs typically display a high proliferative activity with numerous typical and atypical mitoses and high cell proliferation indices. However, there may be considerable intratumoral variation in proliferative potential [168] which has been attributed to nutritional deficiency [104], the presence of areas of better differentiation [168] and cytological heterogeneity with small anaplastic cells show the highest labeling index and gemistocytic astrocytes the lowest [133, 168]. Therefore, in order to improve comparability between studies of cell kinetics, it has been recommended to count areas with the highest density of mitotic figures or labeled nuclei [66, 122, 159].

Due to the difficulty of recognizing mitotic figures [66, 89, 164] and the relative short duration of the mitotic phase [104], other more reliable measures of cell proliferation have been proposed including S-phase markers [13, 104, 105, 134, 183, 191] and cell cycle markers labeling all phases except G0 [37, 60, 108, 116, 117, 125, 126, 146, 169, 191, 203, 32]
234, 250]. Of the latter, Ki-67/MIB-1 has proven to be the most reliable and reproducible [108, 117, 155, 191]. Relative to MIB-1, mean cell proliferation indices have been reported in GBs of 14.6-31.6%, approximately two to six times higher than those found in anaplastic and low-grade astrocytomas respectively (table I-3).

<table>
<thead>
<tr>
<th>Astrocytoma</th>
<th>Anaplastic astrocytoma</th>
<th>Glioblastoma</th>
<th>G/H</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2 (4.1)</td>
<td>7.3 (7.2)</td>
<td>23.9 (13.5)</td>
<td>1/a</td>
<td>169</td>
</tr>
<tr>
<td>6.3 (3.4)</td>
<td>16.6 (13.2)</td>
<td>26.2 (9.6)</td>
<td>2/a</td>
<td>191</td>
</tr>
<tr>
<td>2.0 (2.0)</td>
<td>12.8 (6.3)</td>
<td>14.6 (6.8)</td>
<td>2/b</td>
<td>117</td>
</tr>
<tr>
<td>3.8 (2.7)</td>
<td>18.4 (9.7)</td>
<td>31.6 (12.9)</td>
<td>2/b</td>
<td>235</td>
</tr>
<tr>
<td>4.2</td>
<td>6.7</td>
<td>29.7</td>
<td>2/a</td>
<td>203</td>
</tr>
</tbody>
</table>

Table I-3. Mean (SD) of MIB-1 proliferating cell index in astrocytic gliomas. (G = grading system: 1 = UCSF, 2 = WHO-2. H = choice of HPP: a = highest labeling, b = at random.)

**Spread and metastasis**

The topographical nature of GB has been a matter of great dispute. While some investigators considered the GB a localized disease, based on the observation that most GBs recur within a 2 cm margin of the primary tumor site [16, 50, 99, 142], others considered the GB a diffusely infiltrating tumor defying any form of local therapy [10, 114, 206]. Currently, the GB is thought of as a diffusely infiltrating tumor consisting of a solid tumor component and isolated tumor cells infiltrating intact parenchyma [33, 70, 115, 119].
The GB has a high propensity for local intraparenchymal spread which can be related to the profile of its infiltrating tumor cells i.e. poorly differentiated, often bipolar cells; to the mechanical growth pressure ensuing from rapid proliferation and to the constant presence of peritumoral vasogenic oedema dilating the extracellular space [77]. As shown from the studies of Scherer [194, 195, 196], Matsukado [151] and Burger [40], the extent and geometry of the local spread is greatly influenced by the tumor's topographical relationship to adjacent brain structures. Neuroanatomical white matter tracks and basement membrane lined surfaces such as the subependymal space and lamina glia limitans superficialis or perivascularis are preferential routes of migration of infiltrating tumor cells [137, 151, 152].

Extraneural spread by spontaneous metastasis i.e. without defense-altering procedures such as craniotomy or shunting operations is on the other hand rare in GBs [5, 100, 173]. This confinement to the CNS has been related to an inability of the tumor cells to penetrate the vascular basement membrane [18] and to the fact that most GB patients die rapidly, long before the detection of clinical metastases [143, 228].

Intraneural spread by way of the cerebrospinal fluid resulting in meningeal gliomatosis and ependymitis blastomatosa has been reported in 6-27% of cerebral GBs at autopsy [6, 167, 188, 248]. Long postoperative survival [6, 228, 249] and poor differentiation [167] have been identified as promoting factors for ventriculoleptomeningeal spread.
6. Treatment

The poor survival results of GB patients treated only with surgery on one hand and the neurotoxicity and limited radio- and/or chemosensitivity on the other, is the rationale for the multimodality therapy of GB for almost 30 years ([75, 180]. Currently, conventional management of patients with newly diagnosed GB consists of maximal feasible surgical removal, limited field external beam irradiation with 55 to 60 Gy with or without adjunctive chemotherapy with nitrosoureas [14].

Except for locations in the frontal, temporal or occipital pole allowing more generous partial lobectomies [4, 111], surgery primarily addresses the solid tumor tissue probably out of fear of inflicting additional damage to a patient with already a grim prognosis [226]. Whereas its role in providing adequate tissue samples for definitive histopathological diagnosis and in alleviating symptoms resulting from mass effect and intracranial hypertension has been established for many years [4, 17, 51, 111, 227], the role of cytoreductive surgery remains to some extent unclear [161]. Although many studies have reported a clear benefit of gross total removal over partial resection or biopsy [4, 14, 64, 69, 111, 165, 226] others did not [52, 68, 132]. Besides not taking into account or controlling other factors influencing the survival rate [74, 161] such as age, preoperative functional status, use of radiotherapy, tumor location, prior low-grade tumor, there is another major impediment in the determination of the effect of aggressive surgery on survival namely the assessment of the extent of tumor removal. Until very recently this was solely based on the neurosurgeon’s peroperative impression, which was - seen the infiltrative mode of tumor growth - often imprecise, as he had to rely upon differences in consistency and color between tumor and adjacent brain tissue [1, 17].
When performed within three days after surgery, contrast enhanced neuroimaging (CT and in particular MRI) has proven to be a more objective tool in the evaluation of postsurgical tumor burden [1, 14, 51, 247]. Neuronavigational systems, which are currently being applied, will undoubtedly facilitate gross total removal by providing permanent visual feedback [131]. At present, the view of performing maximal feasible surgery is gaining more acceptance [17, 189].

External beam irradiation constitutes the other cornerstone in the therapeutic management of GB and remains the most effective adjunctive therapy to surgery [244]. Already in 1967, Jelsma and Bucy [111] reported a 31% increase in survival at 9 months following radical surgical removal and radiation therapy compared to extensive surgery only. Since then many randomized clinical trials confirmed the beneficial effect of postoperative irradiation on survival [14, 132, 138, 165, 238].

The somewhat conflicting goals—largely due to the unselectivity of ionizing radiation, causing DNA damage in neoplastic as well as in normal cells [189]—of achieving maximal cytoreduction and of minimizing radiation injury to normal brain tissue raised many questions regarding volume of brain to be treated, optimal total dose and fraction size [68, 75, 138]. Based on the clinical observations that most of the GBs are initially unifocal [15, 50, 68], that more than 90% of GBs recur within a 2 cm margin of the primary tumor site [1, 15, 50, 99, 142] and on the results of dose-survival studies [156, 238], standard radiation therapy currently recommends 60 Gy administered in single daily fractions of 1.8 to 2 Gy to a limited field [68, 138]. The latter is usually defined as a 3 to 4 cm margin of tissue surrounding the contrast-enhancing zone on CT or MRI scans.
[138]. No significant survival benefit has been demonstrated of whole brain irradiation [68, 142] or of accelerated fractionation or hyperfractionation radiation schedules [138]. Moreover, whole brain irradiation and total doses above 60 Gy are related to a higher risk of radiation injury to the normal brain tissue and to the vasculature [54, 68]. As low intratumoral oxygen tension is a major cause of radiation resistance [118] many attempts have been made to increase the oxygen effect including hyperbaric oxygen and hypoxic cell radiosensitizers. Most of them were unfavourable to patients with GB [138].

Interstitial brachytherapy and radiosurgery have the advantage of delivering higher doses of radiation to the tumor bed with relative sparing of the surrounding normal brain [244]. Despite the theoretical benefits and a few preliminary favorable reports [144, 213, 243] their role in the treatment of newly diagnosed GBs awaits further investigation because the eligibility criteria apply to patients with already a more favorable prognosis at the outset, including small, unifocal, radiographically well-defined supratentorial tumors which do not involve the corpus callosum or ependyma. In addition, patients must have a good performance status [189].

The role of post-radiation chemotherapy in the management of GB remains controversial despite more than 20 randomized clinical trials [244]. Confounding variables related to patient heterogeneity (age, histology, extent of resection, performance status, radiation therapy), lack of convincing statistical evidence due to small patient numbers and the lack of uniform response criteria are elements of criticism [75]. The few studies of the possible survival benefit of post-radiation chemotherapy in patients with high-grade gliomas indicate an increase of the mean survival time with
two to three months [74, 86, 97]. A number of new chemotherapeutic agents and new strategies to overcome problems of drug resistance, tumor access and neurotoxicity, are currently being investigated. These strategies include intra-arterial chemotherapy, interstitial chemotherapy, high-dose chemotherapy in combination with autologous bone marrow transplantation and transient disruption of the blood-brain barrier by mannitol or leukotrienes [140].
7. Prognosis

Despite modern vigorous multimodality therapy, the prognosis in patients with GB is poor, most patients die rapidly due to progressive tumor growth with only 10-20\% survivors 18-24 months after diagnosis [189]. Long-term survival has been reported only sporadically throughout the last 40 years with an estimated 5- and 10-year survival of 5\% [48, 160, 229] and 0.5\% [190] respectively.

Besides a ten-fold decrease in peri-operative mortality [165, 189] due to the introduction of glucocorticoids in the early 1960s [78, 111] and improvements in anesthesia, radiology and surgical techniques [189, 245], apparently little has been gained in patient outcome over the last 40 years [153]. In 1950, the mean postoperative survival of patients with astrocytoma, anaplastic astrocytoma and glioblastoma were 63, 32 and 11 months respectively [182]. Almost two decades later, Jetsma and Bucy (1969) reported a mean survival of 10.8 months in a group of 40 “optimally” treated GB patients who underwent extensive tumor resection and postoperative irradiation with the suboptimal radiation dose of 45-50 Gy [112]. At present, mean postoperative survival times of 14 to 17 months in the middle-aged GB patient group belong undoubtedly to the best results reported [14, 17, 67, 165, 176, 183]. Unfortunately, most are still in the range of 9-12 months [64, 97, 132, 138]. Apparently, GB treatment is still facing the same problems, which are essentially related to:

- the mode of growth of the GB, consisting of solid tumor tissue and a corona of isolated tumor cells infiltrating the intact parenchyma. This pattern of tumor growth has two major repercussions:
1) on the surgical procedures. Out of fear of inflicting additional brain damage in patients with already a poor prognosis, resection is usually limited to the solid tumor tissue [1, 120, 226], defined by the zone of contrast enhancement on post-contrast CT or MRI scans, which has been shown not to contain neural parenchyma and can therefore be safely resected [120].

2) on the recognition of tumor margins [4, 51]. Until recently, the neurosurgeon had to rely upon differences in consistency and in color between tumor and adjacent normal brain [17]. Although neuronavigitional systems, by transferring imaging data to the surgical field, greatly improves the feasibility of the surgeon’s task, he is still hampered by the lack of real-time image information, since all data are based on preoperative imaging and brain shift during tumor resection is not accounted for [17]. Ultrasound-controlled navigator-guided brain surgery has been currently proposed to overcome this problem [131].

- the heterogeneous cell population differing in genetics, antigenic expression, kinetics, metabolism, oxygenation, vascular access, resulting in differences in therapeutic sensitivity of the tumor cells [104, 140].
- the sensitivity of the surrounding functional brain to radiation and chemotherapy is related to the non-selectivity of both ionizing radiation and alkylating agents, causing DNA damage in neoplastic as well as in adjacent normal cells [244]. This limits the dose of irradiation and of chemotherapeutic agents well below that needed for complete eradication the tumor [138, 140].
- the problem of tumor access for antineoplastic drugs attributable to the blood-brain barrier [92, 140].

- a persisting degree of treatment nihilism relative to malignant brain tumors [31, 75, 229].

A remarkable feature in the clinical evolution of GB patients is that although GB is uniformly lethal, survival times of individual patients vary considerably ranging from a few weeks to several years after operation [14]. Several patient-, tumor- and treatment-related factors have been identified influencing survival in GB patients, which may account for some of the observed variation in survival time. (Fig. I-3)

Prognostic patient-related factors:

In high-grade astrocytic neoplasms age at diagnosis has been consistently identified as a major prognostic indicator of postoperative survival with young patients faring better than middle-aged and considerably better than elderly patients [14, 38, 64, 68, 69, 97, 121, 132, 138, 165, 210, 212, 226, 229]. In a series of 1265 GB patients mean survival of respectively 6, 9, 12 and 16.8 months were recorded in the 65-84, 55-64, 45-54, and 15-44 age groups [35]. Besides presumed differences in immunocompetence of the host [35, 48, 189, 190] and in biological aggressiveness of the tumor [38, 48, 111, 138, 139, 189], recently Lantos [136] suggested that the better prognosis of young patients is perhaps due to the fact that they have mainly secondary GBs whereas primary GBs occur later in life. In a subsequent paper investigating the relationship between patient age and histological features in GBs, Burger
and Green [38] noted the higher age of patients with GBs containing necrosis, a smaller standard deviation of nuclear size, vascular proliferation, no fibrillary astrocytes and which were of the homogeneous small cell type. However, multivariate analysis made it clear that these histological features were only partly responsible for the observed effect of age on survival.

Recently, it has been suggested that differences in biologic behavior of malignant gliomas in patients of different ages might be due to differences in microvessel density reflecting the tumor's ability to induce angiogenesis [139]. No significant correlation has been reported between the age and the proliferative rate in patients with GB [13, 105].

Another prognostic factor of major importance is the preoperative clinical performance status. In a study of 654 supratentorial GBs, patients with a Karnofsky performance status of less than 70 had a mean survival of only 7.8 months compared to 11.7 months of those with a KPS greater than 80 [212]. Others [17, 48, 64, 68, 69, 139, 156, 165, 229] have reported similar data. Possible explanations for the relationship between high KPS and longer survival are small tumor size and location in non-eloquent brain areas permitting more aggressive therapy. It has also been suggested that performance status may influence patient selection for different therapies [68, 132, 161, 212].

Other pretreatment patient-related prognostic factors which have been observed with less consistency are tumor location [32, 112, 132, 165, 212], duration of preoperative symptoms [156, 220, 246], seizures as initial symptom [64, 236, 246] and prior low-grade glioma [24, 31, 64, 67, 111, 187, 220, 229]. Relative to tumor location, covariance with accessibility to resection [220, 246] and with the extent of tumor resection
is usually suggested, as lesions in non-eloquent brain areas may be more aggressively resected [161]. However, in the study of Simpson et al. [212] no correlation was found between tumor site and extent of surgical resection.

The favorable effect of long duration of preoperative symptoms on survival has been related to earlier detection [156] and to a prior history of low-grade glioma [41, 196, 220].

Seizures as the initial symptom in patients with high-grade gliomas have been found to affect survival positively [64, 206, 236, 246]. In one study of 170 grade IV gliomas [64], patients presenting with seizures survived significantly longer than those with either intracranial hypertension or progressive neurological deficit as first symptom. However, covariance with duration of symptoms [246], superficial tumor location [64] and prior-low grade glioma [246] has been suggested.

The influence of prior low-grade histology on survival has been debated. All eight long-term survivors in the study of Jelsma and Bucy [111] had astrocytomas containing glioblastomatous foci. Three of these showed histological evidence of a prior astrocytoma. However, in this early report no adjustment was made for young patient age and extensive tumor resection. Takeuchi and Hoshino [220] reported longer periods of survival in cases which were initially grade 1-2 changing to grade 3-4 in the terminal period compared with those which started out as grade 4. In a series of 28 patients with anaplastic gliomas (32% GBs), Winger [246] found a clear survival benefit of clinically suspected or biopsy proven prior low-grade glioma, in comparison with those which appeared de novo. By multivariate analysis prior low-grade histology remained a significant
independent prognostic factor when corrected for age, tumor histology, performance status and extent of resection.

Conversely, in the analysis of Devaux et al. [64] no significant survival advantage was noted in the 17 GB patients with prior history of low-grade glioma. However, patients with grade III gliomas with a prior low-grade survived longer than the de novo cases. More recently, Dropeho and Soong [67] conducted a matched case-control study to evaluate the prognostic impact of prior low-grade histology in patients with anaplastic gliomas. 68 pairs of patients were matched for tumor histology (28% GBs), patient age, Karnofsky performance status and type of surgery in order to provide a more valid comparison. No significant difference in survival between patients with anaplastic gliomas arising by transformation of prior low-grade tumors and patients with de novo anaplastic gliomas was found.

Tumor histopathology-related prognostic factors.

Already in the 1912 paper of Tooth [224], tumor necrosis was given a sinister connotation as it was considered “the last stage in the life history of glioma, going on “pari passus” with vigorous vitality elsewhere in the growth”. To Scherer [197, 198], necrosis was the key morphological feature separating primary from secondary GB in necropsy material, accounting for some of the observed difference in clinical duration between the two types. The secondary GB did contain “no or comparatively small necrosis (not over one tenth of the living tumor tissue)” whereas in the primary the necrotic areas were “almost always more extensive than the living tumor tissue.”
More recently, the prognostic importance of tumor necrosis in predicting poor survival in patients with diffuse astrocytic tumors has been clearly established by several large controlled studies [12, 35, 38, 58, 162], so much so that tumor necrosis is considered a principal diagnostic criterion for GB in most of the currently employed histopathological grading systems [43, 58, 128]. A positive correlation with age has been noted for older patients more frequently harboring GBs with necrotic areas [12, 38]. Less agreement appears to exist regarding the effect on survival of the extent of intratumoral necrosis. Whereas Burger et al. [32] found no difference in survival between patients with a high degree or with a low degree of tumor necrosis, Pierallini et al. [176] recently demonstrated a clear correlation between the relative extent of necrosis as assessed radiographically before surgery and survival in 18 patients with GB. In this study, the ten patients with a necrosis/tumor ratio of less than 0.5 had a mean survival of 17.6 months whereas the 8 patients with a necrosis/tumor ratio equal to or more than 0.5 had a mean survival of 10 months.

The prognostic impact of prior low-grade glioma has already been partly addressed in the previous section concerning prognostic patient-related factors. A considerable percentage of prior low-grade gliomas go undetected as diffuse infiltrating low-grade astrocytomas do preserve the preexisting neural parenchyma for quite some time and the clinical symptoms are often aspecific e.g. headache. In illustration of this only three out of eight patients cited by Jelsma and Bucy [111] showed histological evidence of a prior low-grade glioma. On the contrary, when foci of GB are seen on the background of a better-differentiated (low-grade) glioma, an
evolution of the GB from the better-differentiated tumor is generally
assumed [24, 42, 111, 127, 187, 197].
Taveras [221] reported 27% 18 months postoperative survival in 42
patients with secondary GB compared to a 10% survival at 18 months for
383 patients with “pure” GBs.
In a study of 71 GB patients, areas of better differentiation were associated
with longer survival [38].
In contrast, Kleihues [127] found no difference in survival between patients
with secondary GBs and primary GBs in the first 18 months. However,
long-term follow-up revealed a minor survival benefit in less than 10% of
the secondary GB cases. It was concluded that when the histopathological
criteria of GB were present focally in an anaplastic astrocytoma, tumor
biology and clinical outcome were very likely to correspond to those of the
GB. More recently, a similar conclusion was reached by Bouquet et al.
[24] in a review of 87 anaplastic astrocytomas from two clinical trials of
the EORTC Brain Tumor Group. 23 cases were identified containing
highly anaplastic foci characterized by numerous mitotic figures,
endothelial cell proliferation and necrotic areas with perinecrotic
pseudopalisading. It was concluded that highly anaplastic astrocytomas
behave like GBs in their postoperative survival characteristics.
In summary, there appears to be little agreement on the prognostic impact
of prior low-grade histology in GBs. While it might be argued that some of
the older studies suffered from lack of comparability between the de novo
and prior low-grade study group, more recent well controlled studies failed
to demonstrate any significant difference in survival. Therefore, as already
suggested by Rubinstein [186], it seems reasonable that once anaplasia has
progressed to the development of focal necrosis in an astrocytoma, the prognosis becomes that of a glioblastoma.

Prolonged survival has been reported in the giant cell glioblastoma variant which has been attributed to the biological indolence of the predominant cell type [32, 83] and to its better macroscopic circumscription and less infiltrative character allowing more complete removal [32, 112, 136]. Conversely, shorter postoperative survival times have been observed in homogeneous small cell GBs. This has been related to the aggressive biological properties of the constituent small anaplastic and small fibrillated tumor cells [32, 38, 41, 120]. They were virtually the exclusive cell types in lesions with marked mass effect; they freely infiltrated contiguous brain structures and were the predominant or sole cell type in intraneural metastasis [83].

Although tumor cell proliferation indices, such as the bromodeoxyuridine labeling index and the Ki-67/MIB 1 cell proliferation index, seem to be significant predictors of survival in patients with low- and intermediate-grade gliomas [60, 105, 106, 158, 191, 235], no significant prognostic value has been reported in patients with GBs despite the wide range in labeling index [13, 158, 177, 183], nor was there a significant correlation with time of tumor recurrence [13, 183]. Other histological features which have been reported to be positively correlated with survival in patients with GBs are presence of fibrillary astrocytes [38], calcification [220], areas of microcystic degeneration [38, 196, 220, 224] and perinecrotic pseudopalisading [38]. The former three histopathological features have been associated with progression from a prior low-grade glioma [38, 197, 220, 244]. On the other hand, high cellularity in patients treated only with surgery [32], multifocality [50, 183]
and small median nuclear size [38] have been found to affect survival negatively. The prognostic significance of perivascular lymphocytic cuffing in GBs remains controversial [32, 136]. The prognostic significance of the rate of apoptosis is currently being investigated. In the study of Schiffer et al. [203] no significant correlation was noted between survival and apoptotic index.

**Treatment-related prognostic factors**

In the past, the prognostic impact of the extent of tumor resection has been heavily debated [161]. Major causes of this dispute were the lack of objective evaluation of the degree of surgical removal [1] and heterogeneity of the patient populations with regard to prognostic factors [75]. Recently, early postoperative neuro-imaging has been shown to be a more reliable estimator of the extent of tumor removal [1, 12, 17, 69, 247]. In these studies, postoperative tumor size was a significant predictor of survival even after adjustment for other chief prognostic variables such as age, Karnofsky performance status and radiotherapy. Preoperative tumor size on the other hand, did not affect survival significantly [212, 247]. Postoperative radiation therapy has been repeatedly found to prolong survival in patients with high-grade gliomas [13, 64, 111, 121, 156, 165, 189, 226, 236, 246]. Also irradiation dose has been reported to correlate with survival, patients receiving 60 Gy in single daily fractions faring considerably better than patients treated with lower doses [156, 237]. In contrast, no clear survival advantage has been demonstrated of whole brain irradiation over limited field irradiation covering the contrast-enhancing
lesion neither with a 3 cm margin nor of hyperfractionated irradiation over conventionally fractionated radiotherapy [138, 243]. Recently, radiographically assessed response to radiation therapy was shown to be a statistically significant prognostic factor in patients with GB [14, 247]. In the study of Barker et al. [14], 62% of patients with a decrease in enhancing area of more than 50% on early postradiation neuro-imaging scans, were still alive one year after diagnosis compared to the 25% showing a similar increase in enhancing area.

Some authors studied the influence of postoperative performance status on survival and results similar to those of preoperative functional status have been reported meaning that patients with better function live longer [13, 17, 67, 165, 235, 246].

Other treatment-related factors that have been shown to affect clinical outcome favorably in patients with GB include long duration of disease-free interval after multimodality therapy [48] and reoperation as part of a multimodality therapy for tumor recurrence [16, 69, 92, 165, 183, 189].
Fig. 1-3. Multifactoriality of clinical outcome in patients with supratentorial GB.
CHAPTER II

PATIENTS AND METHODS
1. Patient selection

In the laboratory of Neuropathology of the Cliniques Universitaires St.Luc (Dir. Prof. Dr. JM Brucher) new cases of (sub) totally removed supratentorial glioblastoma in adults (> 18 years) without prior radiotherapy or chemotherapy were collected. Only prior biopsy or subtotal resection for low-grade glioma was allowed. For further studies, the material had to be of excellent quality with preserved immunoviability as checked by the vimentin immunostain [43]. In order to study the GB and its topographical relationships in a continuous manner no stereotactic biopsy material could be used and the study was performed on large surgical specimens. Excluded from the study were the less frequent highly anaplastic oligodendrogliaoma, ependymoma and mixed glioma with features of GB as well as the rare gliosarcoma. For concept-10nal [43] and for morphometrical reasons related to the impact of cell size on cell density [2, 35, 197, 248], the giant cell glioblastoma was also excluded.

After thorough selection, 15 cases were retained: eight cases showed considerable amounts of normal brain parenchyma outside the GB and seven were surrounded by a lower-grade astrocytic neoplasm. In order to allow morphometrical comparison, 10 astrocytomas (WHO grade II) as well as 10 anaplastic astrocytomas (WHO grade III) and 3 normal postmortem brains were included in our studies.
2. **Methods**

   Each clinicopathological description was subdivided into clinical, histopathological and morphometrical data.

   A. **Clinical data** of interest were age at diagnosis of GB, gender, location (side and lobe), clinical history with special attention to the duration of the symptomatology defined as the interval between the onset of symptoms and histopathological diagnosis, neuro-imaging data (CT and/or MRI scans), duration of survival calculated from the date of histopathological diagnosis of GB to the date of death (actual survival time) or to the date of last follow-up or date of analysis (censored survival time), the extent of surgical removal as recorded from the operative reports, the existence of a less malignant precursor lesion and type of adjuvant therapy.

   B. For topographical purposes each case was **histopathologically subdivided** into tumor tissue proper (TTP) and periglioblastomatous tissue (PGBT) i.e. tissue surrounding the GB. The latter consisted either of a lower grade glioma (astrocytoma or anaplastic astrocytoma) or of infiltrated brain parenchyma (zone of infiltration, ZI). In the infiltration zone two distinct subzones could be distinguished.
Tumor tissue proper (TTP)

Tumor cells in contact with each other (no intervening normal parenchyma) accompanied by microvascular proliferation.

Zone of infiltration (ZI)

Tumor cells no longer in contact with each other (intervening normal parenchyma) either heavily crowded with associated oedema and microvascular proliferation (zone of dense infiltration, ZDI) or dispersed and usually associated with oedema but without microvascular proliferation (zone of isolated tumor cells, ZITC).

Table II-1. Tumor tissue proper, zone of infiltration: histological criteria (modified from Daumas-Duport [57]).

Special interest was taken in the following features in the tumor tissue proper:

CELL DENSITY was qualified as low, moderate or high, as estimated in the most cellular regions.

CELLULAR COMPOSITION Somewhat modified and extended from Giangaspero and Burger [83] and Burger and Green [38], at least 9 neoplastic cell types were recognized. Admitted the somewhat artificial position of a few of the following cell types, they were thought useful for descriptive purposes and exemplify in their diversity the well-known cellular heterogeneity. The numerically predominant cell type is underlined.

1. Stellate cell (SC): medium-sized cell with a moderate amount of cytoplasm, several cytoplasmic processes in a random stellate configuration. The nucleus is slightly larger, darker and less regular than that of the normal counterparts.

2. Gemistocytic cell (GC): medium-sized cell with a copious amount of cytoplasm demonstrating short stubby processes emanating most clearly
from the abnuclear cell pole. The nucleus is eccentric and its chromatin density and shape resemble that of the SC.

3. Less differentiated cell (LDC): medium-sized stellate cell with less developed cytoplasmic processes and a somewhat larger, more hyperchromatic and irregular nucleus.

4. Pleomorphic cell (PC): medium-sized cell with still visible cytoplasm with small or inconspicuous cytoplasmic processes. The nucleus is darker, larger, and more irregular than that of the SC and GC.

5. Large bizarre cell (LBC): large-to-giant cell with often a prominent amount of cytoplasm and usually displaying multilobulation, multinucleation or other nuclear monstruosities.

6. Small anaplastic cell (SAC): small-sized cell with scanty cytoplasm without visible cytoplasmic processes, with an irregular hyperchromatic nucleus. Regressive events, e.g., vasogenic edema, can give the SAC a somewhat stellate aspect.

7. Large anaplastic cell (LAC): similar to SAC except for the larger size (approximately twice that of the SAC).

8. Small fusiform cell (SFC): only differing from the SAC in the still visible cytoplasm extending into fine bipolar cytoplasmic processes.

9. Large fusiform cell (LFC): large bipolar cell with a considerable amount of cytoplasm with a corresponding large, hyperchromatic nucleus (approximately twice that of SFC).

HISTOARCHITECTURE Following patterns were recognized: amorphous cell arrangement, fascicular pattern, stellate cells in myxoid stroma [43] and astroblastic pseudorosettes.

REGRESSIVE CHANGES Necrosis, calcification and intratumoral hemorrhage. The type (coagulative or colliquative) and geographic extent
of the necrosis were specified. In addition, the presence of perinecrotic pseudopalisading and of cicatriziation (scarring) with or without neoplastic reinvasion was recorded.

VASCULATURE was described as simple endothelial cell (EC) hypertrophy or EC hyperplasia (single layer) or multilayered vascular proliferation (MVP) or glomeruloid multilayered vascular proliferation (GMVP) with designation of the extent noted as present only very focally, in a few low power fields (LPF i.e. 10 x 10), in many LPFs or in most LPFs. Other vascular changes recorded were: thrombosis, collagenous thickening, necrosis, networks of proliferating vessels, abnormal dilated vessels (lacunar ectasia) and perinecrotic, peritumoral or leptomeningeal vascular walls. The presence of perivascular lympho(plasmo)cuffing was recorded.

MITOTIC ACTIVITY The maximum number of mitotic figures in 10 high power fields (HPF i.e. 10 x 40) was recorded. The presence of atypical mitotic figures (multipolar spindles, large and/or puny spindles) was noted. TUMOR BORDER was recorded either as not visible or as well defined or ill defined.

Special attention was paid to following features in the periglioblastoma tissue:

CELL DENSITY was noted as normal cellularity, low or moderately hypercellular.

CELLULAR COMPOSITION: SC, GC, PC, LDC, SAC, SFC, LAC, LFC.
HISTOARCHITECTURE was described as either normal brain parenchyma (cortex, white matter or both) or tumoral tissue pattern when consisting of a low-grade or anaplastic astrocytoma.

The presence of secondary structures as defined by Scherer [194] was noted.

REGRESSIVE CHANGES included microcystic degeneration, calcification.

VASCULATURE was recorded as either unremarkable when no obvious changes were observed or as abnormal: the extent and degree of microvascular proliferation was then noted.

TUMOR BORDER was recorded as not visible, well- or ill defined.

GROWING ACTIVITY was expressed as either SG or focal FG (table 1-2).

MITOTIC ACTIVITY: the maximum number of mitotic figures in 10 HPFs was recorded.

When there was histopathological evidence of a low-grade precursor, following features were studied:

CELL DENSITY was qualified as low or moderate hypercellular.

CELLULAR COMPOSITION: SC, GC, PC, and LDC.

HISTOARCHITECTURE: fibrillary texture, perivascular bushes. The presence of secondary structures was noted.

REGRESSIVE CHANGES: microcystic change, microcalcification.

VASCULATURE was recorded as either unremarkable when no obvious changes were observed or as abnormal: the extent and degree of microvascular proliferation was then described.
MITOTIC ACTIVITY: the maximum number of mitoses counted in 10 HPFs was noted.

GROWING ACTIVITY was expressed as either diffuse SG or focal FG.

TUMOR BORDER was recorded as invisible or as well- or ill defined.

DIAGNOSIS included oncotype and grade of malignancy according to the revised WHO classification [128].
PM.II-1. Cytological heterogeneity in GB: (a) stellate cell; (b) gemistocytic cell; (c) less differentiated cell; (d) pleomorphic cell; (e) large bizarre cell; (f) small fusiform cell; (g) large fusiform cell; (h) small anaplastic cell; (i) large anaplastic cell (MT x 650).
PM II-2. (a) fibrillary texture (MT x 160); (b) feutrage périvasculaire (MT x 160); (c) amorphous cell arrangement (MT x 160); (d) fascicular pattern (MT x 60); (e) stellate cells in a myxoid stroma (MT x 200); (f) astroblastic pseudorosette (MT x 200); (g-i) vascular walls: (g) perinecrotic (MT x 25), (h) peritumoral (MT x 25), (i) leptomeningeal (MT x 60).
PM II-3. (a-b) Coagulative necrosis: (a) small, serpentine pseudopalisading type (MT x 60), (b) large, geographic type (MT x 25). (c) Colliquative necrosis (MT x 25). (d) Cyst due to liquefaction (MT x 25). (e) Cicatization of necrotic area (MT x 60). (f) Reinvasion of organised necrotic area (MT x 60). (g-i) Secondary gliomatous structures: (g) perivascular (MT x 160), (h) perineuronal (MT x 160), (i) subpial (MT x 60).
PM.II-4. Vascular changes in GB. (a) EC hypertrophy (HT) and EC hyperplasia (HP) (MT x 160). (b) MIB-1 immunostain showing proliferating endothelial cells (x 160). (c) MVP (MT x 160). (d) GMVP (MT x 160). (e) High proliferative activity in a glomeroid tuft (MIB-1 x 160). (f) Vascular network (MT x 120). (g) Lacunar ectasia (arrow: desintegrating tumor vessel) (MT x 25). (h-i) Thrombosis (MT x 25).
C. Morphometrical analysis was performed using a Zeiss microscope with following optics: eyepiece KPL-W10x, objective 40/0.65, field of view index 18, and diameter 0.45mm. For counting, a Zeiss eyepiece containing three squares was employed with selection of the largest square, covering a surface of 0.0576 mm² hereafter referred to as high power field (HPF). In this study, all measurements were performed on MIB-1 immunostained tissue sections for reasons of identical HPFs.

In each clinical case, and in both TTP and PGBT, at least one thousand nuclei (N) were counted from areas showing, de visu, the highest density of MIB-1 immunolabeled nuclei. The number of HPFs (n) and of immunopositive nuclei (PN) counted was recorded. Endothelial and haematopoietic cells, when recognized, were excluded from the count. The three recorded data defined the morphometric parameters of interest: (1) cell density (CD): N/n, (2) the MIB-1 cell proliferation index expressed as a percentage (PCI): PN/N x 100%, and (3) a deduced MIB-1 proliferating cell index (PCI/HPF): PN/n. As the latter only reckoned with the number of HPFs, this parameter could also be used when the PGBT consisted of normal brain parenchyma containing isolated tumor cells, whereas the MIB-1 PCI was restricted to tumor tissue as immunonegative nuclei could not always be clearly distinguished from nuclei in normal or reactive glia cells.

The aforementioned data, although ideal for comparison, did not depict accurately the PGBT. The variations from field to field were ruled out by choosing the areas of highest density of PN. Successive HPFs were

---

1 Counting a high number of cells from areas showing the highest density of immunolabeled nuclei, was in view of the regional variation in proliferative potential preferred above at random sampling which may not only lead to an underestimation of the growth fraction by including less active tumor areas, but also reduces the comparability of cell kinetic studies [146, 169].
counted on five randomly chosen tracks as perpendicular as possible to the pial surface\textsuperscript{2} starting from the TTP outwards, expressing the number of nuclei (N) and positive nuclei (PN) as absolute values per HPF (n = 1). The extent of the ZI was morphometrically defined as the highest number of HPF in a single track in which: PN\textgeq1 or stricter PN\textgeq3.\textsuperscript{3}

In the ZDI, the CD is constantly above the upper 95\% reference range limit of normal brain tissue; in the ZITC, CD is normal with PN\textgeq1 or PN\textgeq3. As we were dealing with successive HPFs, the extent of infiltration could be expressed in mm by multiplying the number of HPFs by 0.24 mm, the length of a single HPF. After correction for tissue shrinkage due to fixation and histological processing [33, 184], in vivo estimates of the extent of infiltration were provided.

Quantitative reference data were obtained from 10 astrocytomas (WHO grade II), 10 anaplastic astrocytomas (WHO grade III) and from 3 normal postmortem brains of patients who died from non-neoplastic, non-neurological disease. The astrocytomas and anaplastic astrocytomas were investigated in the same manner as the TTP. As normal brain tissue shows no positive staining for MIB-1, the cortex was studied quantitatively by the method employed for the PGBT with successive HPFs. Three anatomical areas were explored: frontal, parieto-occipital and temporal. With routine staining methods it was not possible to distinguish, reliably and in all cases, neuroglia from neurons, in particular from small non-pyramidal cells, it was decided to count all cells irrespective their neuronal or glial nature.

\textsuperscript{2}Because of the well-known diffuse character of the surrounding lower-grade glioma in SGs counting has been limited to 15 HPFs.

\textsuperscript{3}In the absence of a double GFAP/MIB-1 immunostaining it was impossible to differentiate in all cases neoplastic from reactive PN. Therefore by requiring at least 3 PN/HPF, seen the low proliferative capacity of reactive astrocytes in adults [133, 163], a more accurate depletion of the extent of the ZI was provided.
Thus, total neuronal and neuroglial cell counts were made in successive HPFs of five randomly chosen full-thickness cortical strips cut as perpendicular as possible to the pial surface and passing to the subcortical white matter. CD was expressed as the absolute number of nuclei per HPF (n = 1). In the white matter, which appeared more homogeneous, at least 1000 neuroglia cells were counted at random in each of the anatomical areas of the three postmortem brains with CD = N/n.
3. **Staining methods**

**Routine staining methods**

10% formalin-fixed surgical specimens were embedded in paraffin wax and cut into 5 μm thick sections. These were stained with hematoxylin-eosin (nuclear detail) and Masson Trichrome (cytoplasmic detail).

**Immunohistochemical staining methods**

Several markers were immunostained with common immunoperoxidase techniques using commercial monoclonal antisera for vimentin (DAKO), the Ki-67 epitope (Immunosource Medac) and the polyclonal antiserum provided by the laboratory of Prof. dr. Sindic (UCL) for glial fibrillary acidic protein (GFAP). Ki-67 (Kiel University, Germany, 67th well of the tissue culture plate), a Ig G1 mouse monoclonal antibody generated against the crude nuclear fraction of a Reed-Sternberg derived cell line [81], has been found to recognize a non-histone nuclear protein of a composed bimolecular complex of 345 and 395 kD expressed in all active phases of the cell cycle (G1, S, G2, M-phase) [80, 81, 82] except in nutritionally deprived cells where an inappropriate loss of the Ki-67 defined nuclear antigen has been reported [227]. A major impediment to the routine clinical use of this antibody appeared the extreme sensitivity of the Ki-67 protein for chemical denaturation limiting its application to fresh or frozen tissue [46]. However, microwave [207] or autoclave heating [235] of paraffin-embedded histological sections has shown to reactivate an epitope of the Ki-67 defined nuclear antigen which can be recognized by the newly developed monoclonal antibody, MIB-1 (Institute of Molecular Immunology Borstel, Germany), directed against a recombinant fragment
of the Ki-67 defined antigen [46]. Immunolabeled nuclei are readily identified by this method showing diffuse and/or granular staining.

After deparaffinization in xylene, the histologic sections were rehydrated in graded series ethanol to water. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 minutes. After rinsing with phosphate-buffered saline (PBS), the sections were preincubated for 30 minutes at room temperature with either normal goat serum, diluted 1:10 in PBS containing 1% bovine serum albumin (vimentin, MIB-1 immunohistochemistry) or with normal swine serum diluted 1:20 in PBS (GFAP immunohistochemistry) and subsequently incubated overnight at 4°C with either anti-vimentin antibody, MIB-1 antibody, or anti-GFAP antibody, diluted 1:100 in PBS. After rinsing in PBS, the sections were incubated for 30 minutes with either biotinylated goat anti-mouse antibody, diluted 1:500 in PBS (vimentin, MIB-1 immunohistochemistry) or with swine anti-rabbit antibody, diluted 1:20 in PBS (GFAP immunohistochemistry). Following rinsing in PBS, the sections were labeled for 30 minutes at room temperature with either streptABComplex diluted 1:500 (vimentin, MIB-1 immunohistochemistry) or with horseradish peroxidase rabbit antiperoxidase (PAP) complex diluted 1:50 (GFAP immunohistochemistry) and developed with diaminobenzidine for 7 to 10 minutes. After rinsing, counterstaining was done with hematoxylin of Mayer for five minutes.

4 For MIB-1 immunostaining microwave heating was necessary: the sections were placed in a plastic box containing 250ml 0.01M citric acid monohydrate adjusted to pH 5.7 with NaOH, heated in a microwave oven for 3 minutes at 750W and 4 times 3 minutes at 350W, and cooled down for 15 minutes at room temperature.
4. Statistical analysis

One-way analysis of variance (ANOVA) with unequal sample size was used to analyze differences in normal brain tissue CD between the three anatomical areas studied. Differences in the distribution of patient age and morphometrical parameters between different histopathological grades and between morphological subgroups were evaluated using the Mann-Whitney U test. The relationship between PCI and PCI/HPF in each of the three histopathological grades was evaluated by calculating Spearman’s rank correlation coefficient and tested for significance by comparing with tabulated critical values [3]. Fischer’s exact test and Pearson’s Chi-squared test were respectively employed to determine whether there were significant differences in treatment or anatomical location between morphological subgroups. Survival curves were generated with the Kaplan-Meier product limit estimator and compared using the Mantel-Cox-Savage logrank test. The influence of one or more variables on survival was tested by the univariate and multivariate analyses of Cox’s proportional hazards regression model. All P-values reported are two-tailed and were considered statistically significant when P < 0.05.
CHAPTER III

RESULTS
1. Casuistics

CASE 1 (B17414)

1. Clinical data

Age 66.6.
Sex Male.
Location Left temporal lobe.
Clinical history Seizures, receptive dysphasia.
Duration of symptoms One month (seizures), few days prior to admittance (aphasia).
Neuro-imaging Irregular rim-enhancing mass lesion.
Lower-grade precursor No.
Duration of survival 6 months (04.01.93 - 05.07.93).
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, PC, GC, SC.
Histoarchitecture Amorphous arrangement.
Regression changes Central large area of necrosis with partial perinecrotic pseudopalisading, multiple small-to-medium-sized foci with complete perinecrotic pseudopalisading.
Vascularity EC hypertrophy and EC hyperplasia in most LPF, MVP present in many LPF, GMVP in a few LPF, perinecrotic and peritumoral vascular walls, networks of proliferating vessels, thrombosis, dilated and necrotic tumor vessels.
Mitotic activity > 20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglialblastomatous tissue

Cell density Normal.
Cellular composition SAC, LAC.
Histoarchitecture Cortex. Discrete perivascular structures.
Vasculature MVP in a few LPF (immediate PGBT).
Mitotic activity <10/10 HPF.

3. Morphometrical data

A. Tumor tissue proper

\[
\text{CD} = \frac{1049}{6 \text{ HPF}} = 174.8/\text{HPF}
\]
\[
\text{PCI} = 268/1049 = 25.5\% \ (44.7/\text{HPF})
\]

B. Periglialblastomatous tissue

\[
\text{CD} = \frac{1029}{19 \text{ HPF}} = 54.2/\text{HPF}
\]
\[
\text{PCI/HPF} = \frac{92}{19} = 4.8/\text{HPF}
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Table III-1. Number N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-1. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-1. Outside the TTP, CD progressively decreases as a function of distance, reaching "normal" values in all tracks, 5 HPFs beyond the outer border of the TTP. Comparison between the means of normal CD (46.1, cf. pages 179-180) and PGBT (54.2) indicates however a slight increase, "borderline normocellularity", due to infiltration of isolated tumor cells. The close parallelism between both graphs suggests that much of the "increase" in CD is due to PN, PN are discernable as far as the molecular layer. (Reference lines represent: mean CD and PCI/HPF of AA and A (short and long broken line), 95% upper reference range limit of normal brain tissue CD (solid line)).
PM. III-1. Case 1. (a) GB arising in the background of normal, albeit infiltrated parenchyma. Between the TTP and ZITC, a relatively narrow ZDI is observed (MT x 220). (b-c) MIB-1 immunostains focusing on (b) the TTP and the ZDI, on (c) the ZDI and ZITC (MIB-1 x 220) (arrow: peritumoral MVP).
CASE 2 (B17778)

1. Clinical data

Age 37.3.
Sex Male.
Location Right frontal lobe.
Clinical history Headache, gait difficulties.
Duration of symptoms < 1 month.
Neuro-imaging Irregular rim-enhancing mass lesion.
Lower-grade precursor No.
Duration of survival >44 months (13.05.93 - still alive on 04.01.97).
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

-------------------------------
Cell density High.
Cellular composition SAC, LAC, SFC, LFC, GC, SC, PC, LBC.
Histochmarchitecture Amorphous arrangement.
Regressive changes Several large confluent areas of necrosis with partial perinecrotic pseudopalisading.
Vascuclature EC hypertrophy and EC hyperplasia present in most LPF, MVP in many LPF, GMVP only very focally, perinecrotic vascular wall, networks of proliferating vessels, thrombosis, collagenous thickening, dilated and necrotic tumor vessels.
Mitotic activity > 20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglio-blastomatous tissue

Cell density Low (SG area) - moderate (FG area) hypercellularity.
Cellular composition SC, rare GC (SG area) - SC, GC, PC, LDC (FG area).
Histarchitectue Tumoral tissue pattern (fibrillary texture, perivascular bushes).
Regressive changes Absent.
Vasculature Unremarkable.
Tumor border Ill-defined.
Growing activity Focal FG.
Mitotic activity <10/10HPF (FG area).

3. Morphometrical data

A. Tumor tissue proper

CD = 1034/6HPF = 172.3/HPF
PCI = 379/1034 = 36.7% (63.2/HPF)

B. Periglio-blastomatous tissue

CD = 1069/12HPF(SG) = 89.1/HPF (SG) - 1059/9HPF(FG) = 117.7/HPF (FG)
PCI = 36/1069(SG)= 3.4% (3/HPF)(SG) - 96/1059(FG) = 9.1%(10.7/HPF)(FG)
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Table III-2. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5) starting at the outer border of the TTP (= HPF 1).
Fig. III-2. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-2. Outside the TTP, CD rapidly reaches a "plateau" of low to moderate hypercellularity corresponding morphometrically to astrocytoma. The low PCI/HPF values, representative for astrocytoma, indicate that PN are responsible for only a small fraction of the increase in CD. No FG (anaplastic) area was explored by the tracks.
(a) Hypercellular rim of the TTP surrounding the central large ischaemic necrosis (MT x 120).
(b) Marked proliferative activity in the TTP (MIB-1 x 120). (c-f) Surrounding lower-grade astrocytic tumor: (c) well-differentiated fibrillary astrocytoma (arrow: preserved neuron, MT x 280) showing (d) low proliferative activity (MIB-1 x 280);
(e) anaplastic focus characterized by increased cellularity, nuclear atypia and mitotic activity (MT x 280), (f) moderate proliferative activity in the anaplastic focus (MIB-1 x 280).
CASE 3 (B17941)

1. Clinical data

Age 66.9.
Sex Female.
Location Right temporal lobe.
Clinical history Headache, memory loss, vomiting.
Duration of symptoms One month.
Neuro-imaging Irregular rim-enhancing mass lesion with secondary mass effect (uncal herniation).
Lower-grade precursor No.
Duration of survival 3 months (14.07.93 - 19.10.93).
Extent of surgical removal Subtotal resection.
Adjuvant therapy No.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, SFC, LFC, PC, GC, SC.
Histoarchitecture Fascicular pattern.
Regression changes Large central area of necrosis, multiple small foci with complete perinecrotic pseudopalisading, cicatrization with reinvasion.
Vascularity EC hypertrophy and EC hyperlasia in most LPF, MVP present in many LPF, GMVP only very focally, ectasia, thrombosis, necrosis, collagenous thickening.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglio blastomatous tissue

Cell density Normal.
Cellular composition SAC, LAC.
Histoarchitecture Cortex. Discrete perivascular structures.
Vasculature EC hypertrophy and EC hyperplasia present in a few LPF, MVP only very focally (immediate PGBT).
Mitotic activity <10/10HPF.

3. Morphometrical data

A. Tumor tissue proper

CD = 1007/5HPF = 201.4/HPF
PCI = 293/1007 = 29.1% (58.6/HPF)

B. Periglio blastomatous tissue

CD = 1041/18HPF = 57.8/HPF
PCI/HPF = 92/18HPF = 5.1/HPF
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Table III-3. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of TTP (= HPF 1).
Fig. III-3. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-3. Beyond the ZDI, extending as far as the 7th HPF (T4, T5), CD falls within the reference range of normal cortex. From the close parallelism between both graphs it can be inferred that PN account for a considerable part of the borderline normocellularity. PN are identifiable just beneath the pial surface.
PM III-3. Case 3. (a-b) Low power magnification showing TTP, ZDI and ZITC (MT x 100, MIB-1 x 100). (c) Heavily crowded tumor cell infiltrate in oedematous subcortical white matter (MT x 280). (d) Isolated tumor cells infiltrating the overlying cortex (MT x 280). (e) Astroglisis as demonstrated by the GFAP immunostain (x 100). (f) MIB-1 immunopositive reactive astrocyte. Note the fine granular staining pattern, the enlarged though regular nucleus and the radiating cytoplasmic processes (x 280).
CASE 4 (B18053)

1. Clinical data

Age 50.7
Sex Female.
Location Right occipital lobe.
Clinical history Severe headache.
Duration of symptoms < 1 month.
Neuro-imaging Rim-enhancing mass lesion with secondary mass effect.
Lower-grade precursor No.
Duration of survival >23 months (25.08.93 - last follow-up: 26.07.95)
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, SFC, LFC, PC, GC, SC.
Histoarchitecture Fascicular pattern.
Regressive changes Large central area of necrosis with partial perinecrotic pseudopalisading, a few small foci with complete perinecrotic pseudopalisading.
Vascuature EC hypertrophy and EC hyperplasia present in many LPF, MVP present in a few LPF, GMVP only very focally, perinecrotic vascular wall, networks of proliferating vessels, thrombosis, dilated and necrotic tumor vessels.
Mitotic activity >20/HPF, atypical mitotic figures.
Tumor border Well-defined.

B. Periglio blastomatous tissue

Cell density Normal.
Cellular composition SAC, SFC.
Histoarchitecture Cortex, Subpial structures.
Vascuature Unremarkable.
Mitotic activity < 10/10HPF.
3. Morphometrical data

A. Tumor tissue proper

CD = 1137/4HPF = 284.2/HPF
PCI = 317/1137 = 27.9% (79.2/HPF)

B. Periglioastomatous tissue

CD = 1031/17HPF = 60.6/HPF
PCI/HPF = 88/17HPF = 5.2/HP
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Table III-4. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5) starting at the outer border of TTP (= HPF 1).
Fig. III-4. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-4. This GB shows a relatively broad ZDI extending as far as the 10th HPF (T4). The increased CDs and high number of PN found in HPF's 14, 16 and 17 indicate subpial secondary structures.
PM. III-4. Case 4. (a) TTP and ZDI (MT x 160), (b) ZITC with subpial secondary structure (MT x 160). (c) MIB-1 immunostain demonstrating the TTP and the ZDI (x 160). (d) Scattered PN, accumulating in the subpial zone of the molecular layer (MIB-1 x 160).
CASE 5 (18614)

1. Clinical data

Age 33.4.
Sex Male.
Location Left frontal lobe with extension into left insular and contralateral frontal lobe.
Clinical history Headaches, personality changes (apathy, disinhibition).
Duration of symptoms 6 months.
Neuro-imaging Large, ill-defined hypointense mass lesion with secondary mass effect. Focal enhancement.
Lower-grade precursor No.
Duration of survival > 32 months (12.04.94- still alive on 04.01.97).
Extent of surgical removal Subtotal removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density Moderate.
Cellular composition GC, SC, PC, SAC, LAC, SFC, LFC, LBC.
Histarchitecture Myxoid appearance, astroblastic pseudorosettes.
Regressive changes Small cyst.
Vascularity EC hypertrophy and EC hyperplasia present in most LPF, MVP present in many LPF, GMVP in a few LPF, networks of proliferating vessels, perinecrotic vascular wall, thrombosis, necrosis.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglio blastomatous tissue
----------------------------------
Cell density Low (SGarea) - Moderate (FG area).
Cellular composition SC, GC, LDC, SAC and LAC (immediate periGB area).
Hist架构ture Tumoral tissue pattern (fibrillary texture, perivascular bushes).
Regressive changes Microcystic degeneration.
Vasculature EC hypertrophy and EC hyperplasia present in many LPF with MVP in a few LPF in the immediate periglio blastomatous area.
Tumor border Ill-defined.
Growing activity Focal FG.
Mitotic activity < 10/10HPF (FG area).

3. Morphometrical data

A. Tumor tissue proper
-----------------------
CD = 1065/7HPF = 152.1/HPF
PCI = 414/1065 = 38.9% (59.1/HPF)

B. Periglio blastomatous tissue
-------------------------------
CD = 1012/11HPF = 92.0/HPF (SG) - 1005/8HPF = 125.6/HPF (FG)
PCI = 46/1012 = 4.5% (4.2/HPF) (SG) - 82/1005 = 8.2% (10.2/HPF) (FG)
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Table III-5. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-5. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-5. Outside the TTP, CD rapidly reaches a plateau corresponding morphometrically to astrocytoma. Equally representative PCI/HPF values are noted, accounting for only a small part of the increase in CD.
PM III-5. Case 5. (a) GB arising in the background of a lower-grade astrocytic tumor (MT x 120). (b) Corresponding MIB-1 immunostain highlighting the focal nature of the TTP (x 120). (c-f). Detail of the surrounding lower-grade astrocytic tumor showing in (c-d) a well-differentiated fibrillary astrocytoma (MC: microcystic degeneration, MT x 280, MIB-1 x 280), in (e-f) an anaplastic focus characterized by increased cellularity, mitotic activity and nuclear atypia (MT x 280, MIB-1 x 280).
CASE 6 (18703)

1. Clinical data

Age 56.1.
Sex Male.
Location Right temporal lobe.
Clinical history Headache, seizures, memory disturbances.
Duration of symptoms < 1 month.
Neuro-imaging Irregular rim-enhancing mass lesion with peripheral hypodensity.
Lower-grade precursor No.
Duration of survival 9.5 months (20.05.94 - 07.03.95)
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, SFC, LFC, PC, SC.
Histoarchitecture Amorphous arrangement.
Regressive changes Large central area of necrosis with partial perinecrotic pseudopalising, multiple small-to-mediumsized foci with complete perinecrotic pseudopalising.
Vasculature EC hypertrophy and EC hyperplasia in most LPF, MVP present in many LPF, networks of proliferating vessels, thrombosis, necrosis, ectasia.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglioablastomatous tissue

Cell density Normal.
Cellular composition SAC, LAC.
Histoarchitecture Cortex.
Vascular EC hypertrophy and EC hyperplasia in many LPF with MVP only very focally (immediate PGBT).
Mitotic activity <10/10HPF.

3. Morphometrical data

A. Tumor tissue proper

\[ CD = \frac{1202}{5} \text{HPF} = 240.4/\text{HPF} \]
\[ PCI = \frac{371}{1202} = 30.9\% (74.2/\text{HPF}) \]

B. Periglioablastomatous tissue

\[ CD = \frac{10356}{19} \text{HPF} = 54.5/\text{HPF} \]
\[ PCI/\text{HPF} = \frac{116}{19} \text{HPF} = 6.1/\text{HPF} \]
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Table III-6. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-6. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-6. In this GB showing the narrowest ZDI (2 HPFs beyond the outer border of the TTP), PN could be traced as far as 38 HPFs, eventually halted by the pia mater. Note the close parallelism between both graphs.
PM. III-6. Case 6. (a-b) Overview showing the TTP, the transitional ZDI and the ZITC (MT x 260, MIB-1 x 260).
CASE 7 (18769)

1. Clinical data

Age 61.2.
Sex Female.
Location Left frontal lobe.
Clinical history Expressive dysphasia, personality changes (apathy, emotional lability), contralateral hemiparesis.
Duration of symptoms Progressive deterioration mentally in a few months, aphasia and hemiparesis 2 weeks prior to admittance.
Neuro-imaging Ring-enhancing mass lesion with secondary mass effect.
Lower-grade precursor No.
Duration of survival 8 months (08.06.94 - 09.02.95).
Extent of surgical removal Subtotal resection.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition LAC, SAC, PC, GC, SC, SFC, LFC.
Histoarchitecture Stellate cells in a myxoid stroma.
Regenerative changes Large central area of necrosis with partial perinecrotic pseudopalisading, several small foci with complete perinecrotic pseudopalisading.
 Vasculature EC hypertrophy and EC hyperplasia present in most LPF, MVP present in many LPF, GMVP in a few LPF, networks of proliferating vessels, peritumoral and leptomeningeal vascular wall, thrombosis, necrosis.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglioblastomatous tissue

Cell density Normal.
Cellular composition SAC, LAC.
Histoarchitecture Cortex, white matter.
Vascular EC hypertrophy EC hyperplasia in most of the LPF with MVP in a few LPF (immediate PGBT).
Mitotic activity <10/10HPF.

3. Morphometrical data

A. Tumor tissue proper

CD = 1109/6 HPF = 184.8/HPF
PCI = 401/1109 = 36.2% (66.8/HPF)

B. Periglioblastomatous tissue

CD = 1026/20HPF = 51.3/HPF (cortex) - 1070/16HPF = 66.9/HPF (white matter)
PCI/HPF = 69/20HPF = 3.4/HPF (cortex) - 53/16HPF = 3.3/HPF (white matter)
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Table III-7A. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1). Cortex.
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Table III-7B. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF1). White matter.
Fig. III-7A. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-7 A. At one place, the tumor deeply infiltrated the overlying cortex with PN discernable in the subpial zone of the molecular layer (HPF 12, T4). Outside the TTP, a progressive decrease in CD is noted, reaching normal values in all tracks in HPF 6.
Fig. III-7B. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-7 B. At another place, arcuate fibers seem to temporarily halt the peripheral infiltration, showing a narrow ZDI (3 HPFs) and few PN outside the TTP.
PM III-7. Case 7. Diffuse infiltration of the adjacent cortex. (a) Overview showing the TTP, the ZDI and the ZITC. Note the marked peritumoral oedema (MT x 180). (b-d) Higher power magnification of (b) the TTP, (c) the ZDI and (d) the ZITC (MT x 470) (arrows: reactive astrocytes).
PM III-8. Case 7. (a-d) Circumscribed infiltration in the subcortical white matter showing a narrow ZDI and few ITC (MT x 180 (a), MIB-1 x 180 (b), MT x 470 (c), MIB-1 x 470 (d)).
CASE 8 (19040)

1. Clinical data

Age 35.7.
Sex Female.
Location Left temporal lobe.
Duration of symptoms 12 months (WHO grade II) - 84 months (WHO grade IV).
Duration of survival > 27 months (09.09.94 - still alive on 04.01.97).

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, GC, PC, SC, LBC, SFC, LFC.
Histoarchitecture Amorphous arrangement.
Regressive changes Calcification, hemorrhage.
Vasculature EC hypertrophy and EC hyperplasia present in most LPF, MVP present in many LPF, GMVP in a few LPF, networks of proliferating vessels.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border III-defined.
B. Periglio blastomatous tissue

Cell density Low (SG area) - Moderate (FG area).
Cellular composition SC, PC (SG area) - GC, SC, PC, LDC (FG area).
Histoarchitecture Tumor tissue pattern (fibrillary texture, perivascular bushes).
Regressive changes Calcification.
Tumor border Not visible.
Growing activity Focal FG.
Vascularity EC hypertrophy and EC hyperplasia only very locally.
Mitotic activity <10/10HPF

C. Low-grade precursor.

Cell density Low.
Cellular composition SC, GC.
Histoarchitecture Amorphous arrangement (fibrillary texture, perivascular bushes).
Regressive changes Absent.
Vascularity Unremarkable.
Mitotic activity <10/10HPF.
Growing activity Diffuse SG.
Tumor border Ill-defined.
3. Morphometrical data

A. Tumor tissue proper

\[ CD = \frac{1043}{5} \text{HPF} = 208.6/\text{HPF} \]
\[ PCI = \frac{274}{1043} = 26.3\% (54.8/\text{HPF}) \]

B. Periglialblastomatous tissue

\[ CD = \frac{1038}{11} \text{HPF} = 94.4/\text{HPF} \text{ (SG area)} - \frac{1068}{9} \text{HPF} = 118.7/\text{HPF} \text{ (FG area)} \]
\[ PCI = \frac{50}{1038} = 4.8\% (4.5/\text{HPF}) \text{ (SG area)} - \frac{130}{1068} = 12.2\% (14.4/\text{HPF}) \text{ (FG area)} \]

C. Lower-grade precursor

\[ CD = \frac{1001}{11} \text{HPF} = 91.0/\text{HPF} \text{ (1988)} - \frac{554}{6} \text{HPF} = 92.3/\text{HPF} \text{ (1992)} \]
\[ PCI = \frac{61}{1001} = 6.1\% (5.5/\text{HPF}) \text{ (1988)} - \frac{31}{554} = 5.6\% (5.2)(1992) \]
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Table III-8. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of TTP (= HPF 1).
Fig. III-8. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in Table III-8. Outside the TIP, CDs were found to correspond morphometrically to anaplastic astrocytoma. The PCI/HPF values were somewhat intermediary between those of astrocytoma and anaplastic astrocytoma probably because of the relative high number of MIB-1 immunonegative gemistocytes.
Case 8. (a-b) Low-grade precursor: fibrillary astrocytoma (MT x 280, MIB-1 x 280). (c-d) Recurrent low-grade astrocytoma 4 years later (arrow: preserved neuron, MT x 280, MIB-1 x 280). (e-f) Dedifferentiation into GB 6 years after diagnosis of fibrillary astrocytoma (MT x 120, MIB-1 x 280).
PM.III-10. Case 8. (g-h) Vestiges of the preexisting low-grade astrocytoma. (i-j) Anaplastic focus showing increased cellularity, nuclear atypia and proliferative activity (MT x 280, MIB-1 x 280). (k-l) Microcalcifications in the surrounding low-grade astrocytoma (MT x 280, MIB-1 x 280).
CASE 9 (19041)

1. Clinical data

Age 69.4.
Sex Male.
Location Left temporal lobe.
Clinical history Headache, receptive dysphasia, drowsiness.
Duration of symptoms < 1 month.
Neuro-imaging Irregular rim-enhancing mass lesion.
Lower-grade precursor No
Duration of survival 1 month (09.09.94 - 13.10.94).
Extent of surgical removal Gross total removal.
Adjuvant therapy No.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SFC, LFC, SAC, GC, PC, SC.
Histoarchitecture Largely amorphous arrangement, fascicular pattern focally.
Regenerative changes Central large area of necrosis with partial perinecrotic pseudopalisading, a few small foci with complete perinecrotic pseudopalisading.
Vascularature EC hypertrophy and EC hyperplasia present in most LPF, MVP present in many LPF, GMVP only very focally, networks of proliferating vessels, thrombosis, ectasia, and necrosis.
Mitotic activity 10-20/10HPF.
Tumor border Well-defined.

B. Periglioblastomatous tissue

Cell density Normal.
Cellular composition SAC, SFC.
Histoarchitecture Cortex. Discrete perivascular structures.
Vascularature EC hypertrophy and EC hyperplasia in many of the LPF with MVP in a few LPF (immediate PGBT).
Mitotic activity < 10/10HPF.
3. Morphometrical data

A. Tumor tissue proper

\[ CD = \frac{1169}{6\text{HPF}} = 194.8/\text{HPF} \]
\[ PCI = \frac{207}{1169} = 17.7\% (34.5/\text{HPF}) \]

B. Periglioblastomatous tissue

\[ CD = \frac{1038}{20\text{HPF}} = 51.9/\text{HPF} \]
\[ PCI/\text{HPF} = \frac{109}{20} \text{ HPF} = 5.5/\text{HPF} \]

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Table III-9. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5) starting, at the outer border of TTP (= HPF 1).
Fig. III-9. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-9. Outside the TTP, CD rapidly decreases to normal values, the ZDI extending only as far as the 5th HPF (T3). In this GB, PN probably account for most of the increase in CD seen the relative high PCI/HPF values.
PM III-11. Case 9. (a) Overview showing TTP, ZDI and ZITC (MT x 100). (b-c) Higher power magnification of the TTP and ZDI (MT x 220, MIB-1 x 220).
CASE 10 (19074)

1. Clinical data

Age 35.9.
Sex Male.
Location Left fronto-temporal region.
Duration of symptoms < 1 month (WHO grade II) - 84.5 months (WHO grade IV).
Neuro-imaging Ill-defined area of homogenous low density (1987). Important increase in volume with secondary mass effect (midbrain compromise).
Focal ring enhancement posteriorly (1994).
Lower-grade precursor WHO grade II astrocytoma (primary tumor 1987)
Duration of survival > 10 months (19.09.94 - last follow-up: 10.07.95).

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, SFC, LFC, SC, GC, PC, LBC.
Histoarchitecture Amorphous cell arrangement, astroblastic pseudorosettes.
Regressive changes Calcification.
Vascularity EC hypertrophy present in many LPF, MVP in a few LPF small networks of proliferating vessels, marked perivascular lymphoplasmacellular infiltrates.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border III-defined.
B. Periglioblastomatous tissue

Cell density Low (SG area) - moderate (FG area) hypercellularity.
Cellular composition SC, PC, LDC.
Histoarchitecture Tumoral tissue pattern (fibrillary texture, perivascular bushes).
Regressive changes Calcification.
Tumor border Not visible.
Growing activity Focal FG.
Vascularization EC hypertrophy in a few LPF.
Mitotic activity <10/10HPF

C. Lower-grade precursor

Cell density Low.
Cellular composition SC, GC.
Histoarchitecture Amorphous arrangement (fibrillary texture, perivascular bushes).
Regressive changes Absent.
Vascularisation Unremarkable.
Mitotic activity Absent.
Growing activity Diffuse SG.
Tumor border Ill-defined.
Diagnosis WHO grade II fibrillary astrocytoma (1987)

3. Morphometrical data

A. Tumor tissue proper

\[
\begin{align*}
CD &= 1117/5\text{HPF} = 223.4/\text{HPF} \\
PCI &= 362/1117 = 32.4\% (72.4/\text{HPF})
\end{align*}
\]

B. Periglioblastomatous tissue

\[
\begin{align*}
CD &= 1039/12\text{HPF} = 86.6/\text{HPF} \text{ (SG area)} - 1110/9\text{HPF} = 123.5/\text{HPF} \text{ (FG area)} \\
PCI/\text{HPF} &= 56/1039 = 5.4\% (4.7/\text{HPF}) \text{ (SG area)} - 192/1110 = 17.3\% (21.3/\text{HPF}) \text{ (FG area)}
\end{align*}
\]
C. Lower-grade precursor.

\[ CD = \frac{1017}{12 \text{ HPF}} = 84.7/\text{HPF} \]
\[ PCI = \frac{52}{1017} = 5.1\% \ (4.3/\text{HPF}) \]

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Table III-10. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-10. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-10. The composite nature of the graphs, with CD and PCI/HPF values outside the TTP corresponding morphometrically to either astrocytoma or anaplastic astrocytoma, is due to the fact that tracks 1 and 2 passed through an anaplastic focus (FG area) whereas the other tracks explored more quiescent parts (SG area) of the surrounding lower-grade glioma.
PM.III-12. Case 10. (a-b) Low-grade precursor: paucicellular fibrillary astrocytoma (MT x 280, MIB-1 x 280). (c-d) Dedifferentiation into GB 7 years later. (c) Pronounced lymphoplasmacellular cuffing (MT x 100), (d) high proliferative activity (MIB-1 x 280). (e-f) Surrounding low-grade fibrillary astrocytoma showing a considerable number of gemistocytes (e) and a slightly increased proliferative activity (f) (MT x 280, MIB-1 x 280).
CASE 11 (19400)

1. Clinical data

Age 56.5.
Sex Female.
Location Right frontal lobe.
Clinical history Headache of recent onset increasing in severity and duration, drowsiness.
Duration of symptoms < One month (headache), depressed consciousness a few days prior to admittance.
Neuro-imaging Large, irregular rim-enhancing mass lesion (3.25 x 4.4 cm) with secondary mass effect. Small satellite area of enhancement (0.7 x 0.8 cm). Beginning subfalcine herniation.
Lower-grade precursor No.
Duration of survival 4.25 months (20.01.95 - 26.05.95).
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, SFC, LFC, GC, PC, SC.
Histarchitecture Amorphous arrangement.
Regressive changes Central large area of necrosis with partial perinecrotic pseudopalisading.
Vascularure EC hypertrophy and EC hyperplasia present in most LPF, MVP present in a few LPF, GMVP only very focally, networks of proliferating vessels, ectasia, thrombosis, and necrosis.
Mitotic activity 10-20/10HPF.
Tumor border Well-defined.
B. Periglioblastomatous tissue

Cell density Normal.
Cellular composition SAC.
Histoarchitecture Cortex. Discrete perivascular structures.
Vasculature EC hypertrophy and EC hyperplasia in many LPF with MVP in a few LPF (immediate PGBT).
Mitotic activity < 10/10 HPF.

3. Morphometrical data

A. Tumor tissue proper

\[ CD = \frac{1003}{6} HPF = 167.2/HPF \]
\[ PCI = \frac{253}{1003} = 25.2\% (42.2/HPF) \]

B. Periglioblastomatous tissue

\[ CD = \frac{1031}{19} HPF = 54.3/HPF \]
\[ PCI/HPF = \frac{107}{19} = 5.6/HPF \]
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Table III-11. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-11. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in Table III-11. Outside the TTP, CD progressively decreases. Normal values are reached in all tracks. 7 HPFs beyond the outer border of the TTP, defining the extent of the ZDI. PN are present as far as the subpial zone of the molecular layer. The close parallelism between both graphs suggests that most of the infiltrating tumor cells are proliferating cells.
PM.III-13. Case 11. (a) Low power magnification showing TTP, ZDI and ZITC (MT x 100). (b-c) MIB-1 immunostains focusing on (b) TTP and ZDI, (c) ZDI and ZITC (MIB-1 x 220).
CASE 12 (19443)

1. Clinical data

Age 40.
Sex Female.
Location Left frontal lobe.
Clinical history Unusual headache increasing in severity.
Duration of symptoms < 1 month.
Neuro-imaging Large, irregular rim-enhancing mass lesion (4 cm) with secondary mass effect. Satellite nodulus (2 cm) superiorly with homogeneous enhancement.
Lower-grade precursor No.
Duration of survival > 14 months (03.02.95 - last follow-up: 28.03.96).
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, GC, PC, SFC.
Histopathology Amorphous arrangement.
Regressive changes Large cyst.
Vascularity EC hypertrophy and EC hyperplasia present in many LPF, MVP in a few LPF, GMVP only very focally, networks of proliferating vessels, perinecrotic vascular wall, ectasia, thrombosis.
Mitotic activity < 10/10HPF
Tumor border Well-defined.
B. Periglioiblastomatous tissue

Cell density Low (SG area) - moderate (FG area) hypercellularity.
Cellular composition SC, GC, LDC (FG area).
Histoarchitecture Tumoral tissue pattern (fibrillary texture, perivascular bushes).
Regressive changes Microcystic degeneration (SG area)
Tumor border Ill-defined.
Growing activity Focal FG.
Vascuclature Unremarkable except for the immediate PGBT showing MVP
with focal GMVP.
Mitotic activity <10/10HPF (FG area).

3. Morphometrical data
A. Tumor tissue proper

\[
CD = \frac{1071}{6HPF} = 178.5/HPF \\
PCI = \frac{247}{1071} = 23.1\% (41.2/HPF)
\]

B. Periglioiblastomatous tissue

\[
CD = \frac{1082}{14\ HPF} = 77.3/HPF\ (SG) - \frac{1021}{8HPF} = 127.6/HPF\ (FG) \\
PCI = \frac{56}{1082} = 5.2\% (4.0/HPF)(SG) - \frac{158}{1021} = 15.5\% (19.7/HPF)(FG)
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Table III-12. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-12. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in Table III-12. Outside the TTP, CD rapidly reaches a plateau corresponding to astrocytoma. The higher CD and PCI/HPF values noted in the last three HPFs are due to the presence of a FG area (anaplastic focus) explored by track 2. Comparison between the mean CD of normal brain tissue and of PGBT, indicates that most of the increase in CD is due to non-cycling tumor cells.
PM.III-14. Case 12. (a) Low power magnification showing a highly anaplastic focus (TTP) in a lower-grade astrocytic tumor (MT x 50). (b) High proliferative activity in the TTP (MIB-1 x 280). (c-f) Higher power magnification of the "background" lower-grade astrocytic tumor: quiescent parts (c-d), anaplastic or faster growing parts (e-f) (MT x 280, MIB-1 x 280).
CASE 13 (19449)

1. Clinical data

Age 64.7.
Sex Female.
Location Left temporal lobe.
Clinical history Headache, aggressive behavior, memory disturbances, receptive dysphasia.
Duration of symptoms 3 months (memory disturbances, aggression, headache), aphasia a few days prior to admittance.
Neuro-imaging Rim-enhancing mass lesion surrounded by edema.
Lower-grade precursor No.
Duration of survival 3.8 months (07.02.95 - 02.06.95)
Extent of surgical removal Subtotal resection.
Adjuvant therapy Radiotherapy

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, GC, PC, SC, LFC, SFC, LBC.
Histoarchitecture Amorphous arrangement.
Regressive changes Central large area of necrosis with partial perinecrotic pseudopalisading, multiple small foci with complete perinecrotic pseudopalisading.
Vascularity EC hypertrophy and EC hyperplasia present in most LPF, MVP in a few LPF, networks of proliferating vessels, necrosis, thrombosis, ectasia, collagenous thickening.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglioblastomatous tissue

Cell density Normal.
Cellular composition SAC, LAC.
Histoarchitecture Cortex Perivascular and subpial structures.
Vascular EC hypertrophy and EC hyperplasia in many LPF with MVP only in a few LPF (immediate PGBT).
Mitotic activity <10/10 HPF.

3. Morphometrical data

A. Tumor tissue proper

\[
CD = \frac{1009}{6\text{ HPF}} = 168.2/\text{HPF}
\]
\[
PCI = \frac{262}{1009} = 26\% \ (43.7/\text{HPF})
\]

B. Periglioblastomatous tissue

\[
CD = \frac{1003}{19\text{ HPF}} = 52.8/\text{HPF}
\]
\[
PCI/\text{HPF} = \frac{99}{19\text{ HPF}} = 5.2/\text{HPF}
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Table III-13. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-13. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-13. Outside the TTP, CD progressively decreases. The ZDI extends as far as the 6th HPF whereas PN are present just beneath the pia mater, defining the extent of the ZI. The relative high CD and PCI/HPF values noted in HPF 10, indicate the presence of subpial secondary structures.
PM III-15. Case 13. (a) Low power magnification showing the TTP and the ZDI (MT x 180). (b) ZITC displaying a subpial accumulation of tumor cells (MT x 180). (c-d) Corresponding MIB-1 immunostains (x 180).
CASE 14 (19667)

1. Clinical data

Age 59.6.
Sex Female.
Location Left parietal lobe.
Clinical history Receptive dysphasia.
Duration of symptoms < 1 month.
Neuro-imaging Irregular rim-enhancing mass lesion (3 x 2.5cm) with secondary mass effect.
Lower-grade precursor No.
Duration of survival 12.5 months (11.04.95 - 23.04.96).
Extent of surgical removal Gross total removal.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, LFC, GC, PC, LBC.
Histoarchitecture Amorphous arrangement.
Regressive changes Central large area of necrosis with partial perinecrotic pseudopalisading, a few small foci with complete perinecrotic pseudopalisading, cicatrization with neoplastic reinvansion. Large cyst.
Vascularity EC hypertrophy and EC hyperplasia present in most LPF, MVP in many LPF, GMVP only very focally, networks of proliferating vessels, perinecrotic vascular wall, ectasia, thrombosis, necrosis.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Well-defined.
B. Periglioblastomatous tissue
-------------------------------------
Cell density Low.
Cellular composition GC, SC.
Histoarchitecture Tumoral tissue pattern (fibrillary texture). Subpial structures.
Regressive changes Absent.
Tumor border Not visible.
Growing activity Diffuse SG.
Vascularity Unremarkable.
Mitotic activity <10/10HPF

3. Morphometrical data
A. Tumor tissue proper
-------------------------------------
CD = 1143/6HPF = 190.5/HPF
PCI = 206/1143 = 18% (34.3/HPF)

B. Periglioblastomatous tissue
-------------------------------------
CD = 1055/13HPF = 81.2/HPF
PCI = 42/1055 = 4% (3.2/HPF)
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Table III-14. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-14. Dot plot depicting distributions of CD and PCI/HPF in PGBT as shown in table III-14. Outside the TIP, CD rapidly reaches a plateau of low hypercellularity corresponding morphometrically to astrocytoma. The well-differentiated gemistocytic astrocytoma, which borders this GB, displays only scattered PN.
PM III-16. Case 14. (a) TTP with surrounding low-grade astrocytic tumor (MT x 180). (b-d) Detail of the low-grade astrocytoma showing: (b) well-differentiated gemistocytic astrocytoma (MT x 470), (c) low-proliferative activity consistent with the quiescent nature of the gemistocytic astrocyte (MIB-1 x 470), (d) strong GFAP expression (GFAP x 470).
CASE 15 (19743)

1. Clinical data

Age 64.2.
Sex Female.
Location Right frontal lobe with extension into the right parietal lobe.
Clinical history Unusual headache, increasing gait disturbances.
Duration of symptoms 2-3 months.
Neuro-imaging Irregular rim-enhancing mass lesion with secondary mass effect.
Lower-grade precursor No.
Duration of survival 10 months (08.05.95 - 02.03.96).
Extent of surgical removal Gross total resection.
Adjuvant therapy Radiotherapy.

2. Histopathological data

A. Tumor tissue proper

Cell density High.
Cellular composition SAC, LAC, GC, SC, PC, LBC.
Histoearchitecture Amorphous arrangement.
Regressive changes Large confluent area of necrosis with partial perinecrotic pseudopalisading, small foci with complete perinecrotic pseudopalisading.
Vasculature EC hypertrophy and EC hyperplasia present in many LPF, MVP in a few LPF, GMVP only very focally, networks of proliferating vessels, perinecrotic vascular wall, ectasia, thrombosis, necrosis.
Mitotic activity >20/10HPF, atypical mitotic figures.
Tumor border Ill-defined.
B. Periglio blastomatous tissue

Cell density Low.
Cellular composition GC, SC.
Histoarchitecture Tumoral tissue pattern (fibrillary texture).
Regressive changes Absent.
Tumor border Not visible.
Growing activity Diffuse SG.
Vascularure Unremarkable.
Mitotic activity <10/10HPF

3. Morphometrical data

A. Tumor tissue proper

CD = 1041/6HPF = 173.5/HPF
PCI = 173/1041 = 16.7% (28.8/HPF)

B. Periglio blastomatous tissue

CD = 1058/12HPF = 88.2/HPF
PCI = 60/1058 = 5.7% (5.0/HPF)
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Table III-15. Number of N and (PN) counted in successive HPFs in 5 randomly chosen tracks (T1-5), starting at the outer border of the TTP (= HPF 1).
Fig. III-15. Dot plots depicting distributions of CD and PCI/HPF in PGBT as shown in table III-15. Outside the TTP, CD rapidly reaches a plateau corresponding morphometrically to astrocytoma. The surrounding gemistocytic astrocytoma shows a somewhat higher proliferative activity than in case 14. Nevertheless, most of the increase in CD is due to MIB-1 immunonegative tumor cells.
PM.III-17. Case 15 (a-b) Low power magnification of the TTP and surrounding gemistocytic astrocytoma (MT x 180, MIB-1 x 180). (c) Detail of the "background" gemistocytic astrocytoma illustrating the low proliferative activity, largely confined to tumor cells with a small rim of cytoplasm (MIB-1 x 470). (d) Strong GFAP expression in the gemistocytic astrocytoma (x 180).
2. **Summary of clinical, histopathological and morphometrical data**

The 15 GB cases included 6 men and 9 women, their ages varying from 33.4 to 69.4 years with a mean of 53.2 years (SD 13.2). In 86% of the patients, the tumor was predominantly located in the frontal or temporal lobes (46% and 40% respectively); the parieto-occipital region was only affected in 14% of the patients (7% and 7% respectively).

Headaches were the initial symptom in 33% of patients but were present some time during the clinical course in 73%. 20% of the patients presented with seizures, in two patients the seizures started eight years before GB diagnosis. In 13%, mental status changes were the initial symptom, including memory loss, drowsiness, apathy and disinhibition. Focal neurologic deficits (aphasia, hemiparesis) were usually late symptoms. Intratumoral hemorrhage and rapid deterioration with coma was seen in one patient (#8). In 80% of the cases, the duration of the preoperative symptoms was less than three months. Only three cases showed considerable longer periods of preoperative symptomatology ranging from six months (#5) to four years (#8, #10). The latter two were biopsy proven secondary GBs.

Radiographically, all cases demonstrated marked enhancement following administration of radiographic contrast or gadolinium. Only in three patients there was a more uniform pattern of enhancement, whereas the vast majority displayed the typical irregular, ring-shaped pattern. In two cases (#11, #12) a small satellite area of enhancement was seen.

Two patients (#8, #10) showed clinical and histopathological evidence of an earlier lower-grade astrocytic tumor. In both cases the primary tumor consisted of a WHO grade II fibrillary astrocytoma which was diagnosed
after an initial period of seizures and a non-enhancing hypodensity on CT. The time interval between the diagnosis of the primary tumor and of the GB amounted to 71.5 months in case #8 and 84.25 months in case #10. According to the surgical notes, six patients had undergone subtotal resection; nine patients gross total removal. Except for cases #3 and #9, all cases received postoperative radiotherapy. In addition, cases #8 and #10 also received chemotherapy.

Length of survival following the diagnosis of GB ranged from 1 to more than 44 months including six censored survival times. Three patients (#2, #5, and #8) with follow-up times of more than 44, 32 and 27 months respectively were still alive at the end of the data acquisition period ("withdrawals"). Three other patients (#4, #10, #12) were lost track of, respectively 23, 10 and 14 months after histopathological diagnosis ("losses to follow-up") (Fig.III-18).

The mean age at diagnosis of the ten astrocytoma and ten anaplastic astrocytoma patients was respectively 30.3 years (SD 3.1) and 34.1 years (SD 7.8). The ages at death of the three postmortem brains were 48.4, 56 and 63.5 years.

Except for case #5 in which there was considerable intratumoral vasogenic oedema, all tumors (TTP) were highly cellular neoplasms. The number of neoplastic cell types present, varied from five to eight with most tumors containing six cell types. When subdivided according to the predominant cell type as defined by Zülch [256], the multiform subtype (8/15) clearly outnumbered the fusiform (4/15) and globuliform subtype (3/15).
Histoarchitecturally, a definite fascicular pattern consisting of interwoven bundles of bipolar cells was seen in only three cases (#3, #4, #9). Stellate cells in a myxoid stroma, as described by Burger [43], were encountered in two cases (#5, #7). The vast majority showed no distinct architecture ("amorphous cell arrangement"). Astroblastic pseudorosettes were found in two cases (#5, #8).

Except for cases #8 and #10, which were only subtotally removed, and cases #5 and #12 showing a cystic space due to liquefaction, all other cases displayed coagulative necrosis varying in extent from small, irregularly shaped serpiginous foci to large confluent areas leaving nothing but a narrow peripheral rim of viable neoplasm. Cicatriztion of a necrotic focus with neoplastic reinvansion was seen in two cases (#3, #14). Dystrophic calcification was noted in cases #8 and #10.

Some degree of microvascular proliferation (MVP) was seen in all cases. Simple EC hypertrophy and hyperplasia were present in most of the fields studied. Multilayered vascular proliferation could be observed in a few LPFs in all cases, whereas glomeruloid MVP was only focally present. Vascular walls were encountered in approximately half of the cases showing a clear predilection for perinecrotic areas.

Marked perivascular lymphoplasmacellular cuffing was seen in case #10. Most of the cases displayed a high mitotic activity and atypical mitotic figures were common.

Outside the TTP, in the PGBT, CD appeared either normal or increased. In eight cases (#1, #3, #4, #6, #7, #9, #11, #13), beyond a rather narrow zone of dense infiltration, normal brain parenchyma was seen in which at least a few atypical cells similar to the poorly differentiated tumor cells of the TTP could be identified. Secondary structures (subpial and
especially perivascular) were present in six. Microvascular proliferation, when present was confined to the immediate PGBT. A few mitoses were seen in all eight cases. In the remaining seven cases (#2, #5, #8, #10, #12, #14, #15), well-differentiated neoplastic astrocytes in a fibrillary matrix of interfacing cytoplasmic processes made up the histologic picture. In most of these cases, foci of faster growing activity characterized by increased cellularity, mitotic activity and loss of cellular differentiation were identifiable. Microcystic degeneration (#5, #12) and microcalcification (#8, #10) were seen in four cases.

The CD counted in the most active tumor areas, ranged in the TTP from 152 to 284 cells per HPF with a mean of 194 (SD 34). Likewise, the MIB-1 PCI and the derived MIB-1 PCI/HPF showed considerable variation, ranging from 16.7% to 38.9% and from 28.8 to 79.2 respectively. The calculated means were respectively: 27% (SD 7) and 53 (SD 16).

In the PGBT, two distinct sets of morphometric data emerged (table III-19). Low cell densities were encountered in eight cases (#1, #3, #4, #6, #7, #9, #11, #13) ranging from 50.9 to 60.6 cells per HPF by counting at least one thousand cells from areas showing the highest number of MIB-1 immunolabeled cells outside the zone of dense infiltration. All eight cases had in addition low PCI/HPF values (3.4 - 6.1). Higher CDs were noted in seven cases (#2, #5, #8, #10, #12, #14, #15) ranging from 77.3 to 89.1 cells/HPF in the “quiescent” (SG) parts and from 111.7 to 127.6 cells/HPF in the “anaplastic” (FG) areas. A similar biphasic distribution was recorded for the PCI/HPF values with the lower values between 3 and 7, the higher values between 10.2 and 21.3.
3. Data analysis

Pathological variables

Normal brain tissue

All cells have been counted in successive HPFs in five randomly chosen trajectories, from the pial surface to the subcortical white matter, in three different cortical regions in three normal brains. The mean and standard deviation (SD) of the overall cell density were calculated for the pooled data of the three brains in each of the anatomical areas studied. The mean CD (SD) of normal human frontal, temporal and parieto-occipital cortex was respectively: 45.9 (9.3), 45.7 (10.1), and 46.8 (10.4). As there were no significant differences between the means of the three cortical areas (ANOVA, F = 0.44, d.f. = 2, 435), the mean (SD) of all cell densities was calculated and used to determine the 95% reference interval of normal neocortex\(^5\): 46.1 ± 1.96 x 9.9 (26.7 - 65.5) (23.9 - 64, percentile method).

\(^5\) Blinkov [22] reported that normal human white matter contains about 2000 neurogla cells per 0.01 mm\(^3\) which corresponded after conversion for a surface area of 0.0576 mm\(^2\) and a section thickness of 5 micron to 57.6 per HPF. We found a mean CD of 60.9 (61, percentile method) in a sample of 152 HPFs. More complex calculations were required for the evaluation of our results of normal neocortex due to the fact that in cortex apparently either glial cell density or neuronal cell density have been computed. The same author reported that the number of glia in cerebral cortex was in the range of 989/0.01mm\(^3\) (≈28.5/HPF). Rockel et al. [184] found that with the exception of area 17 of the visual cortex, the absolute number of neurons counted in a volume of tissue of 30 micron (width) by 25 micron (section thickness) through the depth of the cortex was the same (± 110) in all areas and in all species. In that study, the parietal area 7 showed a mean cortical thickness of 2.4 mm with an average of 104.1 neurons. After conversion, the number of neurons per HPF amounted to 16.7 resulting in a total mean CD of 45.2/HPF. We calculated a mean of 46.1 (47, percentile method) in a sample size of 438 HPFs.
At least one thousand neuroglial cells were counted, i.e. several HPFs, in each of the anatomical regions of interest. The mean (SD) was determined for the pooled data of the three brains. The mean CD (SD) for normal white matter in the frontal, temporal and parieto-occipital areas was respectively 60.9 (4.6), 61.5 (5.4), and 60.2 (4.0). As comparisons between means showed no significant differences (ANOVA, F = 0.90, d.f. = 2, 149), the mean (SD) of all neuroglial cell densities was calculated and used to determine the 95% reference interval of normal white matter: $60.9 \pm 1.96 \times 4.7 (51.7 - 70.1)$ (52.8 - 69, percentile method).

Details of the cell counts are listed in table III-16.

No MIB-1 immunoreactivity was observed in sections of normal brain tissue.

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Table III-16. Cell density of normal human brain tissue.

Neocortex (1) measured in successive HPFs (rows) from the pial surface towards the subcortical white matter in five randomly chosen trajectories as perpendicular as possible to the pial surface (columns) in three anatomical locations in three postmortem brains.

White matter (2) counting at least one thousand cells, in several randomly chosen HPFs in three anatomical locations in three postmortem brains.
Astrocytoma WHO grade II and anaplastic astrocytoma WHO grade III

In each of the cases, at least 1000 cells were counted from areas showing the highest density of MIB-1 immunopositive nuclei (table III-17). Astrocytomas showed significantly lower CD6, PCI7 and PCIs/HPF than anaplastic astrocytomas (Mann-Whitney U test: P < 0.002). A statistically significant strong positive correlation was noted between the PCI and PCI/HPF in both the astrocytoma and anaplastic astrocytoma group (rs = 0.9394, rs = 0.9515 respectively) (P < 0.002), whereas no significant relationship was found between CD and these indices of cell proliferation (PCI: rs = 0.224, P > 0.2 (grade II), rs = 0.261, P > 0.2 (grade III); PCI/HPF: rs = 0.503, 0.1 < P < 0.2 (grade II), rs = 0.479, 0.1 < P < 0.2 (grade III)

Tumor tissue proper (TTP)

The mean (SD) of CD6, PCI7 and PCI/HPF for the entire GB group was respectively: 194.3 (34), 27.4 (6.9), 53.2 (16) (table XVIII, fig.16). These values were statistical significantly higher than those of astrocytomas and anaplastic astrocytomas (P < 0.002) A strong positive correlation was found between PCI and PCI/HPF (rs = 0.85, P < 0.002). There was no

6 Despite the general agreement on the prognostic value of CD in brain tumors, apparently little systematic effort has been made to quantify CD. The few authors who did quantitate CD either presented no data [31, 37] or expressed CD per HPF without mentioning section thickness and microscope optics [202, 213]. The latter has been shown to cause a variation in HPF size of up to nearly 600% with current available microscopes [70], making such data difficult to interpret.

7 As seen from table I-3 on page 33, our MIB-1 PCI values fall within the range of those reported previously.
significant relationship between CD and the other two morphometrical parameters (PCI: $r_s = 0.046$, $P > 0.2$; PCI/HPF: $r_s = 0.45$, $P > 0.05$). None of the morphometrical parameters was statistical significant when related to survival (univariate analysis, CD: $P = 0.4729$, PCI: $P = 0.2029$, PCI/HPF: $P = 0.2115$). There was no statistically significant difference in survival between the histological subtypes as defined by the prevailing cell type (logrank test, $P > 0.20$). The presence of microcalcifications seemed unrelated to survival (logrank test, $P = 0.162$). Detail of morphometrical parameters is shown in table III-17.

Fig.III-16. Graphic depiction of the means of age at diagnosis, CD, MIB-1 PCI of 10 astrocytomas, 10 anaplastic astrocytomas and 15 GBs (Numbers in parentheses are the corresponding $P$-values (Mann-Whitney U test)).
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Table III-17. Age at diagnosis, CD and MIB-1 proliferating cell indices in 35 gliomas.
Table III-18. Mean (SD) of age at diagnosis, CD and MIB-1 proliferating cell indices in 35 gliomas.

Periglialoblastomatous tissue (PGBT)

Morphometrically, subgroup A GBs (#1, #3, #4, #6, #7, #9, #11, #13) showed CDs, as measured from areas showing the highest labeling index, that were significantly lower than those of subgroup B (P < 0.002) and those of astrocytomas and anaplastic astrocytomas (P < 0.002).

Compared to the mean normal brain tissue CD, they appeared slightly increased but still fell within the 95% reference interval (the values corresponded to the 70th - 92nd percentile, “borderline normocellularity”). The PCI/HPF values were either significantly higher (P < 0.01) or significantly lower (P < 0.002) than those of subgroup B GBs depending on the proliferative activity of the area studied in subgroup B (SG versus FG). Compared to those of astrocytomas, they appeared significantly increased (P < 0.01), but were significantly lower than those of anaplastic astrocytomas (Mann-Whitney U test, P <0.002).

The eight subgroup A GBs were further characterized by a relatively narrow ZDI (mean 1.52mm, range 0.57 - 2.55 mm). PN, defining the ZI, could be traced as far as the molecular layer in most of the cases, extending on the average 4.64 mm beyond the TTP (range 2.83 - 10.48 mm) (table III-20).
Besides the aforementioned morphometrical relationships with subgroup A, subgroup B GBs (#2, #5, #8, #10, #12, #14, #15) displayed CDs more than three SDs away from the mean normal brain tissue CD and were considered significantly increased ("hypercellularity"). By comparing CD, PCI and PCI/HPF of SG and FG areas of subgroup B GBs respectively with those of astrocytomas and anaplastic astrocytomatas, no significant differences were found (Mann-Whitney U test, P > 0.1).

Details of morphometrical data are listed in tables III-19 and III-21, and depicted in fig. III-17.

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Table III-19. CD and MIB-1 cell proliferation indices of PGBT (In the PGBT of PGBs no MIB-1 PCI could be calculated because it was impossible to differentiate reliably in all cases MIB-1 immunonegative neoplastic nuclei from normal or reactive nuclei. In the PGBT of SGBs, most of the nuclei are presumed neoplastic.)
Table III-20. Extent of the zones of infiltration and dense infiltration as determined in the 8 subgroup A GBs (mm = 0.24 x # HPF, mm = 1.18 x mm i.e. correction for linear shrinkage).

Fig.III-17. Graphic representation of mean age at diagnosis, CD and MIB-1 PCI of GB subgroup A and B patients. (Numbers in parentheses are corresponding P values (Mann-Whitney U test))

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The estimated Kaplan-Meier survival curves for morphological subgroup A and B GB patients are shown in Fig. 19. Subgroup B patients survived significantly longer than those of subgroup A (logrank, $P = 0.004$) and this difference in survival remained statistically significant after correction for age (Cox’s proportional hazards regression, $P = 0.045$). Univariate analysis showed that morphological subtype ($P = 0.0117$), CD ($P = 0.0178$) and PCI/HPF ($P = 0.0491$) were statistically significant predictors of postoperative survival. After adjustment for age, only the morphologic subtype ($p = 0.0454$) and CD ($P = 0.0243$) remained prognostically significant but to a lesser degree (table III-22).

Fig. III-18. Diagram showing follow-up in the 15 patients with GB, entered in this study between 04.01.93 and 02.03.96. ($o =$ death, $|$ = withdrawal, $\Delta =$ lost to follow-up. Numbers in parentheses are months of follow-up)
Clinical variables

Patient age

The mean age at diagnosis of the 15 GB patients in this study was 53.2 years (SD 13.2) which was significantly higher than the mean age of patients with astrocytoma (30 years, SD 3.1) ($P < 0.002$), or anaplastic astrocytoma (34 years, SD 7.8) ($P = 0.0357$). Patients with astrocytomas tended to be somewhat younger than anaplastic astrocytoma patients, but this difference in age was not statistically significant ($\text{Mann-Whitney U test, } P = 0.0588$). Within the GB group, subgroup A patients were significantly older than those of subgroup B (mean 61.5, SD 6.5 versus 43.7, SD 12.6) ($P = 0.0151$). The latter were also significantly older than astrocytoma patients ($P < 0.002$) but did not differ significantly in age with
anaplastic astrocytoma patients ($P = 0.3271$). Age was found inversely related to PCI (TTP) ($r_s = -0.5214, 0.02 < P < 0.05$), CD (PGBT) ($r_s = -0.757, P < 0.002$), PCI/HPF (PGBT) ($r_s = -0.70, 0.02 < P < 0.05$) and to survival (univariate analysis, $P = 0.0104$; multivariate analysis, $P = 0.0114$). Details of age at diagnosis are shown in tables III-17 and III-21.

*Duration of preoperative symptoms*

All patients in subgroup A displayed relatively short periods of preoperative symptoms (less than three months) (table III-21). Although the lengths of preoperative symptomatology were equally short in the majority of patients in subgroup B, three cases showed considerably longer duration of symptoms prior to diagnosis ranging from 6 (#5) months to 4 years (#8, #10). The latter two were biopsy proven secondary GBs. The differences in the duration of preoperative symptoms between subgroups A and B were however not statistically significant ($P = 0.0695$).

*Prior lower-grade glioma*

Two patients (#8, #10) showed clinical and histopathological evidence of an earlier lower-grade astrocytic tumor. In both cases the primary tumor consisted of a WHO grade II fibrillary astrocytoma which was diagnosed after an initial period of seizures and a non-enhancing hypodensity on CT. The time interval between the diagnosis of the primary tumor and of the GB amounted to 71.5 months in case #8 and 84.25 months in case #10. Both cases belonged to subgroup B. None of the patients in morphologic subgroup A had a history of a prior lower-grade glioma.
**Tumor location**

In 86% of patients, the tumor was predominantly located in the frontal or temporal lobe (46% and 40% respectively), whilst the parieto-occipital region was only affected in 14% of patients (7% and 7% respectively). By Chi-squared analysis, there were no significant differences in tumor location between morphologic subgroups A and B (P = 0.6193).

**Treatment**

According to the operative notes, six patients had undergone subtotal resection; nine patients gross total removal. Except for cases # 3 and #9, all cases received postoperative radiotherapy. In addition, cases #8 and #10 also received chemotherapy. Fischer’s exact test showed no significant differences in any of the treatment modalities between morphologic subgroups A and B patients (extent of resection: P = 1; radiotherapy: P = 0.467; chemotherapy: P = 0.20).
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Table III-21. Comparison of the distributions of morphometrical and clinical parameters between GB subgroup A and B patients.

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<th>Univariate P</th>
<th>Multivariate P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.0104</td>
<td>0.0114</td>
</tr>
<tr>
<td>Morphologic subgroup</td>
<td>0.0117</td>
<td>0.0454</td>
</tr>
<tr>
<td>CD (TTP)</td>
<td>0.4729</td>
<td>0.4659</td>
</tr>
<tr>
<td>PCI (TTP)</td>
<td>0.2029</td>
<td>0.1465</td>
</tr>
<tr>
<td>PCI/HPF (TTP)</td>
<td>0.2115</td>
<td>0.1472</td>
</tr>
<tr>
<td>CD (PGBT)</td>
<td>0.0178</td>
<td>0.0243</td>
</tr>
<tr>
<td>PCI/HPF (PGBT)</td>
<td>0.0491</td>
<td>0.7137</td>
</tr>
</tbody>
</table>

Table III-22. Prognostic value of clinical, morphological and morphometrical parameters (Cox's proportional hazards model).
Topographical studies of GB are rare which may be explained by
the fact that most of the 20th century research efforts in neuro-oncology
have been devoted to the classification and grading of brain tumors.
Moreover, prior to the advent of modern imaging techniques in the field of
neuro-oncology, there was no great demand for the exact knowledge of
tumor boundaries as tumor removal was “largely an exercise in eye-hand
coordination” [57] and as one was often dealing with advanced tumor
there is a desperate need of some method of establishing the pathological
diagnosis without an exploratory operation; it would save much futile
labor.” This fatalism persisted for many decades [31].

The few contributions made to the subject, are those of Scherer
[193-200] and more recently, of Burger [33, 39,40] and Daumas-Duport
[57, 119, 120]; the former probably out of revolt against the scholastic
methods and ideas dominating glioma research in the 1920 and 30s, the
latter two out of the renewed interest in stereotactic neurosurgical
procedures due to the advent of computed tomography.
1. **Scherer’s concept of primary and secondary GB: a controversial issue**

The background of this study is the work of Scherer, which is regrettably far from being well known and understood. Scherer is almost exclusively known for his secondary gliomatous structures and concept of primary and secondary GB, whereas in fact he has studied all aspects of modern glioma pathology, from cytology to clinical and biological behavior, often in such detail that even for today’s neuropathology his work\(^8\) contains a wealth of information.

What caused the poor recognition of Scherer’s work? Several explanations emerge. Scherer came between two important neuro-oncological movements: the first being dominated by cytogenetic principles reflected in the numerous cytogenetic glioma classifications of that time [7, 49, 102, 170, 174]; the second, starting soon after his death, focused mainly on tumor grading [123, 182]. Scherer had no classification of his own [198] and his methodology [197], requiring the study of the entire brain, was probably too laborious to be easily accepted. Because of his sharp criticism, Scherer was not a “popular” man. Sayre [192] wrote in this regard: “Perhaps the greatest iconoclast of all was Scherer, who castigated Ribbert and his fellow neuropathologists in language hardly seen before or since”. Finally, his early death, during the Second World War, ended a professional career of barely twelve years.

---

\(^8\) Essential in his work is that the tumor must be studied in all its parts including its growth zone and in its relation to the brain as a whole with equal emphasis on each of the features studied. Consequently, central themes are the intratumoral histological heterogeneity of type and degree of malignancy [193, 197, 200] and the patterns of growth and spread with their practical implications for diagnosis and treatment [194, 195, 196, 199]
Unfortunately, when Scherer is cited, it is often incorrect: there are, for instance, the numerous interpretations given to his concept of primary and secondary GB and the neglect of the fact that he distinguished two pathological subtypes of PGB [197, 198].

For instance, Rubinstein [187] required for acceptance of a tumor as a SGB either “histological evidence of a low-grade astrocytoma at an earlier stage” or the “identification of a substantial portion of the pre-existing and more benign growth”. Furthermore, he drew - probably somewhat annoyed by the relative higher frequency of his PGBs (78/106), at a time when the concept of progressive anaplasia was still unchallenged - attention to the presence in many of the PGBs of recognizable neoplastic astrocytes, interpreting them either as remnants of a pre-existing astrocytoma or as the result of differentiation. In spite of the latter explanation, he nevertheless concluded that most of the GB arise by progression, hereby sacrificing a histological criterion to a pure cytological one.

The same principle was later adopted by Burger [38, 41, 43] defining the PGB as a GB homogeneously composed of small anaplastic cells. About the admixtures of well and poorly differentiated cells in GBs he wrote “there seems little reason to doubt that in such cases the small and poorly differentiated cells arise in transition from obvious astrocytes” [43]. Just as Rubinstein, he is a strong exponent of the theory of tumor progression [154] and questions the existence of PGBs altogether by assuming that in such lesions “the original focus was either small and rapidly obscured by the cellular overgrowth, or destroyed by the typical large central area of necrosis” [41].

Whereas this seemed a possibility to Burger, it became a fact to von Deimling [233]. Referring to the article of Burger and Green [38] he stated
that “although the histopathologic appearance of glioblastoma multiforme is extremely heterogeneous, it has not been possible to differentiate by light microscopy those glioblastomas that arise from previous astrocytomas from those that arise either de novo or rapidly dedifferentiate. Neither immunohistochemical nor ultrastructural analyses have provided evidence for subdividing GB into clinically or biologically distinct entities”. The issue of primary and secondary GB became a molecular genetic one. More recently, clinical criteria have entered molecular biological studies of primary and secondary GB [18, 130, 239], requiring a short clinical history and a histological diagnosis of GB at first biopsy for PGBs, whereas the diagnosis of a SGB necessitates clinical or histological evidence of progression from a low grade or anaplastic astrocytoma.

Let us now consider in more detail Scherer’s concept of primary and secondary GB [197] (table IV-1). In essence, it is a morphological subclassification of GB with clinical and biological implications and not a clinicopathological subclassification. In evidence hereof Scherer wrote “the clear separation of primary and secondary glioblastomas renders also the clinical and biological differences much clearer” [198]. Moreover, about histopathological evidence of prior low-grade astrocytoma he stated: “this alone, however is no proof, since the examination of small biopsy fragments cannot exclude a primary coexistence of both tissues in the same tumor, a possibility which must always be taken in account because of the great frequency of such tumor mixtures” [197]. Nor was the duration of symptoms considered a distinctive criterion: “il va de soi qu’une durée de plusieurs années, par exemple, exclut d’emblée la possibilité d’un glioblastome multiforme primaire. Par contre, une évolution apparemment rapide n’exclut pas avec certitude un astrocytome. Les astrocytomes, ne
mettant pendant très longtemps hors service qu’une minime quantité de parenchyme céphalique, peuvent atteindre un volume important sans donner naissance au moindre symptôme, surtout lorsqu’ils siègent au niveau de la fosse antérieure. En pareille éventualité, les premiers symptômes apparents seront ceux causés par l’hypertension intracrânienne: et ce point atteint, le cas peut évidemment évoluer en quelques mois” [200].

Least of all was it a cytological subclassification, a principle he strongly rejected: “A glioma is a natural phenomenon. A natural phenomenon is characterized by the whole of its morphological and biological characteristics; it would be easy, but arbitrary, to classify an animal according to the number of its teeth alone or a plant according to the form of its leaves only. It is not less arbitrary to subordinate study and classification of gliomas only to their cytology or to their localization” [198].

What did Scherer understand by primary and secondary GB?
Scherer came to this subdivision by studying the tumor in toto and in relation with the surrounding tissue [196, 198]. Whereas some GBs represented only foci in an astrocytoma, others were entirely or predominantly composed of glioblastomatous tissue. Macroscopically, SGBs were large, ill-defined tumors with a brain-like appearance showing no definite tumor unless the foci of dedifferentiation were large enough to be macroscopically visible, emerging as areas of more friable, granular, reddish or multicolored tissue. Microscopically, they were primary diffuse infiltrating tumors showing a typical astrocytoma morphology in most parts i.e. “moderate and uniform cellularity, uniformly amorphous structure, astrocytic character of a high but variable percentage of their cell elements, production of glial fibers in small or extensive areas, preservation of the pre-existent parenchyma, tendency to microcystic degeneration,
absence of necrosis, low vascularity without endothelial or adventitial proliferation”, containing however areas of “definite glioblastomatous nature”. The latter were considered “recent proliferations” by virtue of their size relative to the tumor as a whole and were microscopically characterized by “high cellularity, pronounced cellular and nuclear polymorphism, abundant mitoses, appearance of secondary structures and angioplastic vessel proliferations”, only differing from PGBs in their tendency to necrosis being either absent or present in only very circumscribed areas. Clinically, they behaved like astrocytomas showing a long evolution: “the course being conditioned by the slow growth and feeble destructive properties of the astrocytoma as long as it remained pure”.

On the other hand, PGBs appeared macroscopically as well defined, largely necrotic tumors of generally moderate size showing usually extensive peritumoral brain swelling. Microscopically, the tumors showed an infiltrative pattern of growth, the zone of infiltration being either dense and narrow (circumscribed infiltration) or widespread (secondary diffuse infiltration). They were composed of typical glioblastomatous tissue, either entirely in the “pure” form or predominantly in the rare “combined” form, which contained in addition small zones of typical astrocytoma [197, 198]. Clinically, primary GBs showed a short evolution: “none reached one year, in most cases it was less than 6 months”, which Scherer partly attributed to the almost constant presence in primary GBs of extensive necrosis and peritumoral brain swelling [197].
<table>
<thead>
<tr>
<th></th>
<th>Primary GB</th>
<th>Secondary GB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>75-80%</td>
<td>20-25%</td>
</tr>
<tr>
<td>Limitation</td>
<td>well-defined</td>
<td>ill-defined</td>
</tr>
<tr>
<td>Mode of growth</td>
<td>circumscripted (20%) infiltrative growth primary diffuse infiltrative growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>secondary diffuse</td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>generally moderate (very often &lt;4xΦcm at the moment of death)</td>
<td>generally enormous (more than one lobe)</td>
</tr>
<tr>
<td>Parietal/brain swelling</td>
<td>extensive</td>
<td>absent or minimal</td>
</tr>
<tr>
<td>Cytology</td>
<td>Typical GB aspect (e.g. pronounced cellular and nuclear polymorphism) in all (&quot;pure&quot;) or most (&quot;combined&quot;) parts</td>
<td>Typical astrocytic aspect in most parts, GB aspect in dedifferentiated parts</td>
</tr>
<tr>
<td>Cellularity</td>
<td>high</td>
<td>moderate and uniform in most parts, increased in dedifferentiated parts</td>
</tr>
<tr>
<td>Structure</td>
<td>Amorphous, proper, secondary</td>
<td>amorphous in most parts, secondary structures in dedifferentiated parts</td>
</tr>
<tr>
<td>Degenerative changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuraxis</td>
<td>extensive</td>
<td>no or comparatively small (&lt;1/10 of living tissue)</td>
</tr>
<tr>
<td>Microscopic degeneration</td>
<td>absent</td>
<td>absent or present</td>
</tr>
<tr>
<td>Calcification</td>
<td>absent</td>
<td>absent or present</td>
</tr>
<tr>
<td>Clinical evolution</td>
<td>less than one year (mostly &lt;6 months)</td>
<td>over one year (mostly two to six years)</td>
</tr>
</tbody>
</table>

Table IV-1. Characteristics of primary and secondary GBs according to Scherer.
Histopathological subclassification of GB, primary and secondary GB

Intrigued by Scherer’s morphological subclassification that evoked so much controversy, the primary purpose of this study was to investigate whether primary GBs can be differentiated morphologically from secondary GBs.

Tumor tissue proper

Although there was some variation in cytological composition, in the extent of necrosis, and in the degree and extent of microvascular proliferation between GBs, we could not identify in the TTP any microscopical feature which provided a solid basis for a histopathological subclassification. The differences were either too marginal to provide a non-artificial basis for subclassification (e.g. number of cell types present, degree and extent of microvascular proliferation) or, the feature was of too rare occurrence in our study to be investigated statistically (e.g. calcification, absence or presence of necrosis) or still, the defined subgroups were without prognostic significance. Notably, no significant relationship was found between survival and histological subtype defined by the predominant cell type, nor were any of the morphometrical parameters significantly related to survival even after adjustment for other prognostic factors (table VIII). Relative to the MIB-1 PCI, similar results have been reported by other authors using different markers of cell proliferation [13, 177, 183]. In a large study by Burger et al. [32] investigating the prognostic significance of nine histological variables, the
only histological feature affecting survival in the overall group of 163 unequivocal GBs was giant cell morphology. In addition, a weak, albeit significant, negative correlation was noted between survival time and cellularity in those patients treated with surgery alone, but no correlation was found in those treated with either adjuvant chemotherapy or radiotherapy, and a weak positive correlation in those receiving both. Most of our patients received only postoperative radiotherapy, which may account for the lack of correlation between survival time and CD. Giant cell glioblastomas were excluded from the present study for morphometrical [2, 35, 197, 248] and conceptual reasons [43]. In a later, smaller study on 62 GBs by the same author [38], the presence of fibrillary and of pleomorphic astrocytes, areas of better differentiation, median nuclear size, homogeneously small cell composition and microcystic change were all reported to affect survival, whereas in fact only median nuclear size and the presence of fibrillary and pleomorphic astrocytes achieved statistical significance in the univariate model (P < 0.10), but when corrected for age, only median nuclear size retained its prognostic significance. Somewhat conflicting data have been produced regarding the prognostic value of pseudopalisading when both studies [32, 38] are being compared: whereas in the former, larger study it was of no predictive significance whatsoever, in the latter smaller study, the absence of this feature around necrotic foci was clearly associated with a higher death rate. In our study, some perinecrotic pseudopalisading was present in all cases so that we were unable to confirm either statement.
Periglioblastomatous tissue

The PGBT defined two distinct morphological subgroups with significantly different prognoses. Subgroup A GBs arose in the background of apparently normal brain tissue. On low magnification, the GB appeared rather well circumscribed, but on high magnification, a transitional ZDI was observed between the tumor tissue proper and the normal brain tissue. In the latter, isolated tumor cells (ITCs) permeating the otherwise intact parenchyma could often be identified on the basis of their nuclear and cytoplasmic abnormalities (hyperchromasia, pleomorphism, increased nuclear cytoplasmic ratio, mitotic figures and paucity of cytoplasm) (ZITC). In addition, the periglioblastomatous tissue displayed a clear astrocitosis⁹. Subgroup B GBs arose in the background of a lower-grade glioma into which they either imperceptibly blended (FG area) or from which they appeared well-circumscribed (SG area). Stellate or gemistocytic neoplastic cells were rather evenly distributed in a fibrillary matrix consisting of interlacing cytoplasmic processes with perivascular accentuation (bushes, feutrage périvasculaire) and displaying no or little mitotic activity. In most cases however, foci of faster growing activity were seen, characterized by the presence of less differentiated cells, increased mitotic activity and higher cellularity as observed in anaplastic astrocytomas.

Morphometrical analysis confirmed these “structural” differences by showing CDs - obtained from a large number of HPFs - in subgroup B

⁹ Which as a process is easily identified especially in GFAP or vimentin immunostained sections as a sheet of evenly distributed, well-formed hypertrophic astrocytes extending into long, symmetrically stellate processes often creating a “spidery” appearance. However, their individual distinction from normal or neoplastic astrocytes is often difficult.
that were clearly hypercellular as they deviated more than three SDs from the mean normal brain tissue CD and were found to correspond, together with the PCI and PCI/HPF values, to those of the astrocytoma/anaplastic astrocytoma reference population. The CDs of subgroup A were clearly non-neoplastic, but whereas simple light microscopic examination created the impression of normocellularity, morphometry revealed subtle deviations from the mean normal CD, on the average less than twice the number of MIB-1 immunolabeled nuclei. This relative high proportion of cycling cells was in sharp contrast with the subgroup B GBs where noncycling cells accounted for most of the increase in cellularity as was shown by the PCI, a measure of the growth fraction. In addition, the number of PN was certainly too high - even higher than those found in our astrocytoma series - to be attributed merely to reactive gliosis, so that they could only be explained, seen the minor increase in cellularity, as originating from the TTP by infiltration. A fact perhaps trivial, but never clearly established [39]. This relative high proportion of proliferating cells was fully in accord with the profile of the infiltrating cell type. These were mainly small anaplastic cells, the biological aggressiveness of which has been amply demonstrated by morphological [38, 41, 57, 83, 120] and cell kinetic studies [103, 133, 168].

Logrank test showed our morphological subgroups to differ significantly, but this effect of morphological subtype on patient survival could be spurious since those that survived the longest (subgroup B) were also significantly younger. Other variables such as treatment and tumor location were not found to differ significantly between subgroups and could not account for the difference in survival time. Data on the pre- or postoperative performance status were however not available in this study.
After adjustment for age, morphologic subtype was still prognostically significant. Conversely, when age was corrected for morphologic subtype or CD (PGBT), the principal morphometrical determinant, its P-value was only but little reduced, suggesting that morphologic subtype is responsible for only a small part of the age effect on survival in contradistinction to what is often supposed [129, 136, 239].

Although we were unable to confirm Scherer's statement on the difference in extent of necrosis\(^\text{10}\) between primary and secondary GBs, probably due to the fact that our cases were rather "early" lesions compared to the full-blown postmortem cases of Scherer, with necrosis undoubtedly a time-related event, and although we were often unable to investigate the whole tumor due to incomplete removal, we believe that our subgroup A and B GBs in surgical material correspond as closely as possible to Scherer's primary and secondary GBs. As Scherer, we studied the tumor in all its parts, especially in its growth zone, and in its relationship with the surrounding tissue, employing strict morphological criteria and putting equal emphasis to each of the microscopical features studied. In further support of this resemblance, microcystic degeneration was observed in two of our subgroup B GBs of which Scherer stated: "typical microcystic degeneration in a glioblastoma, on the other hand, is always an important indication that it is dedifferentiated from an astrocytoma. We have never encountered it in a glioblastoma without other definite signs of a primary astrocytoma" [197]. Calcification was also considered to indicate such a derivation [200], it was present in two cases.

\(^{10}\) A recent study by Tohma et al. [223], employing clinical criteria for the diagnosis of PGB and SGB, has reemphasized the differences in extent of necrosis. The small type of necrosis with perinecrotic pseudopalisading was found in similar frequency in PGBs (30%) and SGBs (35%), whereas the large geographical type was encountered predominantly in PGBs (100% versus 35% of SGBs).
He also noted that these secondary features are “more frequently lacking than present “ [197]. From the clinical point of view, our study agrees with that of Scherer: the total length of disease (i.e. from first symptom to death) was significantly shorter in our subgroup A GBs than in those of subgroup B. Fortunately, the statement that “none of the primary GBs reaches an evolution of one year” [197], is rendered out of date by modern therapeutic strategies. Therefore our study contradicts the belief that it is impossible to distinguish primary from secondary GBs morphologically [19, 130, 233, 239].

Our study further demonstrates the low diagnostic value of clinical criteria such as prior lower-grade glioma (i.e. previous operation for astrocytoma or anaplastic astrocytoma) and duration of symptoms to identify primary and secondary GBs since both depend on the production of symptoms by the low-grade precursor. Due to the tendency to preserve the preexisting parenchyma [43, 129, 136, 187] and the existence of so-called functional silent areas in the brain [242], symptoms might be lacking in some astrocytomas, at least temporarily before turning anaplastic. Moreover, the onset of anaplasia will influence the duration of the symptomatic period, being short when anaplasia occurs early in the life history of the tumor and long when anaplasia occurs late [64, 67, 129].

In our study, only two cases showed clinical and histopathological evidence of a lower-grade precursor whereas seven could be classified as SGB using morphological criteria. Similar data have been reported by Jelsma and Bucy [110] (3 out of 8 focally dedifferentiated astrocytomas), by Cornil [55] (1/5) and even by von Deimling [233] (2 out of 18 type 1 GBs). In addition, GBs with clinical or histopathological evidence of a prior low-grade glioma seem on the whole to be rare, accounting for only 3-10% of
all GB cases [64, 67, 111, 165, 246]. Thus absence of evidence of a prior low-grade glioma does not necessarily classify a GB as primary nor can a short clinical duration exclude a priori a SGB. This was illustrated in our study by the fact that five of our seven SGB cases showed equally short periods of symptoms as our PGBs and when considered as a group, there was no statistically significant difference in duration of symptoms. The only two SGBs that displayed considerable longer durations, were the same as those with histological evidence of a prior low-grade, suggesting that evidence of a prior lower-grade glioma is primarily a clinical criterion: only those that produce symptoms are potentially further explored.

It seems also little scientific to base a classification entirely or predominantly on the patient’s recollection of the onset of symptoms especially when dealing with an initially slowly progressing disease, often producing vague and aspecific symptoms such as headache.

With regard to the relative frequency of primary and secondary GBs, it was of course impossible to extrapolate data from this selected study. In a pilot study (personal unpublished data), using material from serial stereotactic needle biopsies, 7 out of 25 GBs (28%) were classified as secondary. Analysis of the available literature showed however wide variations ranging from 5-12% [256] to 26.4% [187] for SGBs. Scherer himself estimated SGB to be three to four times less frequent than PGBs [199]. Recent evidence from molecular genetic studies also points to the relative predominance of PGBs [129, 233].
2. **Extent of peripheral infiltration**

The second aim of this study was to quantitate the extent of peripheral infiltration, which was confined to PGBs because of the diffuse character of the preexisting lower-grade glioma in SGBs. Historically, it was Scherer who drew attention to "the great practical significance of an exact knowledge of the forms of growth and extension of gliomas" as these, together with localization, exerted "a direct and decisive influence on the possibility of complete extirpation" [196]. Although to Scherer it was an open question whether secondary gliomatous structures indicated either selective migration of glioma cells or progressive neoplastic transformation of preexisting glia cells [198], they have been considered since as the principal pathways of local spread of infiltrating tumor cells, along or within white matter tracks ("peri- and intrafascicular growth") or along basement membrane lined surfaces such as bloodvessels ("perivascular growth"), the neuroglial limiting membrane and the subependymal space ("surface growth") [137, 187]. As to GBs, Scherer regarded all as infiltrative tumors, displaying a circumscribed or diffuse infiltration [195, 196]. In the former, the zone of infiltration was so narrow that it approximately coincided with the macroscopically visible tumor limits, creating favorable conditions for total extirpation, whereas in the latter, consisting of "more or less isolated elements over a very large area of tissue", the histological tumor boundaries were always found to exceed considerably the macroscopically visible ones, making a radical removal impossible. The diffuse spread was further subdivided into secondary or primary according to whether the invasion took place from a compact tumor or not. SGBs were primary diffuse infiltrating gliomas as they arose.
from a primary diffuse astrocytoma, whereas primary GBs were either
circumscribed (± 20%) or secondary diffuse infiltrating tumors [196].

It lasted however for more than forty years before attempts were
made to quantitate the amount of tissue infiltration, a demand that came
from the renewed interest in stereotactic neurosurgical procedures for
diagnosis and treatment of brain tumors due the advent of computed
tomography. The major problem encountered in the few topographic
studies addressing this issue - probably also accounting for their paucity -
was the identification of ITCs. The identification problem has been related
to the small size of the ITC often measuring only 20-25 μm in cross-section
[36], to the inadequacy of the standard techniques of tissue processing to
preserve nuclear and cytoplasmic detail [57] and to the absence of solid
criteria to distinguish ITC from normal or reactive neuroglia [57].

Burger, in two partially overlapping postmortem brain studies on
respectively 6 [33] and 15 GBs [40], found the extent and geometry of the
zone of infiltration to be highly variable, being somewhat determined by the
regional anatomy of the preexisting brain. At one end a narrow zone of
infiltration of less than one cm was encountered in four cases which were
positioned rather tangentially to the adjacent compact myelinated fiber
tracts, whereas at the other extreme ITC could be traced as far as 2.7, 3.5
and 5 cm from the outer border of the hypercellular rim infiltrating the
corpus callosum and optic radiation. In view of the experienced difficulties
to identify ITC, he stated that the extent of these neoplasms was probably
underestimated [40, 90].

A major contribution to this issue was made by the work of
Daumas-Duport on stereotactic biopsy material [57, 119, 120] who faced
with the aforementioned problems, started, in imitation of Scherer [196], by
clearly defining both the solid tumor and ITC tumor components. Her definitions have been adopted and somewhat enlarged in our study. In order to cope with the insufficient quality of routine histological techniques for identification of ITC, an improved technique of biopsy specimen preparation was developed which was found to preserve better cytological detail, involving the combined use of glutaraldehyde-fixed and hemalum-phloxine stained sections and alcohol-fixed, hemalum-phloxine stained smears. Finally, morphologic criteria were defined which -although somewhat dogmatically- permitted to distinguish ITC from normal and reactive neuroglia. ITC were conceived of as tumor cells with distinct nuclear atypia and mostly lacking any visible cytoplasm, although it was admitted that in some cases the nuclear abnormalities were minimal and cytoplasm was microscopically visible. Reactive astrocytes were defined by their enlarged, albeit regular vesicular nuclei with most often a single prominent nucleolus showing stainable cytoplasm extending into delicate, symmetrically arranged cytoplasmic processes. With regard to their spatial arrangement, reactive astrocytes were found to be evenly spaced, the intercellular distance being approximately twice that of the length of their cytoplasmic processes. Although no exact data were presented concerning the extent of infiltration but only CT/MRI-pathologic correlations, it has been possible to reconstruct, to some extent, the image-defined volumetric zones by assuming a spherical shape for the central area of low-density and the zone of T2-prolongation and a shell form for the zone of contrast enhancement. By doing so, we calculated a mean extent of peritumoral infiltration of 6.23 mm (SD 3.37) with a range of 2.76-10.78 mm in 6 grade IV astrocytomas.
In two other MRI-histopathologic correlative studies [70, 115], no data at all were given but ITC were found present in biopsy specimens obtained from areas just beyond the area of T2 abnormality in approximately half of the cases.

In the present study, the MIB-1 cell proliferation marker was employed to identify ITC as these have been shown to be highly mitotically active cells [90]. We further subdivided the ZI into a ZDI and a ZITCI depending on the density of the tumor cells found infiltrating the neural parenchyma and on the presence or absence of microvascular proliferation. The ZI was morphometrically defined as the last HPF, starting from the outer border of the TTP, in which PN could still be observed, which was found to correspond in most cases to the subpial zone of the molecular layer. From the literature we adopted the highest figure cited for correction for the linear shrinkage due to fixation and histologic processing [184] - since we were interested in maximal distances - and found the extent of the ZI to vary from 2.83 to 10.48 mm (mean 4.64 mm, SD 2.50).

Of course these data had somewhat limited value as the ITC were at “the edge” before infiltrating the subarachnoid space. Moreover, although there were some differences in nuclear staining pattern (finely granulated versus coarser) and in nuclear configuration (enlarged, regular round to oval versus irregular in outline and size) between reactive and neoplastic PN, and although there was sometimes a helpful cytoplasmic staining revealing the spidery appearance of reactive astrocytes, it was felt that in the absence of a double MIB-1/GFAP immunostaining it was somewhat hazardous to adhere to this morphometrical criterion of PN/HPF ≥ 1 and therefore by requiring at least 3 PN/HPF, seen the limited proliferative capacity of reactive astrocytes in adults [133, 163], a more accurate depiction of the
extent of the zone of infiltration was provided. The recognition of PN of hyperplastic endothelial cells usually posed no problems. Even by applying our more rigid definition, PN could still be traced near or in the molecular layer (mean 3.93, SD 2.09, range 1.98 - 9.63 mm).

The ZDI on the other hand, was defined morphometrically by the CD, which had to lie constantly above the upper 95% reference range limit of normal brain tissue CD with the last HPF determining its extent. This transitional zone appeared rather narrow and measured on the average after correction for tissue shrinkage 1.52 mm (SD 0.64) with a range from 0.57 to 2.55 mm.

Our study thus demonstrates that even PGBs, arising in a background of apparently normal brain tissue are often more diffuse than superficial examination led us to belief. They seem to disseminate rapidly, often at quite a distance from the macroscopically visible tumor, the extent and geometry of which being apparently determined by the regional anatomy of the preexisting brain [40] and the duration of the tumor [143]. Not only basement membranes act as a substrate for migration- perivascular and subpial secondary structures were present in six cases- but also white matter fascicles have been reported to act as a “highway” for migration when the tumor arises at the beginning or at the end of the tract [137, 151, 152]. On the other hand, neuroanatomical structures may also, at least temporarily, halt tumor growth, a phenomenon already well known to Scherer as reflected in his “white matter growth” describing an arrest of the growth of white matter gliomas at the gray matter (cortex, striatum) [194, 196]. White matter tracts have also been shown to serve as a temporal barrier when the tumor extends tangentially to the course of the tract [40]
which is illustrated in our study by case 7 wherein unequivocal PN were present less than 0.5mm away from the outer border of the TTP.

From a therapeutic point of view it seems important, despite the intervening normal parenchyma, to include the ZDI within the resection margins by enlarging the resection cavity by only a few millimeters ($\pm$ 3mm) compared to the preoperative calculated volumes of the contrast enhancing zone. Not that a cure may be hoped for in most of these cases, but at least a meaningful prolongation of life can be expected since most of the recurrences have been reported within a 2 cm margin of the contrast-enhancing rim [1, 16, 50, 99, 142], illustrating the fact that the probability of tumor recurrence is related to the number of residual tumor cells present. It seems therefore of the utmost importance to deal with these tumors aggressively and without any undue delay. In this regard there is an urgent need for neuro-imaging studies addressing the potential radiographic differences between primary and secondary GBs, because only the former, arising on a background of infiltrated, albeit normal parenchyma may be hoped for to be totally eradicated when treated early. Additional homogenous patterns of enhancement, when present could be useful to this purpose as they may indicate less anaplastic foci often present in SGBs, but this awaits further investigation.

In conclusion, although our study was small and encompassed only 15 GBs, it provided some answers to highly controversial issues such as morphological subclassification into primary and secondary GBs, and tumor boundaries in GBs. Our study not only demonstrated the prognostic usefulness of a histopathological subclassification according to the peritumoral environment, but in doing so it contradicts the held belief that primary and secondary GBs cannot be distinguished morphologically. Our
data further suggest that primary and secondary GBs constitute distinct clinical entities differing in age and prognosis.
The diagnostical insensitivity of clinical criteria such as prior low-grade glioma or duration of symptoms was clearly exposed and were found not to correspond with Scherer's original concept.
The TTP proved not to be a fertile soil for histological subclassification and in particular the MIB-1 PCI had no predictive value for survival in the GB group. Other findings were related to the issue of tumor infiltration. MIB-1 immunostaining was shown to be a reliable and objective technique for estimation of the extent of isolated tumor cell infiltration but a double immunostaining with GFAP would undoubtedly have facilitated the distinction of ITC from hyperplastic reactive astrocytes. Nevertheless, PGBs were found to have disseminated rapidly, their extent and geometry being determined by the regional anatomy of the preexisting tissue acting either as a barrier or as a conduit, and by the life history of the tumor. Seen the high frequency of local recurrences it was advocated to include also the ZDI within the resection margins by expanding the excision by a few millimeters compared to the contrast-enhancing rim. Furthermore the need was expressed for neuro-imaging studies to determine radiographical differences between these two morphological subtypes of GB in order to deal swiftly and aggressively with PGBs which seem to offer some hope for prolonged survival.
A brief introduction elucidates the two aims of this thesis: (1) to investigate whether primary glioblastomas can be differentiated morphologically from secondary glioblastomas, and (2) to determine the extent of peripheral infiltration.

The first chapter or "Salient features of glioblastomas" contains a critical review of the literature.

In a brief historical overview, the evolution of the terminology and definition of glioblastoma over the last 150 years is given. Three important periods can be distinguished. The first "descriptive" period (1846-1900) showed a close parallelism between discoveries in the fields of normal neuro-anatomy and tumor pathology. Already in 1865, two subgroups of primary brain tumors were distinguished: the sarcomas, macroscopically well-defined tumors showing a rapid, often apoplecticiform evolution, microscopically characterized by a fusiform tumor cell type, high vascularity and a pronounced tendency towards necrosis; and the gliomas, macroscopically ill-defined, slow growing tumors, microscopically composed of stellate cells. The second "cytogenetic" period (1900-1949) was greatly influenced by the embryonic cell theory of Cohnheim and the glioblastoma was regarded as a tumor derived from spongioblasts, hence the names spongioblastoma (1918) and spongioblastoma multiforme (1925). In 1927, the glioblastoma was given its actual name. The third period, starting in 1949, was dominated by the concept of anaplasia. The glioblastoma was no longer considered to develop from spongioblasts or glioblasts, but from mature astrocytes (Kernohan) or from any of the macroglia cells (Ringertz) through a process of dedifferentiation or
anaplasia. Except for Zülch who considered the glioblastoma a primary
tumor sui generis, the Kernohan view has prevailed to this day.

Then follows a discussion of the concept of malignancy, which has
in the central nervous system both a biological and clinical connotation.
The classical clinicopathological concepts of benignity and malignancy
are difficult to apply to brain tumors. In view of the closed chamber
properties of the skull, the rapidity of tumor growth is one of the foremost
characteristics of biological malignancy in the central nervous system. The
rapidity of tumor growth can morphologically be inferred from the
evaluation of three histopathological variables: cell density, proliferation
activity and degree of cyto-and histoarchitectural differentiation. The
glioblastoma is thus defined as a very fast growing glioma denoting high
cellularity, high proliferative activity and poorly differentiated cells.
Necrosis and microvascular proliferation are considered indirect signs of
accelerated growth.

Subsequently, some basic facts concerning the epidemiology are
considered. In adults, glioblastomas account for approximately one fifth of
all primary brain tumors amounting to 2-3 new cases per 100000
inhabitants per year in most of the industrialized countries. Although
glioblastoma can occur at any age and in any part of the central nervous
system, most commonly it affects adults and the most frequent localization
is in the cerebral hemispheres.

After a brief review of the principal clinical and neuroradiological
features it is noticed that glioblastomas often present initially with non-
specific symptoms which may create undue delay in therapy and that
modern imaging techniques, although highly sensitive, are often imprecise
in depicting the tumor boundaries.
Most of the grading schemes currently in use for the diagnosis of glioblastoma require three histopathological criteria: marked hypercellularity or mitotic activity, nuclear atypia or poorly differentiated cells, necrosis or microvascular proliferation. Glioblastomas typically display a high proliferative activity, but there may be considerable inter- and intratumoral variation. In order to improve comparability between cell kinetic studies, it is recommended to count the areas with the highest density of mitotic figures or labeled nuclei. Cell proliferation markers have been shown to be a more reliable measure of proliferative activity than mitotic figures.

Glioblastomas have a high propensity for local intraparenchymal spreading; most frequently along the basement membrane lined surfaces and the neuro-anatomical white matter tracks. Extraneural spread is unusual and has been correlated to the short survival of patients and to the inability of the tumor cells to penetrate the vascular basement membrane. Intraneural spread by way of the cerebrospinal fluid pathway has been reported in 6-27% of cerebral glioblastomas at autopsy.

The different standard therapeutic modalities with their successes and failures are described. Whereas the roles of surgery and radiotherapy in the management of patients with glioblastoma are well established, the role of post-irradiation chemotherapy remains controversial.

The prognosis in patients with glioblastoma is poor; most patients die within the year of diagnosis. Apparently, glioblastoma treatment is still facing the same problems which can be related to: the infiltrative mode of tumor growth limiting surgical removal to the solid tumor; the genetic, biochemical, immunological and morphological heterogeneity of the tumor cell population resulting in differences in therapeutic sensitivity; the
sensitivity of the surrounding functional brain tissue for adjuvant therapies; the problem of tumor access by antineoplastic drugs; and the persisting degree of treatment nihilism relative to malignant brain tumors. Finally the multifactoriality of prognosis in patients with glioblastoma is discussed, pointing out several patient-, tumor- and treatment related prognostic factors.

**Chapter II** deals with patient selection and methods employed in the present study. 15 cases of untreated, supratentorial glioblastoma in adults were studied.

For each case clinical, histopathological and morphometrical data were listed. Each glioblastoma was subdivided into tumor tissue proper and periglioblastomatous tissue. The tumor tissue proper was defined according to Daumas-Duport as the tumor component in which the tumor cells are in close contact, without intervening normal parenchyma and associated with microvascular proliferation. For the zone of infiltration, the criteria established by Daumas-Duport were felt to be inaccurate. Instead, two subzones were defined: the zone of dense infiltration in which the tumor cells, although no longer in contact, are still heavily crowded, associated with oedema and microvascular proliferation, and the zone of isolated tumor cells in which the tumor cells are dispersed in the usually oedematous parenchyma without microvascular proliferation. In both the tumor tissue proper and periglioblastomatous tissue, several histological variables were studied: cell density, cytology, histoarchitecture, regressive changes, vascularisation, tumor border and mitotic activity. Morphometrically, two methods of counting were used. The first method, counting a minimum of 1000 cells from areas - in both the tumor tissue
proper and periglialblastomatous tissue - showing the highest density of MIB-1 immunolabeled nuclei, proved to be an ideal method for internal comparison, whereas the assessment of the extent of peripheral infiltration was best served by the second method: counting successive high power fields (HPFs) along randomly chosen tracks as perpendicular as possible to the pial surface, starting at the outer border of the tumor tissue proper. Three morphometrical parameters were defined: (1) cell density = N/n, (2) MIB-1 cell proliferation index = PN/N x 100%, (3) MIB-1 cell proliferation index per HPF = PN/n. Morphometrical equivalents were defined of the zone of infiltration: PN > 1(3), the zone of dense infiltration: N/HPF > 95% upper reference range limit of normal brain tissue cell density and the zone of isolated tumor cells: cell density normal and PN > 1(3). For morphometrical comparison, 10 astrocytomas, 10 anaplastic astrocytomas and 3 normal postmortem brains were studied: the astrocytic tumors by counting areas with the highest density of MIB-1 immunolabeled nuclei, the postmortem brains either by successive HPFs along randomly chosen tracks as perpendicular as possible to the pial surface (cortex), or at random (white matter).

The last two paragraphs deal with the statistical and staining methods employed in this study.

In Chapter III “Results” the 15 cases are described, followed by a summary of the clinical, histopathological and morphometrical data. The pathological and clinical variables are statistically analyzed. No significant differences in cell density were found in the normal cortex or in the normal white matter between the frontal, parieto-occipital and temporal regions. A mean cell density of 46.1 and 60.9 was calculated for
normal cortex and normal white matter respectively. Glioblastomas (tumor tissue proper) showed statistically significant higher cell densities (mean 194.3), MIB-1 cell proliferation indices (mean 27.4) and MIB-1 cell proliferation indices per HPF (mean 53.2) than anaplastic astrocytomas (116.2, 13.6 and 16 respectively) which in turn were significantly higher than those of astrocytomas (84.3, 4.25 and 3.6 respectively). A strong positive correlation was noted between the MIB-1 cell proliferation index and the MIB-1 cell proliferation index per HPF. None of the morphometrical parameters of the tumor tissue proper was statistically significantly related to survival.

The periglioiblastomatous tissue showed two distinct histoarchitectural patterns: infiltrated parenchyma in eight (subgroup A), tumoral tissue in seven (subgroup B). Morphometrically, subgroup A glioblastomas showed cell densities that were significantly lower than those of subgroup B and those of astrocytomas. Compared to normal brain tissue, they appeared slightly increased to the mean cell density, but still fell within the 95% reference interval, and were termed “borderline” normal. The MIB-1 cell proliferation indices per HPF were significantly higher than those of astrocytomas. The cell densities in subgroup B glioblastomas were clearly hypercellular, and were found to correspond, together with the MIB-1 cell proliferation index and the MIB-1 cell proliferation index per HPF values, to those of the astrocytoma or anaplastic astrocytoma depending on the area studied.

The glioblastoma patients were significantly older (mean 53.2) than the patients with astrocytoma (mean 30.3) and with anaplastic astrocytoma (mean 34.1). Within the glioblastoma group, subgroup A patients were significantly older (mean 61.5) than subgroup B patients (mean 43.7).
No significant differences were found in the duration of the preoperative period, nor in tumor location, or in treatment between the morphological subgroups. Postoperatively, subgroup B patients survived significantly longer than subgroup A patients. The Cox regression model proved the morphological subtype and the cell density of the periglioblastomatous tissue to be of significant prognostic value.

The first part of Chapter IV “Discussion” deals with the controversy whether primary glioblastomas can be distinguished morphologically from secondary glioblastomas, a concept raised by Scherer in 1940.

The origin of this controversy can be traced back to the unreceptivity of the scientific climate at that time, being dominated at first by cytogenetic principles regarding the glioblastoma as derived from spongioblasts, and later by grading implying a derivation through anaplasia from mature astrocytes. Clinical and cytological criteria have reinforced the controversy, so much so that it is of current belief that only molecular genetics can reliably distinguish primary, de novo glioblastomas from those arising through malignant progression of astrocytoma or anaplastic astrocytoma (secondary glioblastoma).

The present study contradicts this belief by demonstrating that glioblastomas which develop de novo, arise in the background of normal, albeit infiltrated parenchyma, whereas those developing through progression appear as foci in the preexisting lower-grade glioma which are often large, diffuse tumors and therefore unlikely to be totally obscured by the secondary glioblastoma. Patients with secondary glioblastomas were significantly younger and showed a better prognosis which was independent of the age effect on survival.
Our study further demonstrates the low diagnostic value of clinical criteria such as evidence of prior lower-grade glioma and duration of symptoms to distinguish primary from secondary glioblastomas because both criteria depend on the production of symptoms by the lower-grade precursor which may be lacking in some astrocytomas in view of their tendency to preserve the preexisting parenchyma and the existence of so-called functional silent areas in the brain. Moreover, the onset of anaplasia, being an early event in some, a late in others may also influence the duration of the symptomatic period prior to the diagnosis of the secondary glioblastoma. A long clinical duration excludes a primary glioblastoma, but a short clinical duration may be due to an asymptomatic astrocytoma becoming symptomatic when turning anaplastic or to a rapid onset of anaplasia in a symptomatic astrocytoma. Both criteria lead to an overestimation of primary glioblastoma. It must also be emphasized that how convincing histological evidence of a prior lower-grade glioma may be, in essence it is a clinical criterion since only low-grade gliomas producing symptoms are likely to be further explored.

Other important findings of this study were that within the glioblastoma group, the MIB-1 cell proliferation index has no prognostic value even after adjustment for other prognostic factors, and that isolated tumor cells originate from the tumor tissue proper by infiltration.

The second part of this chapter deals with the extent of peripheral tumor infiltration in primary glioblastomas.

MIB-1 immunostaining appeared to be an easy and objective method for identification of isolated tumor cells. GFAP/MIB-1 double
immunostaining would have improved its reliability by differentiating reactive from neoplastic MIB-1 immunopositive nuclei.

The regional anatomy i.e. the tumor's topographical relationship to surrounding neuro-anatomical white matter tracks (barrier/conduit), adjacent gray matter structures (barrier) and basement membrane lined surfaces (conduit), and the life history of the tumor are important determinants of the extent of peripheral infiltration.

Primary glioblastomas were more diffuse than superficial examination led us to believe with MIB-1 immunopositive nuclei present in or near the molecular layer, often - in view of the small size of isolated tumor cells - at quite a distance from the macroscopically visible tumor suggesting rapid dissemination. The zone of dense infiltration, constituting a zone of transition between the tumor tissue proper and the zone of isolated tumor cells on the other hand measured less than 3 mm. As the probability for local recurrence is determined by the number of residual tumor cells, this zone should ideally be included in the tumor resection volume.

In conclusion, we proved that primary glioblastomas can be differentiated morphologically from secondary glioblastomas on the basis of the periglioblastomatous tissue. We found that primary glioblastomas, arising in a background of normal brain parenchyma, are often more diffuse than superficial examination would led us to believe due to the rapid dissemination of isolated tumor cells, the extent of which is determined by the regional anatomy and the life history of the tumor.
A narrow zone of dense infiltration, which could be prognostically important, can be demonstrated in between the tumor tissue proper and the zone of isolated tumor cell infiltration.
SAMENVATTING
In een korte **inleiding** worden de twee doelstellingen van dit proefschrift **uiteengezet**: (1) na te gaan of primaire glioblastomen kunnen worden onderscheiden van secondaire glioblastomen en (2) bepalen van de omvang van de locale infiltratie in glioblastomen. De achtergronden van de aangevatte studies zijn de blijvende controversen omtrent Scherer's concept van primair en secondair glioblastoom en de begrenzing van het glioblastoom.

Het **eerste hoofdstuk** betreft een kritische analyse van de literatuur.

In een kort **historisch overzicht** wordt de evolutie van de terminologie en van definitie van het glioblastoom geschetst. Drie grote periodes worden onderscheiden. De eerste periode (1846-1900) is die van de macro- en microscopische beschrijving. Twee subgroepen van intrinsieke centraal zenuwstelsel tumoren worden onderscheiden: (1) de sarcomen, macroscopische circumscripte tumoren met een snelle klinische, vaak apoplectiforme evolutie, microscopisch gekenmerkt door een fusiform celtype, hoge cel- en vaatrijkdom en uitgesproken necroseneiging; en (2) de gliomen, diffuus groeiende tumoren met trage klinische evolutie, microscopisch opgebouwd uit stervormige glia cells.


Onder invloed van Bailey (1927) die het unipolair spongioblastoma beschrijft, verkrijgt het glioblastoom zijn huidige naam. De derde periode (1949- ) is gedomineerd door het concept van de anaplasie. Het glioblastoom wordt niet langer beschouwd te ontstaan uit spongioblasten of
glioblasten maar door dedifferentiatie uit mature astrocyten (Kernohan) of één der macrogliacellen (Ringertz). Het Kernohan concept krijgt grote aanhang.

Vervolgens komt het concept van maligniteit in het centraal zenuwstelsel aan de orde. De klassieke clinicopathologische concepten van benigniteit en maligniteit zijn moeilijk toe te passen op hersentumoren. Niet alleen zijn alle onbehandelde hersentumoren vroeg of laat fataal door hun groei binnen de rigide schedel, maar bovendien metastaseren primaire hersentumoren zelden spontaan buiten het centraal zenuwstelsel en is de grote meerderheid van de intrinsieke hersentumoren invasief. De groeisnelheid van de tumor laat daarentegen wel een onderscheid toe en is, gezien de gesloten-doos eigenschappen van de schedel, één van de voornaamste determinanten van biologische maligniteit met als histologische parameters de celdichtheid, de proliferatieactiviteit en de graad van cyto-en histoarchitecturale differentiatie. Het glioblastoom wordt gedefinieerd als een zeer snelgroeien glioma. Dit impliceert: hoge cellulariteit, hoge proliferatieactiviteit en aanwezigheid van anaplastische cellen. Necrosis en vaatproliferatie zijn indirecte tekenen van snelle tumorgroei.

Daarna worden enkele basisgegevens over de epidemiologie van het glioblastoom vermeld. Het glioblastoom is bij volwassenen de meest frequente primaire hersentumor met een jaarlijkse incidentie van 2-3 per 10^5 capita in de meeste Europese en Noord-Amerikaanse landen. Alhoewel het glioblastoom voorkomt op alle leeftijden en in alle delen van het centraal zenuwstelsel, ligt de piekincidentie tussen de 45-65 jaar en zijn de tumoren vooral in de cerebrale hemisferen gelokaliseerd.
Het klinisch beeld is initieel vaak vaag en aspecific. Hoofdpijn is de meest voorkomende klacht. Het trias hoofdpijn, epileptische insulten en hemiparesis doet zich voor in minder dan de helft van de patiënten. Ongeveer 35% van de patiënten hebben alleen epilepsie en 30% alleen hoofdpijn. Het klinisch verloop is meestal kort: minder dan 6 maand in de helft van de gevallen.

Neuroradiologisch tekent het glioblastoom zich doorgaans af als een onregelmatige ringvormige zone van contrastcaptatie rond een centrale zone van hypodensiteit. Het geheel is omgeven door een variabele zone van peritumoraal oedeem.

Het merendeel van de actueel gebruikte graderingscriteriën vereisen voor de histopathologische diagnose van glioblastoom drie criteria: (1) hoge cellulariteit of hoge mitotische activiteit, (2) kernatypie of geringe celdifferentiatie, (3) necrose of microvasculaire proliferatie. Alhoewel het glioblastoom een hoge proliferatieactiviteit vertoont, zijn er toch aanzienlijke intratumorale variaties. Om de vergelijkbaarheid van celkinetische studies te verbeteren is het nodig om de zones met de hoogste labelingdensiteit of mitotische activiteit te tellen. Cel proliferatie merkers laten een nauwkeuriger inschatting toe van de proliferatie activiteit dan de mitotische index.

Het glioblastoom is “berucht” om zijn locale intraparenchymateuze spreiding vooral langs het basale membraan beklede structuren en neuroanatomische witte vezelbundels. Extraneurale metastasering daarentegen is weinig frequent en wordt in verband gebracht met de korte overlevingsduur en het onvermogen van glioblastomcellen om de vasculaire basale membraan te doorboren. Spreiding via de liquor wordt gezien in 6-27% van de glioblastomen bij autopsie. Lange postoperatieve overleving en geringe
differentiatie blijken belangrijke determinanten van intraneurale metastasering.

De standaardbehandeling van het glioblastoom is multimodaal: maximale tumorresectie, gevolgd door radiotherapie tot 60 Gy en chemotherapie. De rol van de chemotherapie is controversieel.

De prognose is inafzit ondanks agressieve behandeling. De meeste patiënten overlijden binnen het jaar. De infiltratieve groeiwijze, de heterogeniteit van de tumorcellen, de radio- en chemosensibiliteit van het omgevende hersenparenchym, het probleem van de bloedhersenbarrière en een persisterend therapeutisch fatalisme ten aanzien van maligne hersentumoren liggen aan de basis. Ten slotte wordt de prognostische multifactorialiteit van het glioblastoom besproken en worden talrijke patiënt-, tumor-, en therapie gerelateerde factoren onderscheiden.

Hoofdstuk II behandelt de patiënten selectie en de methodologie.
De studie omvat 15 onbehandelde cerebrale glioblastomen en beperkt zich tot de astrogliale cellijn. Iedere casus omvat klinische, histopathologische en morfometrische data. Ieder glioblastoom wordt voor topografische doeleinden opgedeeld in eigenlijke tumor (het solide tumorweefsel) en periglioblastomateus weefsel i.e. astrocytوم/anaplastisch astrocytوم of normaal maar geïnfiltreerd hersenparenchyma. De eigenlijke tumor wordt gedefinieerd volgens Daumas-Duport als de tumor component waarin de tumorcellen in dicht onderling contact liggen zonder tussenliggend normaal parenchym, vergezeld van microvasculaire proliferatie. De zone van infiltratie wordt, in tegenstelling tot Daumas-Duport die enkel spreekt over geïsoleerde tumorcellen, opgedeeld in een zone van dense infiltratie.
gekenmerkt door een dicht tumorcel infiltraat in een oedematous parenchyma met microvasculaire proliferatie, en een zone van geïsoleerde tumorcel infiltraat waar de tumorcellen verspreid liggen in een veelal oedematous parenchym zonder tekenen van vaatproliferatie. Verschillende histologische variabelen worden bestudeerd zowel in de eigenlijke tumor als in het periglioiblastomateus weefsel: cellenhedigheid, cytologie, histoarchitectuur, regressieve veranderingen, vascularisatie, tumorbegrenzing en mitotische activiteit.

Morfometrisch worden met betrekking tot het glioblastoom twee meetmethoden gebruikt: enerzijds worden ten minste 1000 nuclei geteld in areas die de visu de hoogste densiteit van MIB-1 gelabelde nuclei tonen (voor onderlinge vergelijking), anderzijds worden opeenvolgende gestandaardiseerde microscopische velden geteld langsheen vijf banen zo loodrecht mogelijk op de pia mater en beginnend vanuit de eigenlijke tumor (voor de bepaling van de uitgebreidheid van de perifere tumor infiltraat). Het aantal nuclei (N), het aantal gestandaardiseerde microscopische velden (n) en het aantal MIB-1 immunopositieve nuclei (PN) wordt genoteerd. Drie morfometrische parameters worden gedefinieerd: (1) cellenhedigheid = N/n, (2) MIB-1 cel proliferatie index = PN/N x 100% en (3) de MIB-1 cel proliferatie index per veld = PN/n. Vervolgens wordt de extensie van de zone van infiltraat, de zone van dense infiltraat en de zone van geïsoleerde tumorcel infiltraat morfometrisch omschreven als het laatste microscopische veld op een bepaald traject waarin: PN/veld > 1(3) = zone van infiltraat, N/veld > 95% bovengrens van normale cellulariteit = zone van dense tumorcel infiltraat, cellenhedigheid normaal met PN/veld > 1(3) = zone van geïsoleerde tumorcel infiltraat. Voor duiding van de gevonden waarden worden 3 normale postmortem hersenen in drie anatomische
localisaties morfometrisch onderzocht evenals 10 astrocytomen en 10 anaplastische astrocytomen. De astrocytaire tumoren worden volgens de eerste meetmethode onderzocht, de cortex van de normale hersenen volgens de tweede, en de witte stof door het “at random” tellen van tenminste 1000 kernen.

In de twee laatste paragrafen worden de histologische technieken en gebruikte statistische methoden besproken.

**Hoofdstuk III** omvat het eigen werk. Na beschrijving en illustratie van de 15 casussen, worden de klinische, histopathologische en morfometrische data samengevat. Vervolgens worden de pathologische en klinische variabelen geanalyseerd. Gemiddeld bedraagt de totale cel dichtheid van cortex 46.1 (SD 9.9) en die van witte stof 60.9 (SD 4.7). Er worden geen significante verschillen aangetroffen in cel dichtheid tussen de frontale, parieto-occipitale en temporale regio’s. Glioblastomen vertonen significant hogere cel dichtheden (gem. 194.3), MIB-1 cel proliferatie indices (gem. 27.4) en MIB-1 cel proliferatie indices per veld (gem. 53.2) dan astrocytomen (84.3, 4.25 en 3.6 respectievelijk) en anaplastische astrocytomen (116.2, 13.6 en 16 respectievelijk). De MIB-1 cel proliferatie index en de MIB-1 cel proliferatie index per veld zijn onderling sterk positief gecorreleerd. De morfometrische parameters zijn geen prognostische betekenis in de eigenlijke tumor.

In het periglio blastomateus weefsel worden twee verschillende histoarchitecturale patronen beschreven: normaal, geïnfiltreerd parenchyma in 8 glioblastomen (subgroep A), tumorweefsel in 7 (subgroep B). De cel dichtheid in subgroep A is significant lager dan die in subgroep B en die van het astrocytoom, maar is ten opzichte van de gemiddelde normale
celdichtheid licht verhoogd doch valt nog steeds binnen de 95% bovengrens van normale celdichtheid: borderline normocellulariteit. De MIB-1 cel proliferatie indices per veld van subgroep A glioblastomen zijn significant hoger dan die van het astrocytoom. De celdichtheden van subgroep B zijn duidelijk hypercellulair en corresponderen met die van het astrocytoom of anaplastisch astrocytoom afhankelijk van de bestudeerde area. Patiënten met een glioblastoom zijn statistisch significant ouder (gem. 53.2) dan die met een anaplastisch astrocytoom (gem. 34.1) of astrocytoom (gem. 30.3) en subgroep A glioblastoma patiënten zijn significant ouder (gem. 61.5) dan die met een subgroep B glioblastoom (43.7). Er blijken geen significante verschillen te bestaan tussen de morfologische subgroepen inzake tumor lokalisatie, behandeling en duur van de preoperatieve periode. Subgroep B glioblastoma patiënten vertonen een significant langere postoperatieve overleving die onafhankelijk is van het waargenomen leeftijdsverschil. Het Cox regressie model toont dat het morfologisch subtype en de celdichtheid van het periglioblastomateus weefsel statistisch significante prognostische factoren zijn.

Het eerste deel van de discussie, hoofdstuk IV, behandelt het vraagstuk van de morfologische differentiatie van primaire en secundaire glioblastomen, één der voornaamste controverses uit de neuro-oncologie. De oorsprong van deze controverse is terug te voeren tot het toenmalige wetenschappelijk klimaat dat eerst beheerst wordt door cytogenetische classificatiesystemen waarin het glioblastoom een tumor van spongioblasten/glioblasten is en later door gradering met het glioblastoom een graad 3-4 astrocytoom. Beide denkwijzen laten weinig ruimte voor Scherer’s concept van primair en
secondair glioblastoom. De stempel gedrukt door het Kernohan schema is zo diep dat zelfs vijftig jaar later het glioblastoom nog steeds zijn vermeende oorsprong heeft in astrocyten. Ondertussen infiltreren cytologische (Rubinstein, Burger) en klinische criteria (Burger) Scherer's concept en versterken de opvatting dat vermoedelijk alle glioblastomen secondair zijn. De komst van de moleculaire genetica brengt hierin uiteindelijk verandering en bevestigt dat er tenminste twee genetische subtypen van glioblastoom zijn die evenwel morfologisch niet van elkaar te onderscheiden zijn: het de novo of primair glioblastoom essentieel gekenmerkt door een amplificatie van het epidermal growth factor receptor gen en het secondair glioblastoom dat onstaat door maligne progressie vanuit een astrocytoom of anaplastisch astrocytoom, moleculair genetisch gekenmerkt door een mutatie van het p53 tumor suppressor gen. Opnieuw doen klinische criteria hun intrede en vereisen voor diagnostiek van een primair glioblastoom een korte klinische duur en histologische tekenen van full-blown glioblastoom in de eerste biopsie, en voor een secondair glioblastoom klinische of histologische evidentie van een laaggradige precursor.

Gebruik makend van Scherer's methodologie waarbij de tumor in zijn topografische relatie tot het omgevend weefsel wordt bestudeerd, wordt aangetoond dat het primair d.i. de novo ontstane glioblastoom zich bevindt op een achtergrond van normaal, maar geïnfiltreerd parenchyma, terwijl het secondair glioblastoom dat onstaat door progressie vanuit een astrocytoom of anaplastisch astrocytoom haardvormig aanwezig is in de lager-gradige tumor. Patiënten met een secondair glioblastoom zijn beduidend jonger dan die met een primair glioblastoom en hebben een ook betere prognose die statistisch onafhankelijk is van de leeftijd.
Verder toont onze studie de lage diagnostische waarde aan van criteria zoals de duur van de preoperatieve symptomatologie en evidentie van een precursor-laesie. Een lange klinische duur sluit een primair glioblastoom uit, maar een korte klinische duur sluit geen secondair glioblastoom uit daar deze kan berusten op hetzij een asymptomatisch groeiende laaggradige tumor (functioneel stille hersenzones, preservatie van het preëxistente parenchyma) of vroegtijdige anaplasie in een symptomatische precursor. Beide criteria leiden tot een overschatting van het primair glioblastoom. Overigens moet benadrukt worden dat histologische evidentie van een voorafbestaande laaggradige tumor in essentie een klinisch criterium is: enkel symptomatische laaggradige gliomen worden chirurgisch geëxploreerd.

Andere belangrijke bevindingen van deze studie zijn dat de MIB-1 cel proliferatie index in de glioblastoma groep geen prognostische waarde heeft en dat de geïsoleerde tumorcellen afkomstig zijn uit het solide tumorweefsel door infiltratie.

Het tweede deel van de discussie betreft het probleem van de tumorgrenzen in het glioblastoom. Terwijl sommige auteurs het glioblastoom onder de diffuus groeiende astrocytaire tumoren rekenen, beschouwen andere auteurs het glioblastoom als een eerder circumscripte tumor hierin gesteund door het locaal recidiefpatroon van het glioblastoom. De oorzaak van dit dispuut ligt in de moeilijkheid geïsoleerde tumorcellen te herkennen en het ongenuanceerd gebruik van de term glioblastoom voor zowel het primair als secondair glioblastoom.

In deze studie wordt gebruik gemaakt van de MIB-1 proliferatie merker voor de identificatie van geïsoleerde tumorcellen en spits het onderzoek
zich toe op het primair glioblastoom. Het secondair glioblastoom is immers door de laaggradige precursor diffusus. MIB-1 immunokleuring en morfometrie tonen aan dat het primair glioblastoom diffuser is dan routine lichtmicroscopisch onderzoek laat blijken: tumorcellen zijn aanwezig in of nabij de moleculaire laag in de acht primaire glioblastomen, vaak op een relatief d.i. in verhouding tot de tumorcel grootte, grote afstand van de eigenlijke tumor wat een snelle verspreiding suggereert. De uitgebreidheid van de perifere infiltratie wordt evenwel sterk bepaald door de regionale anatomie waarbij aangrenzende structuren de infiltratie kunnen bevorderen of integendeel (tijdelijk) remmen. Van potentieel groot prognostisch belang-gezien de kans op recidief in of nabij het tumorbed bepaald wordt door de hoeveelheid overblijvende tumorcellen- is de relatief smalle zone van dense infiltratie (minder dan 3 mm in alle gevallen).


30. Brucher JM. Personal communication.


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215. Stefanik DF, Rizkalla LR, Soi A. et al. Acidic and basic fibroblast
growth factors are present in glioblastoma multiforme. Cancer
216. Stewart P., Hayakawa K., Farrell C. et al. Quantitative study of
microvessel ultrastructure in human peritumoral brain tissue: evidence
for a blood-brain barrier defect. Journal of Neurosurgery 1987; 67:
697-705.
217. Storch H. Uber die Pathologischen-anatomischen Vorgänge am
218. Strauss L., Globus J. Spongioblastoma with unusually rapid growth
Beitrage zur pathologischen Anatomie 1911; 51: 1.
220. Takeuchi K, Hoshino K. Statistical factors affecting survival after
221. Taveras J., Thompson H., Pool J. Should we treat glioblastoma
multiforme? A study of survival in 425 cases. American Journal of
Roentgenology 1962; 87: 473-479.
222. Thapar K., Rutha J., Laws E. Brain edema, increased intracranial
pressure, vascular effects, and other epiphenomena of human brain
tumors. In: Brain Tumors: an encyclopedic approach. Kaye A.,
223. Tohma Y., Gratas C., Van Meir E. et al. Fas/Apo-1 (CD95)
expression in primary and secondary glioblastoma. Journal of
224. Tooth H. Some observations on the growth and survival period of
225. Vandenbergh S. Current diagnostic concepts of astrocytic tumors.
Journal of Neuropathology and Experimental Neurology 1992;
226. Vecht C., Avezaat C., Putten W. et al. The influence of the extent of
surgery on the neurological function and survival in malignant glioma.
A retrospective analysis in 243 patients. Journal of Neurology
matrix associated proliferation related antigen. Localization in mitotic
cells and association with chromosomes. Journal of Cell Science
1989; 92: 531-540.
228. Vertosik F., Selker R. Brain stem and spinal metastases of
supratentorial glioblastoma multiforme: a clinical series.


