TUMOR CHARACTERISTICS AND BIOLOGICAL BEHAVIOR OF OLIGODENDROGLIOMA

13 Mei 1930

Dirk D

Tumor cerebri

Aanwezig: Prof., Oljenick, Diemond, van Dijk, Portheine, Hemhof, Krause, Co's.
Dr. Portheine stelt voor Dirk Dornbos, oud 13 jr. opgenomen 8 Mei '30 op advies van Prof. van der Hor (opname in Valeriuskliniek op 16 April '30)
Anamnese: Vorig jaar op school blijven zitten, terwijl te voren redelijk goed kon leeren. Sinds Oct. '29 optreden van aanvalletjes waarin dwangstand van het hoofd naar links, zonder bewustteloosheid, duur 2 min, frequentie tot op heden 1x. In Dec. '29 af en toe duizelig. Later ook bewusteloos af en toe heeft hierbij aanrechtsrijk eens met de l. hand ge trokken en de ogen naar l. gedraaid. Vervolgens aanvalletjes van niet kunnen spreken. Sinds April 1930 hoofdpijn r. in het voorhoofd met braken.
Status: 1) zeer fraai "bruit de pot fêlé" rechts pariëtaal.
2) Bdr. stuwingspapil
3) Peesreflexen l. hooger, buikreflexen l. lager.
4) X foto: dilatatie van de naden; kalkschaduw rechts fronto-pariet. l.
Conclusie: glioma cerebri met verkalking, ondanks het feit, dat dit bij jeugdige personen zeldzaam is. Voor één tuberkel in 't geheel geen aanknopingspunten.
Advies: operatie

14 Mei 1930
Dr. Oljenick Speciele opmerking.
De diagnose bood in dit stadium geenerlei moeilykheid meer
De calcificatie in het onderste gedeelte van den rechter
voorhoofdskwab naby de ala magna minor van het Os Sphenoidale kwam goed uit met de zy het ook geringe symptomen van de linker zyde,nl. hoogere voetreflex (Achillespees) met een geringe neiging tot clonus, een wellicht eenigszins hoogere kniepeesreflex en een uiterst geringe en niet constante emotionele Facialis, die alleen vermeld wordt omdat het by de rechtszydige diagnose zou passen.
Bovendien was er in de anamnese sprake van een Jackson bestaande uit trekkingen van het hoofd naar links.
Moeilyker was het te beoordeelen in hoeverre ik in staat zou zyn iets voor den jongen te doen ,nu de calcificatie zoo diep lag op minstens 6 cM. van de schedeloppervlakte.
Gelukkig bleek het vermoeden dat wy hier te maken hadden met het goedaardig type glioom juist en bleek de calcificatie in een wandstandige tumor te zitten van een groote gliocyste, die tot vlak onder het hersenoppervlak reikte
De drukvermindering die de ontlediging van de cyste met zich brachtmaakte het werken betrekkelyk gemakkelyk, zoodat ik wel meen te mogen beweren dat het wandstandig gedeelte zwel geheel verwyderd is.
Behalve een eenmalig braken dat tegen het einde der operatie optrad , vermoedelyk tengevolge van een beetje veel drinken, werd de operatie zonder eenige stoornis ten einde gebracht en het mag wel vermeld worden dat patient aan het einde der operatie op de operatietafel genoegelyk lag te zingen en te fluiten.Dit is een nieuwe ervaring voor my.

Uit de status van patientje Dirk D., geboren 1917, opgenomen in de neurochirurgische kliniek van het Wilhelmina Gasthuis te Amsterdam op 8 mei 1930.

In de loop van 1932 volgden tekenen van recidief, resulterend in een heroperatie in november van dat jaar. Na een subtotale extirpatie overleed patientje op 6 november 1932.
Einddiagnose: oligodendroglioma rechts fronto-parietaal.

TUMOR CHARACTERISTICS AND BIOLOGICAL BEHAVIOR OF OLIGODENDROGLIOMA

Tumor Eigenschappen en Biologisch Gedrag van Oligodendroglioma

PROEFSCHRIFT

ter verkrijging van de graad van Doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.Dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 27 mei 1992 om 15.45 uur

door

JOHAN MARINUS KROS

geboren te Apeldoorn

universiteits
drukkerij

PROMOTIECOMMISSIE

Promotor:	Prof.Dr. F.T. Bosman
Co-promotor:	Dr. Th.H. van der Kwast
Leden:	Prof.Dr. S.Z. Stefanko
	Prof.Dr. F.C. Stam
	Prof.Dr. C.J.J. Avezaat

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Kros, Johan M.

"Tumor Characteristics and Biological Behavior of Oligodendroglioma"
Thesis E.U.R. (Rotterdam) - with ref. - with summary in Dutch
ISBN 90-9003787-X

Subject Headings:
** oligodendroglioma * glioma * grading*
** glial fibrillary acidic protein * immunohistochemistry*
** DNA-ploidy * electron microscopy * immunoelectron microscopy*

영희 에게 드림니다.

aan mijn ouders

TUMOR CHARACTERISTICS AND BIOLOGICAL BEHAVIOR OF OLIGODENDROGLIOMA

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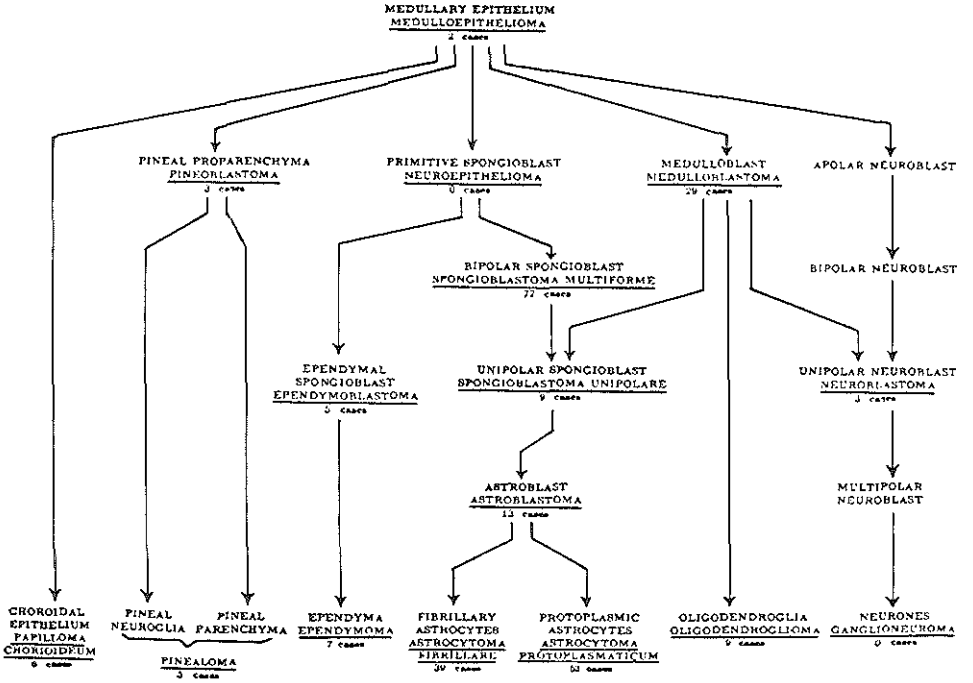


Figure 1A. Scheme of evolution of certain elements of the central nervous system serving as key to the terminology (after Bailey and Cushing) for neuro-ectodermal tumors. (From: P. Bailey: "Further remarks concerning tumours of the glioma group", Bull Johns Hopkins Hosp 40:354, 1927).

GENERAL INTRODUCTION

CLASSIFICATION AND GRADING OF GLIAL TUMORS

During the end of the nineteenth century it was believed that cancer was the result of defective embryogenesis, or more specifically, of the outgrowth of an overshoot of cells representative of a particular developmental phase (Cohnheim 1889). Subsequently, the morphologic differences of glial neoplasms would be caused by the outgrowth of embryologic remnants of various stages in glial development (Ribbert 1907, 1918). Because Ribbert did not believe in differentiation or anaplasia of tumor cells, all glial tumors would consist of cells with counterparts known from normal embryology. Hence, the first classifications of glial tumors were based on the resemblance of the neoplastic cells with cells of normal developing glia (Bailey and Cushing 1926, 1930, Penfield 1931, Hortega 1931) (**Fig. 1A and 1B**).

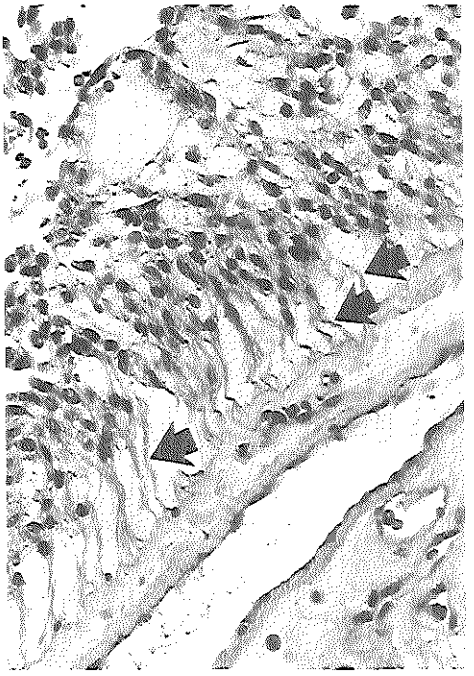


Figure 2A. Spongioblastic differentiation. This example of spongioblastic differentiation was found in a case of ependymoma. The tumor cells are connected with the endothelial cells by thin cytoplasmic processes (arrows). (hematoxylin-eosin, x150).

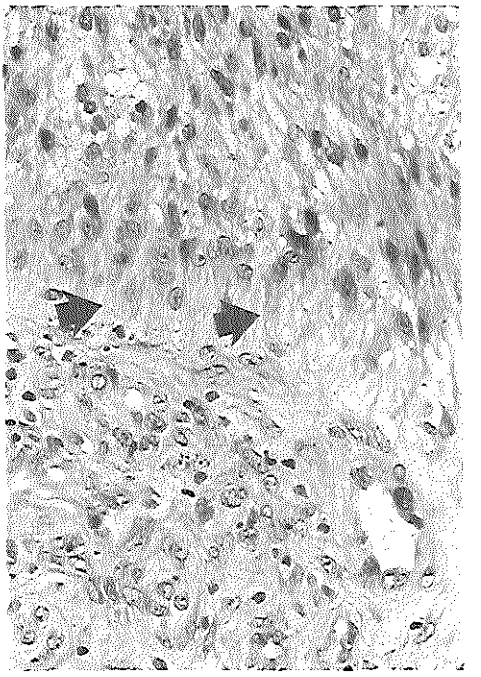


Figure 2B. Astroblastic differentiation. The "astroblastoma" as separate tumor entity has been removed from most modern classifications. In some astrocytomas focal "astroblastic differentiation" may be encountered. In contrast to the spongioblastic differentiation, plump cell processes (arrows) touch the vessel walls. (hematoxylin-eosin, x150).

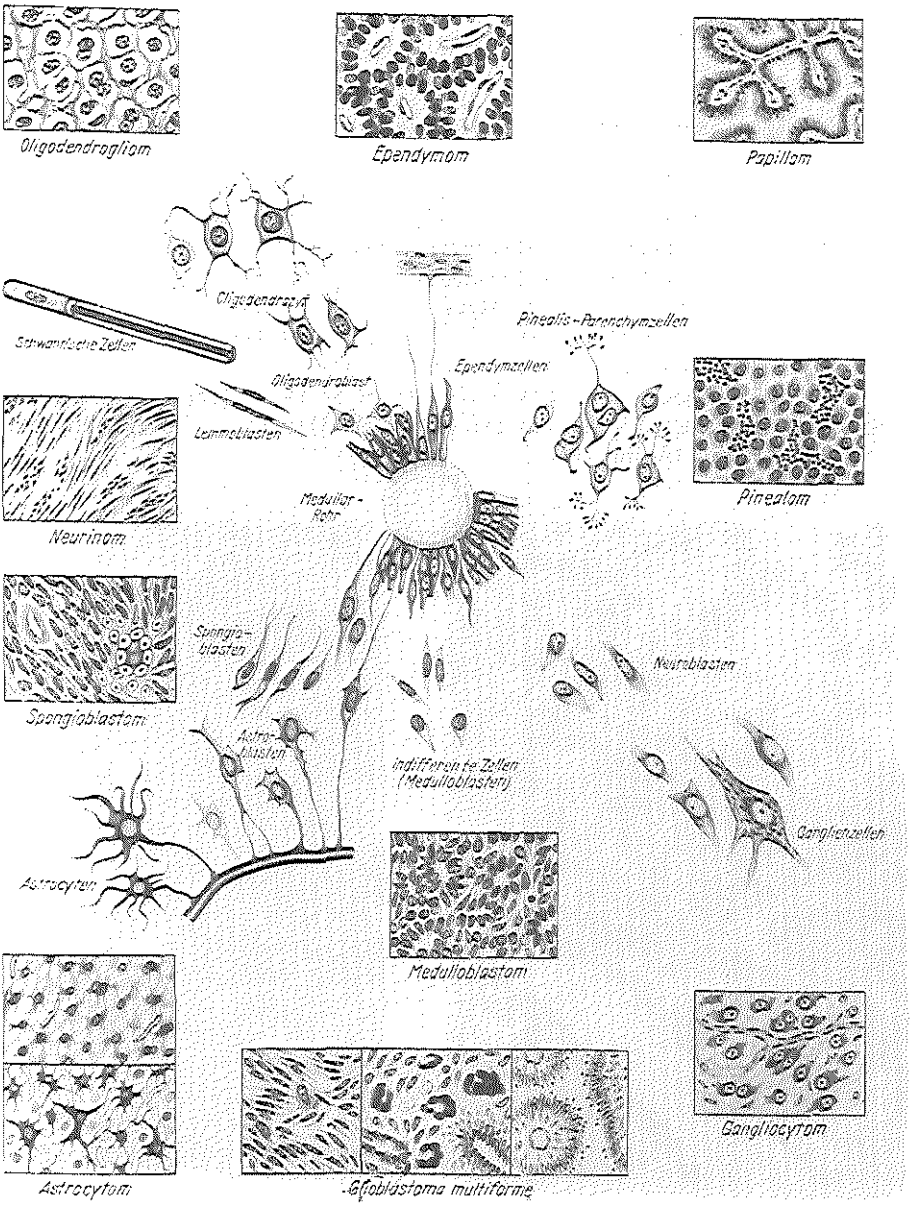


Figure 1B. "Die Entwicklung der reifen Zellen und ihrer Reifungsstufen aus dem Medullarrohr mit den dazu in Vergleich gesetzten Hirngeschwülsten". (From: "die Hirngeschwülste", Joh. Ambr. Barth, 1951, Handbuch der Neurochirurgie. Ed. H. Olivercrona and W. Tönnis. 3. Band: Pathologische Anatomie der raumbeengenden intrakraniellen Prozesse. Bearbeitet von K.J. Zülch and E. Christensen. Springer Verlag - Berlin - Göttingen - Heidelberg, 1956, p. 15).

The "pinealoma" of the scheme is drawn as a germ cell tumor, which is frequently found in the pineal region. In figure 9 the "true pinealoma" is represented. Only two astrocytic variants are shown; the pilocytic variant (Figure 4) is represented by (part of) the obsolete entity of "spongioblastoma" (Figure 2C), while the gemistocytic variant is missing in the scheme.

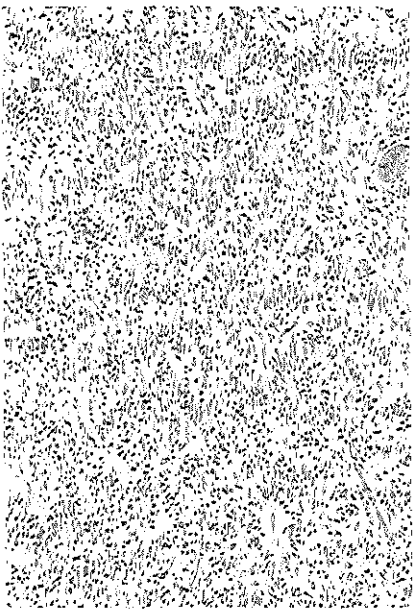


Figure 2C. Spongioblastoma. The term "polar spongioblastoma" was used for a tumor with elongated cells arranged in parallel rows. The term should not be confused with the old term "spongioblastoma multiforme", that was later changed into "glioblastoma multiforme", and used for the small-cell anaplastic glioblastomas. In fact, the occurrence of thin, elongated cells within various tumors repeatedly elicited the term "spongioblast", the cause of much confusion in terminology. (hematoxylin-eosin, x60).

Based on morphologic differences revealed by impregnation methods of Golgi, Bielschowsky, Cajal, and del Río-Hortega, several different tumors of the nervous system were described. In old terms as "astroblastoma", "polar spongioblastoma", and "medulloblastoma", developing neuroglial cells are recalled (**Fig. 2A, 2B and 2C**).

Unfortunately, glial tumor cells were not always succesfully matched with developing glial cells, simply because not all embryologic counterparts seemed to exist, while also there was confusion about the taxonomic order of the various developing glial cells. For instance, the medulloblast was not identified as a stem cell, and it was shown that the unipolar spongioblast does not arise from a bipolar spongioblast. Furthermore, the stem cell of the oligodendrocyte remained obscure. Gradually the embryogenetic theory was overruled by the theory that tumors are the result of anaplastic changes overtaking a well differentiated tissue (Bailey 1927, Roussy 1928, Cox 1932, Scherer 1935, 1940).

Whereas terms presently used for less differentiated tumors as "glioblastoma" and "medulloblastoma" remind to the early embryologic theories, only few cerebral tumors have been convincingly related to germinal zones of the developing human brain (Globus 1953, Lewis 1981).

Scherer is considered as the founder of the modern classification of glial tumors, although he never published a new classification. Supported by experimental evidence (Deelman 1923) he rejected the embryogenetic principle for the genesis of gliomas (Scherer 1933). Now, the tumors were not only characterized by the cytology of the individual cells, but by the structure of the tumor tissue as well. Glial neoplasms would develop from (end-)differentiated glial cells, showing anaplastic changes. In addition to characteristic growth patterns ("primary structures") of cerebral tumors so-called "secondary structures" were distinguished, histologic phenomena that were the result of tumor growth into the preexistent brain tissue. Reactive mesenchymal proliferation due to growth of neoplastic cells in the meninges, or organization of necrotic areas, led to formation of "tertiary structures". Influenced by Scherer's view the embryogenetic schemes were replaced by new classifications (Table 1, page 32) (Bailey 1927, Carmi-

chael 1928, Roussy and Oberling 1931, Hortega 1932). In 1949 Kernohan and coworkers introduced a new classification based on the phenotypes of normal end-differentiated glial cells (**Table 2**, page 33). They considered the astrocytomas, astroblastomas and glioblastomas as variants of the same group.

> *"It soon became evident that there is within this tumor-group-complex a gradual transition from least malignant to most malignant, throughout which the histologic features of the former can be traced uninterrupted into the latter. We of course saw the same cells which Bailey and Cushing considered to be astroblast, and the variegeted polymorphic cells and the mitotic figures which are said to characterise the glioblastoma multiforme. However, rather than attempt to relate these various cells to certain stages of the development of the central nervous system of the embryo, we interpreted them as anaplastic transformations of normal astrocytes. Therefore on the basis of dedifferentiation or anaplasia that is, pleomorphism, hyperchromatism and mitotic figures, we believe that these three tumor groups the astrocytoma, astroblastoma and glioblastoma multiforme are merely malignant variants of normal astrocytes"* (Kernohan 1949).

Many glial tumors consist of mixtures of cells resembling astrocytes, oligodendrocytes or ependymal cells. Furthermore, lipidization of tumor cells, myxoid and chondroid changes and forming of cellular whorls are phenomena that may complicate characteristic histopathological pictures, and may cause difficulties in proper classification (Kepes 1987). Because of the confusing terminology and the vast variability in histopathology of glial tumors, simplification of the nomenclature is still proposed (Armstrong 1990).

The classification schemes were not designed to provide any prognostic information. Scherer had been unable to propose a classification scheme for glial tumors by himself, because he realized that besides differences in morphology large differences in biologic behavior should be taken into account. Analogous with the practice of grading epithelial tumors (Broders 1926), grading of cerebral neoplasms was introduced. Increasing tumor grade should parallel more aggressive biologic behavior. Kernohan and Sayre and almost simultaneously Ringertz developed grading schemes in order to predict the biologic behavior of the gliomas (**Table 2**) (Kernohan 1952, Ringertz 1950). In a grading procedure morphological varieties are ranked according to so-called "dedifferentiation" (lack of differentiation or anaplasia), noticed when a tumor cell is compared with its putative non-neoplastic counterpart. To summarize, first the homology in differentiation of tumor cells with non-neoplastic cells is established by classifying a tumor. By subsequent attribution of a malignancy grade, the degree of dissimilarity of the tumor cells with the cells they putatively descent is expressed. Prognostic information is implicitly provided by the tumor category: for instance, ependymomas behave less malignant than astrocytomas, and pilocytic astrocytomas are more benign than any of the other members of the astrocytoma group. The tumor grade ranks the relative degree of malignancy within the category. As a general rule, gliomas are assigned the malignancy grade of their least differentiated parts.

The value of grading of gliomas has been disputed since glial neoplasms often display strongly heterogeneous differentiation in different areas (Globus 1953, Paulus 1989). Proper grading (and even proper classification) would only be possible when the entire tumor could be investigated, a situation only feasable at autopsy, since surgery seldom results in total tumor removal (Scherer 1940, Russell 1977). Particularly grading of mixed

gliomas is problematic. Whether grading these tumors should be limited to the predominant cell type, or alternatively, all components should be taken into account, remains open for discussion (Hart 1974).

About 50 percent of the glia-derived tumors are glioblastomas (**Fig. 3A and 3B**). In its "primary form" this tumor is built by small spindle-shaped cells (reflected in the old term "polar spongioblastoma") (**Fig. 3A**). Primary glioblastomas generally are small, ovoid tumors with extensive necrotic areas, appearing without less malignant precursors. On the contrary, considerable pleomorphism may be found in secondary glioblastomas, which would represent - according to some authors - the end-stage of various gliomas (**Fig. 3B**).

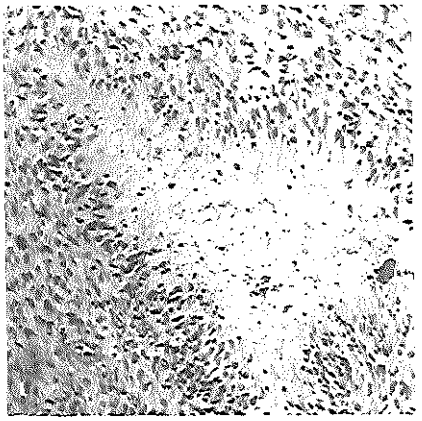


Figure 3A. Small-cell anaplastic glioblastoma ("primary glioblastoma"). (hematoxylin-eosin, x60)

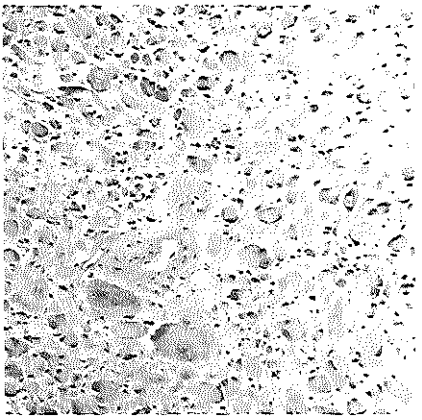


Figure 3B. Pleomorphic glioblastoma ("secondary glioblastoma"). (hematoxylin-eosin, x60).

The group of astrocytomas accounts for about 30 percent of the total glioma group. Subtypes of astrocytomas, based on cytological differences, are distinguished (**Fig. 4**).

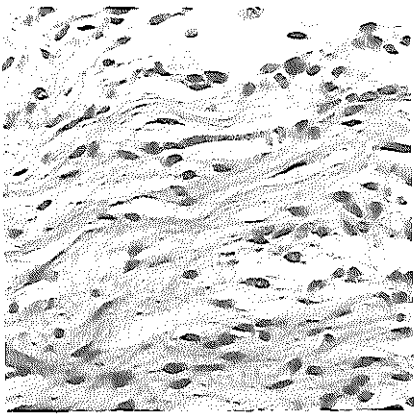


Figure 4A. Astrocytoma, pilocytic variant. (hematoxylin-eosin, x150)

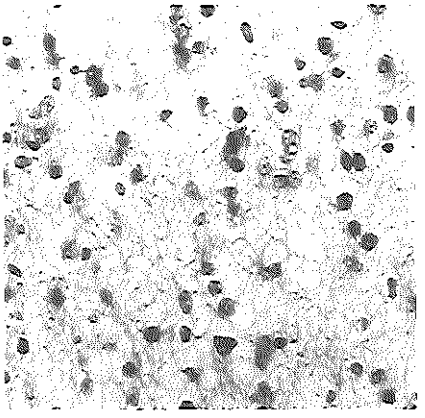


Figure 4B. Astrocytoma, fibrillary variant. (hematoxylin-eosin, x150)

OLIGODENDROGLIAL TUMORS

variants of well-differentiated oligodendrogliomas

As consequence of the assumed parallelism between normal development and neoplasia, speculations on the existence of tumors resembling developing oligodendroglial cells were already made by Bailey and Hiller in 1924. A short time later Bailey and Cushing (1929) introduced the term "oligodendroglioma" for a tumor with oligodendrocyte-like cells. In 1929 Bailey and Bucy published the first small series of this neoplasm. Oligodendrogliomas encompass 2 to 14 percent of primary brain tumors in the subsequent literature. The monotonous picture of the classic oligodendroglioma is reflected in the term "oligodendroglioma isomorphe". Analogous to the description of normal oligodendroglia by Del Rio Hortega and Penfield the neoplastic oligodendroglial cells were described as

> *"small, with scanty cytoplasm and round nuclei containing abundant chromatin. Mitotic figures were very rare. Between the nuclei was a delicate fibrillary material which could not be impregnated nor stained differentially by any method then at our disposal. When degenerated it formed a honeycomb around the nuclei so that the cross section had much the appearance of a section of a woody plant. Scattered astrocytes were usually present"* (Bailey and Bucy 1929).

In silver impregnation preparations the oligodendroglial cells were identified by their short processes.

In the routine hematoxylin and eosin staining, the classical oligodendrogioma shows a monotonous architecture of cells with a perinuclear halo, caused by (delayed) fixation. The typical honey-comb structure arises when the cells with their perinuclear halos are packed closely together (**Fig. 12**).

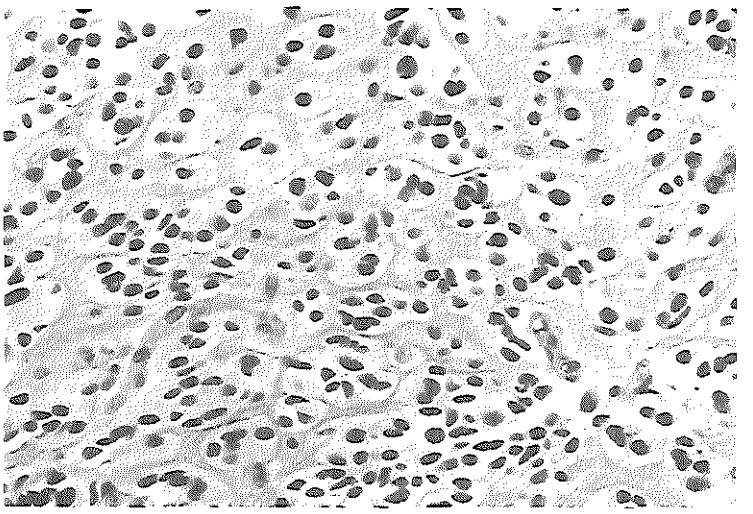


Figure 12. The classical picture of the oligodendroglioma. The classical "honeycomb" structure is due to the perinuclear halos, caused by shrinkage of the cytoplasm during fixation. (hematoxylin-eosin, x150).

Cell borders are clearly visible between the optically empty cytoplasmatic spaces. Characteristic arcuate capillaries are found between the patternless sheets of neoplastic oligodendrocytes. Calcospherites may be encountered, and may be a diagnostic clue in less typical oligodendrogliomas, since they occur less fre-

The pilocytic astrocytoma (**Fig. 4A**) is not only characterized by its cellular constituents, but by its infratentorial localization as well. Whereas the fibrillary, protoplasmic and gemistocytic astrocytomas (**Fig. 4B, 4C, 4D**) do not differ too much in clinical behavior, the pilocytic variant matches a much better prognosis.

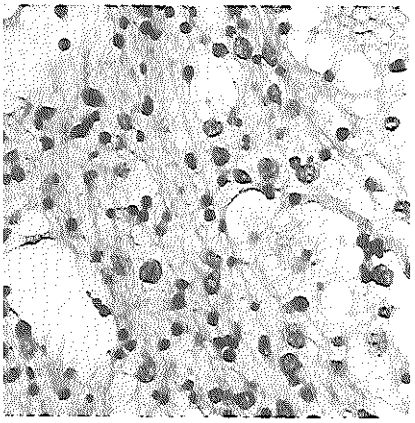


Figure 4C. Astrocytoma, protoplasmic variant. (hematoxylin-eosin, x150)

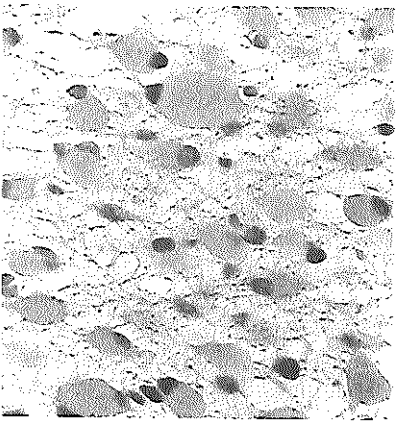


Figure 4D. Astrocytoma, gemistocytic variant. (hematoxylin-eosin, x150)

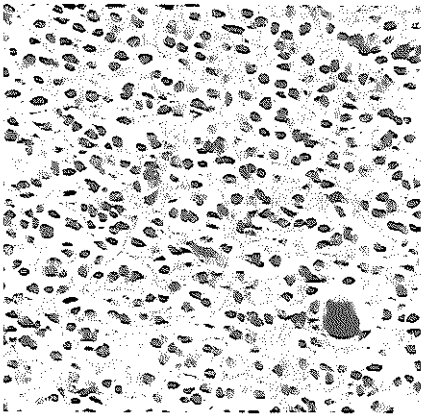


Figure 5. Oligodendroglioma. (hematoxylin-eosin, x150).

The oligodendrogliomas (**Fig. 5**) are relatively uncommon neoplasms, accounting for 2% to 14% of intracranial gliomas. In most schemes the ependymomas (**Fig. 6**) were recognized as separate group. About the histogenesis of these tumors much debate existed. The ependymal spongioblast (**Fig. 2A**) would be closer in structure to the astrocytic series than to the adult ependyma (Cox 1932, Willis 1948). The choroid plexus papilloma (**Fig. 7**) often is indistinguishable from normal choroid plexus tissue. A congenital origin of this highly differentiated tumoris suggestive, although it is not exclusively a tumor of childhood.

The medulloblastoma (**Fig. 8**) is a tumor of the cerebellum that is mainly found in children. It is considered as a true embryonal tumor with naked hyperchromatic nuclei with a tendency to form rosettes. This tumor is closely related with the group of the socalled primitive neuroectodermal tumors PNET).

quently in astrocytomas or ependymomas. When invading cortical gray matter, neoplastic oligodendroglia was often seen crowded around neurons, a phenomenon termed "perineuronal satellitosis" (**Fig. 13**).

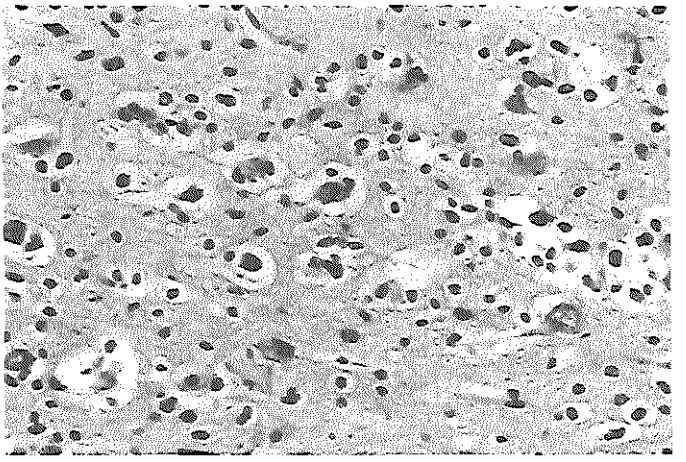


Figure 13. Perineuronal satellitosis. Cortical neurons are surrounded by neoplastic oligodendrocytes. (hematoxylin-eosin, x150).

Subpial infiltration and tumor progression along the surface of the brain are other characteristics of oligodendrogliomas.

Several variants of the classical picture are met. Mucinous material may be found within or between the neoplastic cells, commonly interpreted as degeneration. Sometimes so called "signet-ring cells" are found in clusters, often in combination with mucoid degeneration (Burger 1982, Rubinstein 1989) (**Fig. 14**). Signet ring cells were described since long in oligodendrogliomas, and they were defined by Burger as

> *"Cells with nuclei that were similar in shape and conformation to adjacent neoplastic oligodendroglia and had perinuclear halos, but in which prominent eosinophilic cytoplasm could be seen. This usually was an eccentrically placed, hyalin mass, but sometimes displayed a distinct fibrillarity"* (Burger 1987).

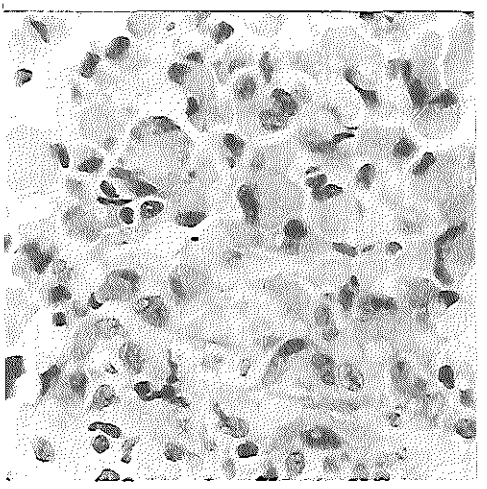


Figure 14. Signet ring cells. The nucleus of these cells is peripherally displaced, due to the ballooned cytoplasm filled with mucoid material. (hematoxylin-eosin, x380).

The origin of signet-ring cells remains obscure. Another variant oligodendroglioma is entirely or partly composed of eosinophilic granular cells (Escalona-Zapata 1981) (**Fig. 15**). It was shown by electron microscopy that the cytoplasm of these granular cells

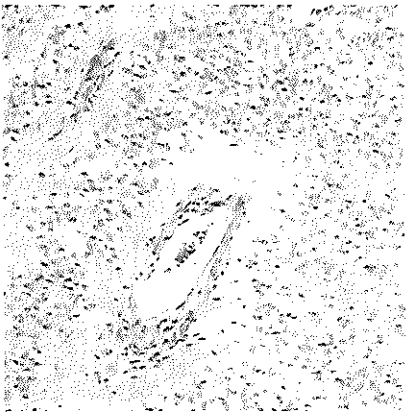


Figure 6. Ependymoma. (hematoxylin-eosin, x60). The epithelial properties are witnessed in perivascular pseudorosettes ("kernfreie Höfe") and clefts ("Ependymschläuche").

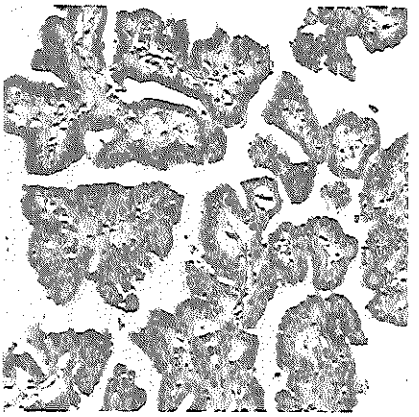


Figure 7. Choroid plexus papilloma. (hematoxylin-eosin, x60).

Sometimes differentiation is encountered in tumors of this group. The pineoblastoma is a highly cellular variant of the pineocytoma consisting of primitive cells that closely resemble the medulloblastoma. The (true) pineocytoma (or pinealoma) (Fig. 9) consists of primitive undifferentiated cells, and should also be considered as embryonal tumor, although it is largely a neoplasm of adolescence and adulthood.

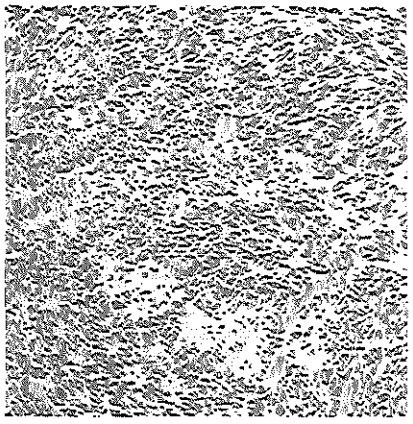


Figure 8. Medulloblastoma. (hematoxylin-eosin, x60). Rosettes (Homer Wright rosettes) are specific but not often seen.

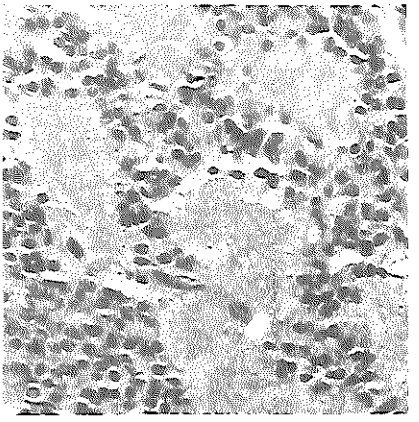


Figure 9. Pineocytoma. The "true pineocytoma" or "pinealoma" is built by small cells arranged in strands around acellular zones of fibrillarity. The tumor that was drawn in Figure 1B probably represents a germinoma of the pineal region. (hematoxylin-eoxin, x150).

is filled with membrane-bound autophagocytic vacuoles (Takei 1976). Sometimes parts of oligodendrogliomas consist of fusiform cells. With respect to histoarchitecture, cellular arrangement in parallel rows, mimicking the pattern of the "polar spongioblastoma" may be seen in rare cases (**Fig. 16**). Occasionally, the presence of perivascular pseudorosettes in an oligodendroglioma may cause confusion with ependymoma (**Fig. 17; compare Fig. 6**).

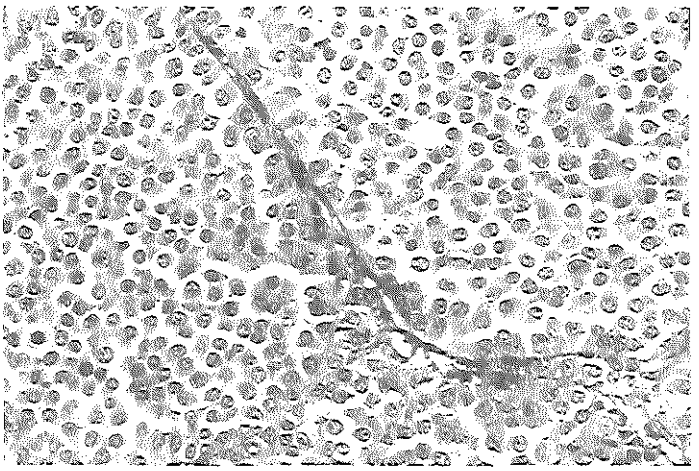


Figure 15. Eosinophilic granular cells. Voluptuous eosinophilic cytoplasm is seen at one side of the nucleus. The nuclei are not flattened. The cytoplasm is filled with autophagocytic vacuoles. (hematoxylin-eosin, x150)

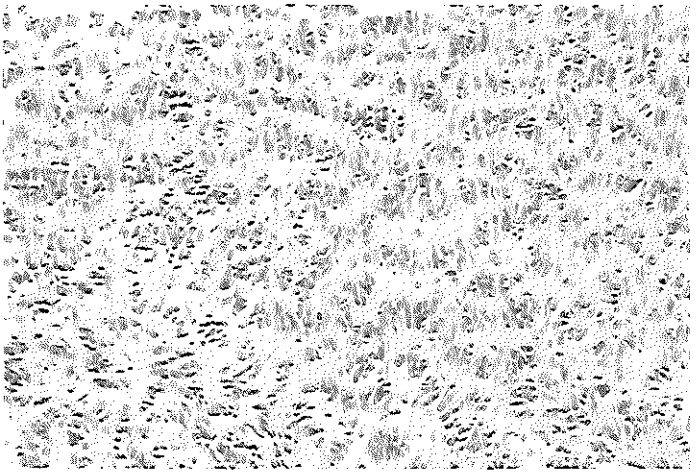


Figure 16. Oligodendroglioma mimicking a polar spongioblastoma. In this oligodendroglial variant the tumor cells show a palissading pattern analogous to the growth pattern seen in polar spongioblastoma (Figure 2C). (hematoxylin-eosin, x60).

The intracranial schwannoma (**Fig. 10**) has a predilection for sensory nerves, the acoustic nerve in particular. It is believed to arise from the cells of Schwann.

For the diagnosis of the rare ganglion cell tumors (**Fig. 11**) neoplastic neuronal cells are required. These cells are distinguished from non-neoplastic ganglion cells by their distribution and cytologic characteristics.

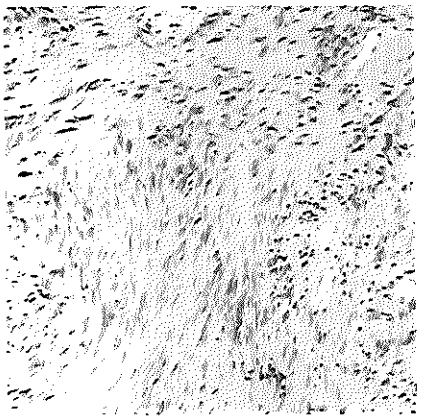


Figure 10. Schwannoma (neurilemmoma, neurinoma). The "unipolar spongioblastoma" was described as "neurinome centrale" by Josephy (1924), because the elongated cells resemble the cells of Schwann. Roussy and Oberling believed that it was composed of oligodendroglial elements (Roussy 1931). In fact, many tumors indicated by "spongioblastoma" were pilocytic astrocytomas. (Figure 4A) (hematoxylin-eosin, x150).

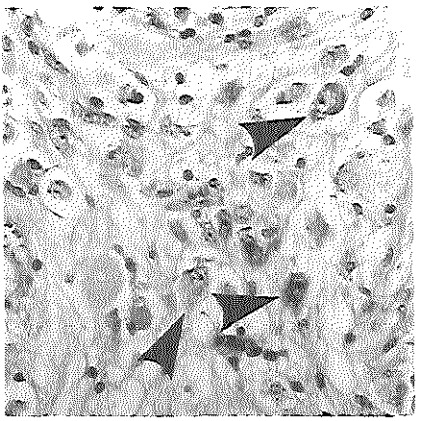


Figure 11. Ganglion cell tumor. The neoplastic ganglion cells (arrowheads) lack polarity, are sometimes binucleated, and vary in shape and size. (hematoxylin-eosin, x150).

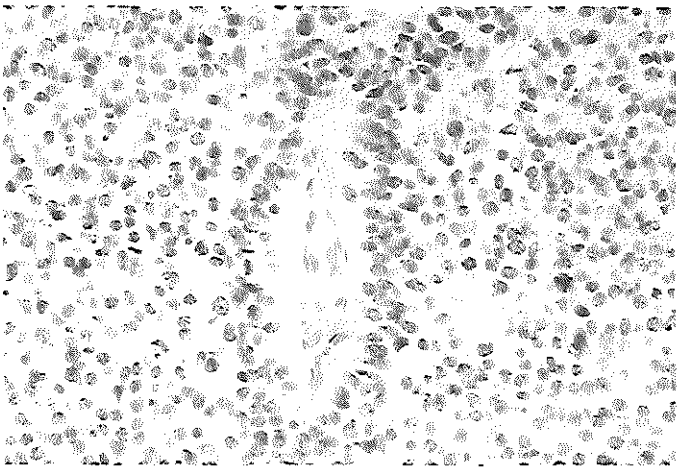


Figure 17. Perivascular pseudorosettes in an oligodendroglioma. Illustrative for the overlap in histologic configuration between different glial tumors. (hematoxylin-eosin, x150).

Anaplastic and polymorphous oligodendrogliomas

The existence of anaplastic oligodendroglial tumors was ignored in the earliest reports, because the developmental scheme of normal glial cells that was used by Bailey and Cushing did not include a glial precursor cell positioned between the medulloblast and the oligodendrocyte (Bailey and Cushing 1926).

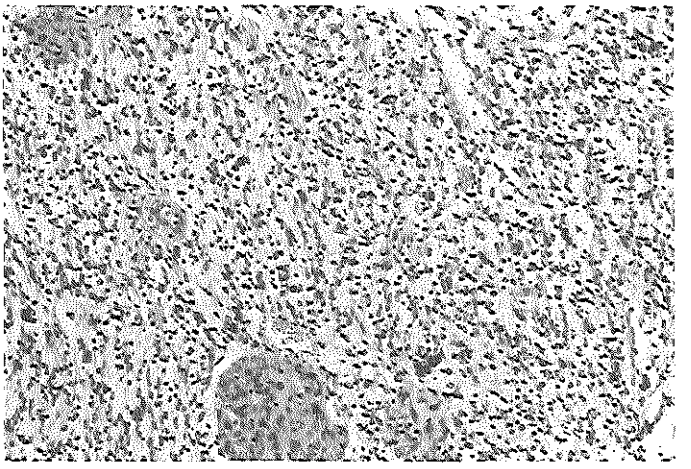


Figure 18. Anaplastic oligodendroglioma. High cellularity, prominent nuclear pleomorphism, high mitotic rate, presence of necrosis, and vascular and endothelial proliferation are required for an anaplastic oligodendroglioma. (hematoxylin-eosin, x60)

Hence, less differentiated oligodendrogliomas were not described. Nevertheless, anaplastic gliomas with oligodendroglial traits were encountered (**Fig. 18**). Frustrated by the low discriminating power of his grading system in oligodendrogliomas, Kernohan simply divided this neoplasm into two subgroups (Kernohan 1938, 1949). Zülch distinguished the classic oligodendrogliomas, the mixed oligo-astrocytomas and the anaplastic (malignant) oligodendrogliomas (Zülch 1968). The anaplastic oligodendrogliomas characteristically had areas of anaplasia. When an entire tumor had been transformed into a highly anaplastic glioma the distinction with the glioblastoma (especially the microcellular form) was hardly possible. Anaplastic oligodendrogliomas, however, should be intermediate between the better differentiated variant and the glioblastoma with respect to the survival of the patients (Zülch and Wechsler 1968, Schuier 1976).

According to Rubinstein the term "anaplastic oligodendroglioma" should be reserved for a rapidly growing, highly cellular and poorly differentiated oligodendroglioma, with high mitotic rate and presence of necrosis (Rubinstein 1989). In the meantime, mitotic count and necrosis are identified as the most useful prognosticators in oligodendrogliomas indeed (Burger 1987). Scherer's distinction of primary and secondary glioblastomas, the latter represented by anaplastic oligodendroglial tumors, was quoted by Rubinstein:

> *"In the case of a highly cellular and poorly differentiated round-cell glioblastoma, an oligodendroglial origin may be hard to establish, but when in such a tumour many microscopic fields are present that closely resemble those of an oligodendroglioma it becomes entirely reasonable to assume that the glioblastoma has arisen in a pre-existing oligodendroglioma or, more precisely, that the resulting picture of cellular density and anaplasia represents the development of rapidly proliferating clonogenic subpopulations within an oligodendroglioma"* (Rubinstein 1989).

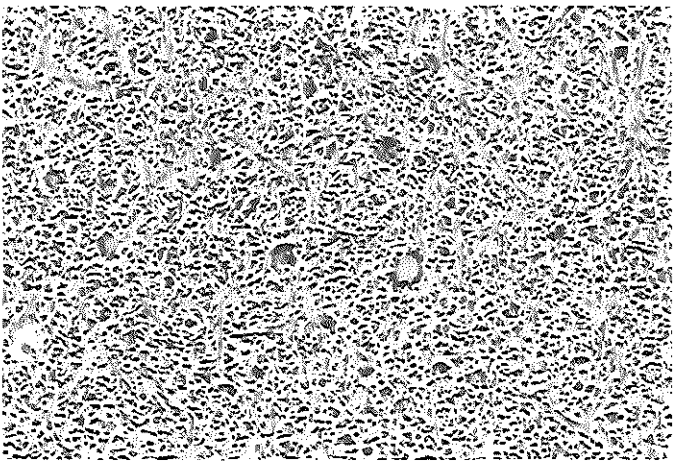


Figure 19. Polymorphous oligodendroglioma. Characteristic for a polymorphous oligodendroglioma is the presence of large, multinucleated giant cells dispersed within a monotonous oligodendroglial ground structure (hematoxylin-eosin, x60)

Mechanisms of selection of aggressive subpopulations are believed to result in a rapid deterioration of clinical course, following a long period of slow progression (Schmitt 1983).

The term "polymorphous oligodendroglioma" is reserved for an oligodendroglial tumor in which uni- or multinucleated giant cells are seen interspersed with typical oligodendroglial cells (**Fig. 19**). This unusual variant forms a subgroup of the anaplastic or malignant oligodendrogliomas according to Zülch (Zülch 1979). The polymorphous oligodendroglioma is, although undoubtedly genuine, only exceptionally seen (Rubinstein 1989).

CLINICOPATHOLOGICAL FINDINGS

age and gender

Most cases of oligodendroglioma are diagnosed at ages varying from 35 to 45 years (Mørk 1985, Ludwig 1986, Earnest 1950, Chin 1980, Horrax 1954). In our own studies on two separate patient series, viz. those of the University Hospitals of Amsterdam and Rotterdam, we indeed found peaks around the age of 35, but also a second peak around the age of 55. Chin et al. reported an additional peak between 6 and 12 years (Chin 1980). In our two studies this childhood peak was represented by only two and five patients, respectively. A predominant occurrence of the oligodendroglioma in males was almost invariably found in previous studies (Bailey and Bucy 1929, Shenkin 1947, Reymond 1950, Earnest 1950, Horrax 1954, Roberts 1966, Mansuy 1967, Weir 1968, Chin 1980, Mørk 1985). There is no explanation for a possible sex predominance.

site

A predilection for the frontal lobes was reported in most larger studies on oligodendrogliomas. The first reference of tumor site influencing prognosis was made by Martin in 1931, who noted a shorter survival in patients with medially located tumors compared with tumors that were located laterally (Martin 1931). Two years later Greenfield and Robertson studied five cases and did not mention localization as a relevant factor for prognosis; they pointed to the fact that medially located tumors may block the exit of cerebrospinal fluid, but not necessarily do so (Greenfield 1933). Although localization has been related to symptomatology (Chin 1980), the relation between localization and histopathological picture has not been subject to previous research.

therapeutic modalities

Surgery

Oligodendrogliomas were characterized clinically by Bailey and Bucy as

> *"slow-growing with the appearance of encapsulation, and unless they happen to produce focal and irritative lesions they may attain a large size before they make their presence known - so large a size that their removal is well nigh impossible"* (Bailey and Bucy 1929).

According to Horrax the benign character of a brain tumor was defined by the possibility for radical removal (Horrax 1954). Of course most oligodendrogliomas - low grade cases included - could not be operated radically and consequently they should be considered malignant tumors. Although Elvidge noted a dicrepancy between the long duration of preoperative period and the short postoperative survival of many patients suffering from oligodendroglioma (Elvidge 1937), the conclusion that surgery would stimulate the tumor growth was by no means proven (Weir 1968). Obviously, complete removals (read: removals as radical as possible) yielded favorable survival rates (Earnest 1950, Reymond 1950). Nevertheless, high recurrence rates after surgery that was supposed to be radical were reported as well (Roberts 1966, Neumann 1978, Chin 1980, Sun 1988).

Radiation therapy

The success of radiation therapy in glial tumors has been generally disappointing, because of the relatively radioresistancy of these neoplasms, and the limited radiation tolerance of the surrounding brain tissue (Brada 1989). The unacceptability of permanent neurological deficit makes it impossible to irradiate brain tissue beyond radiation tolerance.

A randomized clinical trial evaluating radiation therapy for oligodendrogliomas has never been executed untill now. While some authors reported beneficial effects of radiation (Sheline 1977, Chin 1980, Leibel 1987, Lindegaard 1987), others disputed usefulness of additional radiation therapy (Bouchard 1960, Neumann 1978, Afra 1978, Reedy 1983, Bullard 1987). All studies mentioned have been retrospective and non-controlled. In our own clinicopathological studies (papers 1, 2, 3, 5) about half of the patient group had been treated with radiation therapy. Since the latter patient series extends over periods of about two decennia, different radiation dosages and modes of application had been used. Therefore, a conclusion about the possible effect of radiation therapy on the time of tumor recurrence is by no means justified.

More accurate delineation of tumor localization and subsequent more effective local irradiation may yield more satisfying therapeutic results in patients with circumscript gliomas. It is expected that the results of radiation therapy in the oligodendroglioma will improve because this tumor is often circumscript and seldom appears multifocal, circumstances that would make this glioma suitable for more accurate delivery of interstitial or external beam radiotherapy (Hochberg 1980, Lyman 1985, Halperin 1988).

INTRODUCTION TO THE PAPERS

BIOLOGICAL BEHAVIOR OF OLIGODENDROGLIOMAS

Controversial opinions with respect to the biological behavior of the oligodendroglioma exist. Traditionally, the oligodendroglioma was considered as a slowly growing, more "benign" glioma, with better survival than most tumors of the astrocytic group. The capricious biological behavior of oligodendrogliomas yielded many case reports of patients with a favorable clinical course (Freeman 1962, Solitare 1967, Aebi 1978). However, with the increasing number of reports on larger series this opinion has been changed, and the notion of the oligodendroglioma being a relative benign tumor is by no means justified.

In early studies disproportionally short postoperative survival, compared to the long preoperative period, was reported (Elvidge 1938, Shenkin 1947), and no correlation between these two time intervals could be shown (Reymond and Ringertz 1950). Straightforward malignancy, i.e. short preoperative as well as postoperative periods, was also reported (Martin 1931, Eisenhardt 1937, Beck and Russel 1942, Blumenfeld 1945). Although rarely found outside the cerebral hemispheres, in many case reports malignant behavior as reflected in arachnoidal seeding and even hematogenous metastasis of oligodendrogliomas was described (Beck 1942, Blumenfeld 1945, Polmeteer 1947, Shenkin 1947, James 1951, Berkheiser 1956, Korein 1957, Best 1963, Daum 1963, Spataro 1967, Minauf 1968, Jellinger 1969, Kernohan 1971, Voldby 1974, Kummer 1977, Fortuna 1980, Macdonald 1989). Surprisingly, in most cases of oligodendroglioma with metastases the tumors showed a well-differentiated histological appearance: the potency of metastatic behavior seems to have no relationship with the histopathological appearance. Thus, when a tumor is classified as isomorphous oligodendroglioma the possibility of malignant behavior is not ruled out.

The lack of metastatic propensity and the absence of regional lymph nodes in the brain have invalidated staging procedures in glial tumors. Reports on the relation between tumor size and clinical course are scarce with respect to gliomas (Norman 1976, Pay 1976, Lunsford 1985). Data on the prognostic impact of tumor size of oligodendrogliomas in particular are entirely absent. Therefore, in the series of oligodendroglioma patients of the University Hospital of Rotterdam the tumor volume was calculated from available CT-scans. The tumor volume was correlated with the histopathology and grade as well as with the survival time. Since removal of a substantial amount of tumor tissue may influence recurrence, the patients who had undergone only a stereotactic biopsy were evaluated separately. The results of this retrospective investigation are described in paper 1.

GRADING OF THE OLIGODENDROGLIOMAS

In the astrocytomas, the ependymomas and the neuro-astrocytomas, Kernohan distinguished four degrees of malignancy (Table 2) (Kernohan 1949). Whereas his grading system for the gliomas yielded satisfying results in astrocytomas, the scheme was without success with respect to the oligodendrogliomas. Relatively small series might have been responsible for the bad grading results. Because of this poor prognostic impact, Kernohan made a rough division into the oligodendrogliomas or oligodendrocytomas, and the oligodendroblastomas or juvenile oligodendrogliomas. The cells of oligodendroblastomas characteristically had larger nuclei and more cytoplasm, and showed a higher mitotic index (Kernohan 1938). The distinction between the two groups remained, however, vague. Zülch also distinguished basically two groups of oligodendroglioma with regard to biological behaviour: the oligodendroglioma isomorphe with "semibenign" behavior, and the "semimalignant" oligodendroglioma polymorphe. In an early series no group of intermediate malignancy was distinguished (Davis 1950). Later a third intermediate group was recognized, termed "oligodendroglioblastomas" by Kernohan (Kernohan 1952).

Ringertz introduced a three-step grading system for gliomas based on pleomorphism, cellularity, mitoses, vascularity, proliferation of vessel walls, infiltration zones, and necrosis (Ringertz 1950). Grade 1 was composed of astrocytomas, oligodendrogliomas and ependymomas in their classical form, followed by an intermediate grade with cellular polymorphism and high mitotic count. The glioblastoma formed a common third grade of malignancy for all three tumor types. The scheme was tested on more than 300 cases of astrocytoma, oligodendroglioma and ependymoma. Only between the grades 1 and 2 a significant difference in survival was found (Ringertz 1950).

Other grading schemes suffered from inaccuracy in definition of the respective grades, and hence remained without acclaim (Horrax 1951, Afra 1978). Neumann developed a grading system for oligodendrogliomas in which the grades were descriptively defined. Third grade tumors should still be very well recognizable as oligodendrogliomas, and were considered not to represent glioblastomas with focal oligodendroglial differentiation (Neumann 1978). The system was tested on 99 cases of oligodendroglioma and the authors claimed a good correlation between grades and clinical courses. Unfortunately, figures to substantiate this success are not found in Neumann's report.

It would take until 1983 before a new grading scheme for oligodendrogliomas was presented by Smith and coworkers (Smith 1983). This grading system consisted of only five histopathological features, which all had already been used in earlier schemes. These particular features were acknowledged because they were *"easily recognizable"* and were *"among the basic characteristics of malignancy"* (Smith 1983). Mitotic count was not included in the grading system because of *"anecdotal experience"* and *"the poor correlation of this feature with other indicators of malignancy"*. A novelty of the scheme was the way of scoring the features in a simple on-off way. While endothelial proliferation and necrosis were rated present or absent, the features maximal nuclear/cytoplasmic ratio, maximal cell density, and pleomorphism were either high or low, according to the predominant appearance in the sample. Subsequently, four grades of malignancy were defined. The lowest grade was attributed if all features were judged absent or low, the highest grade if all were present or high. Whereas grade C was exactly defined, several histopathologic pictures fitted within grade B. The system of Smith promised reduction of the inter-observer error because of its simplicity. Its practical use was tested in large group of oligodendrogliomas, correlating the grading results with survival of the patients. The grading results were compared with the results of conventional grading according to

Kernohan. This retrospective study is reported in **paper 2.**

HISTOPATHOLOGICAL FEATURES AND PROGNOSIS

Essential differences between tumors of the central nervous system and tumors of other organs exist. A histopathologically benign tumor located within the cranium can kill the patient simply because of expansion. Whereas most gliomas lack well-defined margins, and infiltration in the leptomeninges is frequently seen, these features not necessarily indicate less favorable prognosis (Rubinstein 1972, Gilles 1977, Hedley-Whyte 1978, Burger 1982). In fact, infiltration in surrounding brain tissue is an invariable finding in glial tumors. On the contrary, metastatic behavior, a feature that is always associated with malignancy in epithelial tumors, is only exceptionally encountered in gliomas. Nevertheless, most histopathological features that are associated with malignancy in non-glial tumors are ominous features in glial tumors as well. Due to tumor sampling and tumor heterogeneity not all variables related to malignancy will always be present in the same specimen, particularly in the small biopsies obtained by stereotactic surgery (Kleihues 1984, Scerrati 1987, Paulus 1989). Moreover, histopathological features are not entirely specific for a given grade; for instance, necrosis is always found in tumors of the highest grade, but may be found in tumors of lower malignancy grades as well (paper 2).

Histopathological features which are undoubtedly ominous for prognosis in astrocytic tumors do not always have the same impact in the oligodendrogliomas (Rubinstein 1989). In the last decade various investigators have searched for histopathological features, either individually or clustered, with prognostic significance for oligodendroglioma. In the largest study, consisting of 323 cases of the Armed Forces Institute of Pathology (AFIP), only cellular pleomorphism was identified as significant factor in prognosis (Smith 1981, Ludwig 1986). Pleomorphism in terms of variability in cellular as well as nuclear size and shape, was simply scored as present or absent. In the relatively small study of Wilkinson nuclear pleomorphism was also found correlating significantly with the clinical course (Wilkinson 1987). Surprizingly, in the second large survey in the literature, concerning the Norwegian population, pleomorphism was not among the features with independent significance for prognosis (Mørk 1986). As result of this study, presence of necrosis and high cellularity correlated with shorter survival, while the presence of microcysts had a favorable influence. Using univariate analysis in a study of the patients of the Neurosurgical Department of the University Hospital of the Duke University, the presence of necrosis, nuclear atypia, vascular proliferation and vascular hypertrophy as well as the mitotic count were found to be significant factors (Burger 1987). In a stepwise regression analysis only the mitotic count and the presence of necrosis retained significance, while almost all features showed inter-dependency. Necrosis was the only feature with independent significance. Necrosis was identified as the most important prognostic feature in astrocytomas (Nelson 1983).

Although in the first description of oligodendrogliomas mitoses seemed to be rarely encountered (Bailey and Cushing 1926), in later series mitoses were commonly seen (Elvidge 1937, Reymond 1950, Earnest 1950, Horrax 1951, Roberts 1966, Rousta 1972, Smith 1983, Mórk 1985). Nevertheless, only in one recent study mitotic count was identified as an independent factor influencing the survival (Burger 1987).

proliferation markers and DNA-flow cytometry

Although counting of mitoses is still honoured as the oldest, easiest, fastest and cheapest way of assessing proliferation (Montironi 1988), differences in growth rate nowadays can be studied also by visualization of proliferation associated proteins as Ki-67

(Gerdes 1985), and proliferating cell nuclear antigen (PCNA) by using of monoclonal antibodies (Detta 1990). Alternatively, incorporation into DNA during S-phase of Bromodeoxyuridine or ^{3}H-thymidine visualized by immunohistochemistry and autoradiography respectively, yield parameters for proliferation. In addition, the S-phase fraction in samples used for DNA-flow cytometry is used to estimate proliferation of the cell population. Whereas the incorporation techniques are done in short-term tissue culture, and immunohistochemical detection of Ki-67 is performed on fresh frozen samples, techniques detecting PCNA, and DNA-flow cytometry can be applied to formalin-fixed and paraffin-embedded material. Therefore, DNA-flow cytometry is feasible for retrospective studies.

From a clinico-pathological point of view, the investigation of differences in proliferation between various brain tumors, say, meningioma versus glioma, or glioma versus ependymoma (Burger 1986, Giangaspero 1987), seems to be redundant, since differences in the clinical course are well-known from histopathology. Nevertheless, it was claimed that

> *"Measuring the proliferative potential of individual gliomas is essential because histopathologically similar tumors may show large differences in labeling. Measuring proliferative potential of individual gliomas is crucial for accurate prognostic predictions"* (Hoshino 1972, 1979, 1985, 1986, 1989).

It was suggested that differences in labeling index can be used to separate tumors of astrocytic lineage in an objective way, since this distinction is not always possible in small biopsies used for histology (Raghavan 1990).

Scepticism about conclusions drawn from proliferation indices in biopsy material is still warranted. In 38 astrocytic tumors no correlation between labeling index for ^{3}H-thymidine and survival was found (Bookwalter 1986). An explanation was sought in theoretical determinants of tumor behavior, i.e. altered ability for growth arrest and differentiation, constantly evolving mutant sublines, genetic instability, and ever-changing metabolic and vascular environment. Sometimes correlations between labeling indices and presence of histopathological features were made (Germano 1989). Since different brain tumors were used in these studies, the results should be carefully interpreted. A good correlation between Ki-67 labeling and mitotic index in gliomas of different malignancy grades was reported by Schröder (Schröder 1991).

Examining differences in proliferation between tumors of the same histopathological category could have value in particular when grading results are insufficient. Thymidine and bromodeoxyuridine incorporation studies should be performed on prospectively collected material. The application of Ki-67 immunostaining requires freshly frozen tumor specimens. Hence, gathering a substantial group of relatively uncommon tumors as oligodendrogliomas would take considerable time. An attractive option in order to obtain a global impression of the proliferative behavior of cell populations is an estimation of the S-phase fraction by DNA-flow cytometry on archival paraffin-embedded tumor material.

In several non-cerebral tumor categories the ploidy of the tumor cells correlated nicely with tumor progression (Aardal 1979, Costa 1981, Barlogie 1982, Raber 1982, Wolley 1982). In studies on the proliferation rate of brain tumors oligodendrogliomas were only sporadically included. Since flow cytometry can be done on paraffin-embedded tumor material, we were able to study its prognostic value in a retrospective study concerning 85 cases of oligodendroglioma (**paper 3**).

THE PROBLEM OF THE MIXED GLIAL TUMORS

As early as in the first report on oligodendroglial tumors intermingled astrocytic cells were noticed (Bailey and Bucy 1929). For tumors composed either of oligodendroglial cells with foci of astrocyte-like cells, or astrocytomas with oligodendroglial parts, the term "oligo-astrocytoma" was proposed (Cooper 1935, De Buscher 1942). The occurrence of gliomas consisting of cells with various morphology was explained by the "field theories" based on results of early experimental oncology (Berenblum 1947, Willis 1948): the carcinogenic stimulus affects the various cell types within a preexisting tissue, resulting in different phenotypes of the tumor cells. To the opinion of Willis (1948) most gliomas were mixed gliomas, while pure astrocytomas or oligodendrogliomas were an exceptional finding. Rubinstein defined mixed gliomas as tumors

> *"with a diversity in cell population which cannot reasonably be explained by a process of anaplastic degradation of the more differentiated glial element. The supervention of anaplasia in a glioma with an originally mixed cell population results in a further blurring of the neat boundaries of taxonomic conventions"* (Rubinstein 1964).

The eventual dedifferentiation and subsequent malignant behavior of mixed oligo-astrocytomas would be mainly a sequel of loss of differentiation of the astrocytic component (Wislawski 1970, Rubinstein 1989). Hart divided mixed gliomas into a compact and a diffuse subtype, depending on the arrangement of the astrocytic, oligodendroglial and eventual ependymal component, but these subtypes remained without practical significance (Hart 1974).

Originally the term "transitional cells" was used for cells with an astrocytic morphology in oligodendrogliomas and mixed oligo-astrocytomas. Following the introduction of immunohistochemistry, positivity for glial fibrillary acidic protein (GFAP) became the new criterion for astrocytic lineage. The putative transformation of normal developing as well as neoplastic oligodendroglial into astrocytic cells was suggested in various schemes (Penfield 1924, Ravens 1955, Herpers 1984) (**Fig. 20 and 21**). The potential of differentiation into astrocytic or oligodendroglial cells of non-neoplastic glial precursor cells was shown in vitro (Raff 1983). Furthermore, recently the astrocytes in mixed oligo-astrocytomas have been found to express an oligodendroglia-specific surface marker (Bishop 1989), suggestive for "oligodendroglial lineage" of these neoplastic astrocytes.

A problem in the study of oligodendrogliomas is the acceptable proportion of interspersed cells with astrocytic properties. While Mørk as well as Burger reject oligodendrogliomas with more than 25% astrocytes, Smith accepts up to 49% of astrocytic elements, although 85% of his cases remained below 14% astrocytes (Mørk 1986, Smith 1983, Burger 1987). It was suggested that the presence of astrocytic differentiation in oligodendrogliomas is associated with shorter survival, and when malignancy ensues this would be mainly due to the anaplasia of the astrocytic component (Wislawski 1970, Müller 1977, Barnard 1968, Rubinstein 1989).

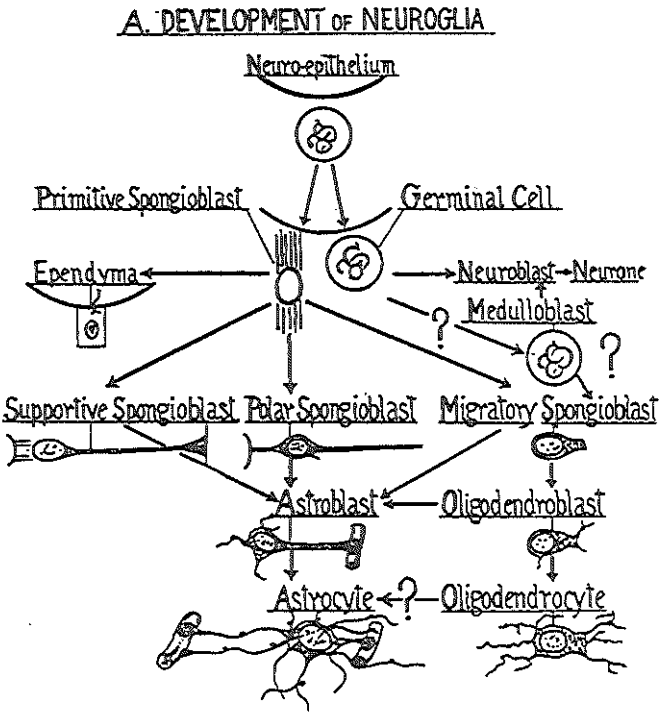
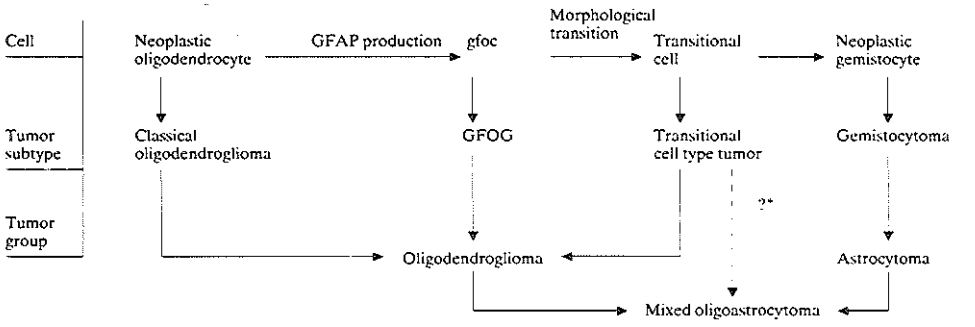


Figure 20. Hypothetical transformation of glial cells. The putative conversion of an oligodendrocyte into an astrocyte is indicated with a questionmark. (From: "Cytology & Cellular Pathology of the nervous System". Vol. 2, page 450, Ed. Wilder Penfield. Hafner Publishing Company, New York, 1965).



?* = development of a mixed oligoastrocytoma from a transitional cell type tumor is conceivable but not supported by this study.

Figure 21. Hypothetical transformation of an oligodendroglioma into an astrocytoma. (From: M.J.H.M. Herpers and H. Budka: "Glial fibrillary acidic protein (GFAP) in oligodendroglial tumors: Gliofibrillary oligodendroglioma and transitional oligoastrocytoma as subtypes of oligodendroglioma", Acta Neuropathol (Berl.) 64:265-272, 1984, with permission).

IMMUNOHISTOCHEMISTRY IN OLIGODENDROGLIAL TUMORS

glial fibrillary acidic protein and transitional cell types

Because the anti-GFAP-antibody, raised against 9 nm intermediate filaments of astrocytes (Eng 1971), was found to be strongly and specifically reactive in astrocytic neoplasms, GFAP soon became established as a marker for (neoplastic) astroglial cells. Now, in addition to the demonstration of cell processes in routine stains, astrocytic differentiation was defined by immunohistochemical demonstration of glial filaments. Anti-GFAP antibody could be used for assessing the extent of the astrocytic population in mixed oligo-astrocytomas (Nakagawa 1986). Concerning gliomas, a correlation between tumor grade and GFAP-content was suggested in several - but not all - studies on astrocytic neoplasms (VanderMeulen 1978, Jacque 1979, Delpech 1980, Velasco 1980, Duffy 1980, Tascos 1982, Kunz 1986, Nakopoulou 1990). In a few mixed oligo-astrocytomas the astrocytic cells reacted strongly, while the oligodendroglial elements remained unstained.

Surprisingly, in some oligodendrogliomas also typical oligodendroglial cells and cells with the morphology of small gemistocytes (minigemistocytes) showed reactivity with anti-GFAP. The minigemistocytes - considered as astrocytic cells, and noted since long in oligodendrogliomas (Penfield 1931, Zülch 1968, Gluszcz 1972) - resembled the well-known classic gemistocytes, which were seen as third GFAP-positive cell type in oligodendroglial tumors. Differentiation between an oligodendroglioma with minigemistocytes and a gemistocytic astrocytoma may be difficult, particularly in small (stereotactic) biopsies (Rubinstein 1989). Some authors recommended silver impregnation techniques for their distinction (Escalona-Zapata 1981). The immunohistochemical and ultrastructural idiosyncrasies of minigemistocytes and classic gemistocytes are described in **paper 4.**

The GFAP-positive oligodendroglial cells were called "gliofibrillary oligodendrocytes (GFOC)" (Herpers 1984). As a sequel of the identification of GFAP positive cells with oligodendroglial phenotypes, the old concept of transitional cell types revived (Meneses 1982, Herpers 1984, Liao 1984, Ishida 1989). The term "transitional cell types" was originally used exclusively for the minigemistocytes, but now it was used for GFOCs as well. While in some studies the finding of GFAP in oligodendroglial cells was reproduced (Hamaya 1985), in other studies GFAP was only found in astrocytic elements and never in cells with oligodendroglial morphology (Eng 1978, Velasco 1980, Tascos 1982). These contradictory results are simply explained by the fact that GFAP-positive oligodendroglial cells are not always present in oligodendroglial tumors. Wilkinson studied the occurrence and arrangement of the GFAP-positive cells in oligodendrogliomas more specifically (Wilkinson 1987). Different patterns of distribution of these cells within the oligodendroglial tumors were discerned. No data concerning the influence of the transitional cells on the biological behavior of the oligodendrogliomas in which they occur were known yet. A possible difference in biological behavior between oligodendrogliomas with and without transitional cells was studied in **paper 5.**

other markers

In addition to immunoreactivity for GFAP antibodies some reports on reactivity with oligodendroglial tumor cells other immunohistochemical markers have been made. The myelin-associated glycoprotein (MAG) was claimed to be a selective marker for neoplastic oligodendroglial cells, corresponding with their degree of anaplasia (Szymas 1985).

This finding, however, was disputed by the results of another study (Nakagawa 1986). Among seven markers tested in 28 oligodendrogliomas, MAG, carbon anhydrase C (CA C) and neuron specific enolase (NSE) were only positive in some tumor cells. In addition, more than 90% of the oligodendrogliomas reacted with anti-leu-7. Other neuroepithelial tumors were also reactive with anti-leu-7, violating its presumed specificity for oligodendroglial tumor cells. Nevertheless, this antibody might have some value in differentiating oligodendroglioma invading the meninges, and syncytial meningioma containing large numbers of cells with clear perinuclear cytoplasm (Rubinstein 1989). Reactivity with an antibody to myelin basic protein (MBP) remained negative in one study (Nakagawa 1986), while in another study invariable positivity was reported, and MBP was advertised as delineator of the oligodendroglial component in mixed gliomas (Figols 1985).

Recently the monoclonal antibody A2B5, raised against a glial precursor cell, was tested on mixed astro-oligodendrogliomas in addition to the oligodendroglial marker galactocerebroside, and the astrocytic marker GFAP. Positivity of astrocytic cells for A2B5 led to the conclusion that the mixed gliomas basically have an oligodendroglial lineage (Delamonte 1989).

Incubation of oligodendrogliomas with the monoclonal antibody Pm43 was done in order to trace a possible production of myelin by neoplastic oligodendrocytes. Pm43 was raised against melanocytes, but coincidentally reactive with myelin sheaths of the peripheral nervous system (Van Dijk 1986). No positivity in oligodendroglial cells was found, however. By surprise, the gemistocytic cells were reactive with anti-Pm43. Classic gemistocytes, which are believed to be astrocytic in nature (although oligodendroglial origin is by no means excluded) reacted with Pm43. Seemingly the gemistocytes contain unidentified cross reacting antigens reactive with Pm43 (**paper 4**).

ULTRASTRUCTURAL FINDINGS IN OLIGODENDROGLIAL TUMORS

Various electron microscopical features are encountered in oligodendrogliomas. The typical neoplastic oligodendrocytes have a pale cytoplasm filled with many mitochondria, some with an atypical morphology. Besides variable quantities of aspecific cell organelles, concentric laminar structures (sometimes seen as part of the cell membrane), crystalline structures as well as amorphic irregular inclusions were described (Robertson 1962, Tani 1969, Hossmann 1971, Baloyannis 1981, Sarasa 1990). In the cells of less typical oligodendrogliomas autophagic vacuoles were found (Takei 1976, Escalona-Zapata 1981).

In the first electron microscopic studies of the cells of classical honey-comb oligodendrogliomas intracytoplasmic filaments were seen, which were referred to as protogliofibrils (Raimondi 1962), or perikaryal microtubules (Garcia 1970). These fibrils within neoplastic oligodendrocytes had not been seen in an earlier study by Luse (Luse 1960), and should not be confused with the fibrillated cytoplasm of interspersed reactive or neoplastic astrocytic cells (Hossmann 1971, Baloyannis 1981).

Since immunoreactivity for GFAP became the hallmark of astrocytic cells, visualization of these filaments was considered to support an astrocytic origin of a particular glioma. A major problem of electron microscopic investigations is the representativity of the tumor cells that are selected for ultrastructural processing. This problem was apparent in two ultrastructural studies on the different cell types in oligodendrogliomas by Kamitani (1987, 1988). A few cytoplasmic fibrils were seen within so-called light-shaded and medium-shaded cells, both considered as representing oligodendroglial tumor cells. Interspersed astrocytes were dark-shaded, and contained an abundancy of glial filaments. The presence of glial filaments and of perivascular end-feet in some of the cells would suggest an astrocytic nature of all oligodendroglial tumor cells. In other studies confusing discrepancies between the immunohistochemical demonstration of GFAP at light microscopy and the finding of intermediate filaments at the ultrastructural level exist (Jagadha 1986, Luse 1960, Cervós-Navarro 1981a, Cervós-Navarro 1981b).

Using a technique of processing adjacent semithin immunostained sections, the various GFAP-positive cells in oligodendrogliomas could be unequivocally identified and compared at the electron microscopic level (paper 6).

Bailey and Cushing	Bailey (1927)	Roussy and Oberling (1931)	
1. Medulloblastoma	1. Medulloblastoma	Neurospongiome	Neuroblastoma (Wright) Neurogliocytome embryonaire (Masson) Glioma sarcomatoides (Borst)
2. Pineoblastoma			
3. Neuroepithelioma	2. Neuroepithelioma		Neuroépithéliome (Flexner) Retinocytome à stephanocytes (Mawas) Blastome ependymale (Marburg)
4. Spongioblastoma multiforme (glioblastoma)	3. Spongioblastoma multiforme (glioblastoma)	Glioblastome	Spongioblastoma multiforme (Globus and Strauss) Glio-sarcoma (Ewing, Borst) Gliome à petites cellules (Masson) Gliome polymorph (Roussy, Lhermitte and Cornil)
5. Pinealoma	4. Pinealoma		Pinealoma (Krabbe) Chorioma (Askanazy) Compound pineal gland type (Strong)
6. Spongioblastoma unipolare	5. Spongioblastome unipolare	Oligodendrocytome fasciculé	Neurinome centrale (Josephy-Macpherson)
7. Astroblastoma	6. Astroblastoma	(Included in astrocytomas)	Neuroblastoma? (Greenfield)
8. Ependymomas 9. Ependymoblastoma	7. Ependymoma	(*a*) Ependymocytome (*b*) Ependymoblastome (*c*) Ependymogliome	Ependymal glioma Glio-ependymome (Masson)
10. Astrocytoma fibrillare	8. Astrocytoma	Astrocytome	Astrocytoma (Ewing)
11. Astrocytoma protoplasmicum			Sternzellen (Stroebe)
12. Oligodendroglioma	Oligodendroglioma	Oligodendrocytome	Gliome à petites cellules rondes (Roussy, Lhermitte and Cornil)
13. Ganglioneuroma	Ganglioneuroma		Neurogliome ganglionaire

Table 1. Classification scheme of neuro-ectodermal derived tumors of the central nervous system. The number of categories was reduced to ten at a later time. The astroblastomas have been added to the group of astrocytomas, and the pineoblastoma has been included in the medulloblastoma. (From: L.B. Cox: "The cytology of the glioma group; with special reference to the inclusion of cells derived from the invaded tissue", Am J Pathol 9:847, 1932).

New	*Old with new in parentheses*
Astrocytoma, grades 1–4	Astrocytoma (astrocytoma, grade 1) Astroblastoma (astrocytoma, grade 2) Polar spongioblastoma (obsolete) Glioblastoma multiforme (astrocytoma, grades 3 and 4)
Ependymoma, grades 1–4	Ependymoma (ependymoma, grade 1) Ependymoblastoma (ependymoma, grades 2–4) Neuroepithelioma (obsolete) Medulloepithelioma (ependymoma, grade 4)
Oligodendroglioma, grades 1–4	Oligodendroglioma (oligodendroglioma, grade 1) Oligodendroblastoma (oligodendroblastoma, grades 2–4)
Neuro-astrocytoma, grades 1–4	Neurocytoma Ganglioneuroma Gangliocytoma Ganglioglioma } (Neuro-astrocytoma, grade 1) Neuroblastoma Spongioneuroblastoma Glioneuroblastoma And others } (Neuro-astrocytoma, grades 2–4)
Medulloblastoma	Medulloblastoma

Table 2. Grades mentioned in a simplified classification scheme for the gliomas by Kernohan and co-workers (1949). By the introduction of their grading system the glioma types were reduced to five main groups, discarding various forms as obsolete. An outline of the actual grading system is drawn in paper 2, Table 1. (From: "J.W. Kernohan et al.: "A simplified classification of the gliomas", Proc Staff Meet Mayo Clin, 24:71-75, 1949).

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PAPER 1

"OLIGODENDROGLIOMA: RELATIONSHIP BETWEEN TUMOR SIZE, HISTOPATHOLOGY AND SURVIVAL"

J.M. Kros, H. Pieterman, C.G. van Eden, F.T. Bosman, Th.H. van der Kwast

(Submitted for publication.)

OLIGODENDROGLIOMA: RELATIONSHIP BETWEEN TUMOR SIZE, HISTOPATHOLOGY AND SURVIVAL

Johan M.Kros, MD. (*)
Herman Pieterman, MD. (**)
Corbert G. van Eden, PhD. (***)
Fred T. Bosman, MD., PhD. (*)
Theodorus H. van der Kwast, MD, PhD. (*)

(*) Department of Pathology and
(**) Department of Radiology
University Hospital Rotterdam-Dijkzigt
Dr. Molewaterplein 40
3015 GD Rotterdam
The Netherlands
(***) Netherlands Institute for Brain Research
Meibergdreef 33
1105 AZ Amsterdam
The Netherlands

ABSTRACT

In order to investigate the prognostic impact of tumor size on the survival of patients suffering from oligodendroglioma, the tumor volume was calculated from CT-scan images of 43 patients and was compared with the overall survival and the histopathologic grade of the tumor. To circumvent a possible effect of debulking on tumor progression the relationship between tumor size and survival time was tested separately in those patients who had undergone only a diagnostic biopsy. Neither for these patients, nor for the whole group of patients a significant correlation between tumor volume and survival time was found. No correlation existed between tumor size and histopathologic grade. On the other hand, gross tumor localization was found to be an important factor in the clinical courses of patients suffering from oligodendrogliomas.

Acknowledgments

The authors thank Prof.Dr. S.Z. Stefanko for providing materials for this study. Dr. Ch.J. Vecht and Dr. W.C. de Bruin are thanked for fruitful discussion. Mrs. C.W.J. Sorber and Mr. A.L. Nigg are acknowledged for their cooperation and instruction in the use of the IBAS 2000 and integral software. Mr. G. van der Meulen is thanked for the photography.

INTRODUCTION

Whereas for almost all cancers clinical staging is the most important factor for prediction of tumor progression and survival, histopathologic typing and grading are the most important predictors for the clinical course in patients with a tumor of the central nervous system. This difference can be attributed to the lack of metastatic propensity of brain tumors and the absence of regional lymph nodes. Since the early days of clinical application of CT imaging, correlations between the CT findings and histology of the tumors were sought[1,2]. However, staging according to tumor size, relation to the tentorium cerebelli, the midline and the ventricular spaces never gained practical importance[3].

Although it has been known for a long time that consistent histopathological grading of gliomas yields patient groups with comparable survival times[4], the selection and the order of importance of histopathological features in grading schemes are disputed, especially with respect to the grading of oligodendrogliomas[5-8]. For oligodendrogliomas the traditional grading system derived from Kernohan's grading system for the gliomas, yields poor correlations with clinical course[5]. Recently, a more practical grading system for oligodendroglial tumors has been developed, and the value of individual histopathological features has been evaluated in multivariate analyses[7,8].

In a few studies close correlations between the size of gliomas and the clinical course of the patients have been reported[9-11]. However, the role of tumor size or volume in the clinical course of oligodendrogliomas is not known. Intraventricular oligodendrogliomas show even better survival than those at other localizations[12], a finding that makes a clinical staging scheme even more controversial. In the present study the tumor sizes of 43 oligodendrogliomas were assessed from CT-scans, and were correlated with the clinical course. The tumor size was also compared with histopathological grade. Since it was anticipated that the prognosis might be dependent on the residual tumor mass after debulking, the relationship between tumor size and survival of the subgroup of patients, who had only undergone a diagnostic biopsy, was tested as well. Furthermore, the relationship between tumor localization and tumor size was investigated.

MATERIAL AND METHODS

clinical data

The preoperative CT-scans of 43 patients with histologically verified oligodendrogliomas of the brain were obtained from the files of the Department of Radiology of the University Hospital Rotterdam-Dijkzigt. The group consisted of 33 men and 10 women. The patients had been admitted between the years 1979 and 1986. In 26 cases an internal decompression was performed, while in 17 cases surgery was limited to a diagnostic biopsy. Seventeen patients had been treated with additional radiation therapy. No information about the doses and modes of application of the radiation therapy could be obtained from the clinical records. The age at admission, the age at death and the survival frequencies are represented in Fig. 1. The survival times were calculated from the time of the first operation. Six patients were still alive at the end of this study. The median age at first operation was 42.5 years and the median age at death was 46.3 years (Figure 1). Estimations of the preoperative period were as accurate as the anamnestically obtained data about onset of symptoms. A reliable assessment of preoperative conditions or

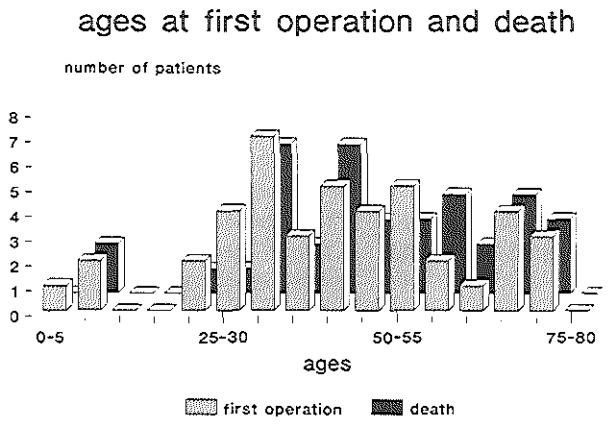

Figure 1. Ages at the time of first operation and death of the patients. The biphasic curve with peaks at 35 and 55 years for age of first surgery is characteristic for the oligodendroglioma.

Karnofsky scores of the patients could not be obtained from the neurosurgical records.

The localization of the oligodendrogliomas is listed in Table 1. The tumors were divided into those predominantly localized in the hemispheres, and those localized in deeper regions such as basal ganglia, thalamus and brainstem (Table 1). The hemispheric tumor sites were subdivided into a frontal and a non-frontal group.

TABLE 1
Localizations of the oligodendrogliomas

	left	medial	right
frontal	6	2	7
frontoparietal	1		1
parietal	4		5
parietotemporal	2		3
temporal	3		
occipital	1		
parietooccipital	1		1
basal ganglia, thalamus	1	1	1
brainstem		3	

histopathological grading of the oligodendrogliomas

The oligodendrogliomas were graded according to the revised grading scheme of Smith (6,8). In this grading scheme endothelial proliferation, necrosis, nuclear-cytoplasm ratio, cell density and pleomorphism are scored in a simple on-off scheme. In grade A tumors all histopathological features were low or absent, while in grade D all were present or high. The difference between grade C and grade D was the presence of necrosis in the latter. Grade B+, i.e. grade B with the presence of necrosis (6), was added to grade B because of the relative small patient groups.

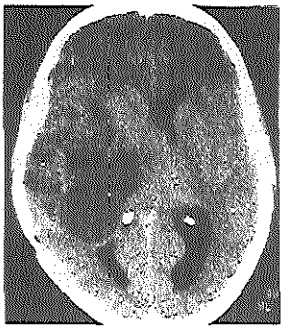

Figure 2A. A large partly cystic temporoparietal tumor pushes the midline structures to the left. There is a considerable amount of perifocal edema.

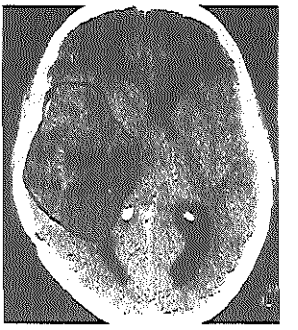

Figure 2B. This particular cross section of the tumor is delineated on the scan that was taken without contrast.

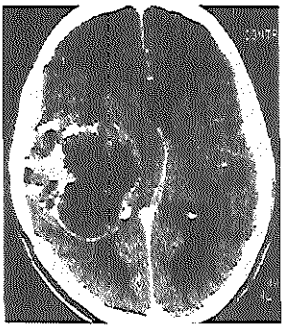

Figure 2C. The same oligodendroglioma from Figure 2A and 2B after contrast injection. The outline of the tumor became more clear.

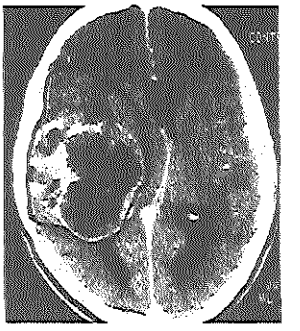

Figure 2D. In this particular case the tumor looked more extensive after contrast enhancement, and the areal fraction is larger than that on the precontrasted scan.

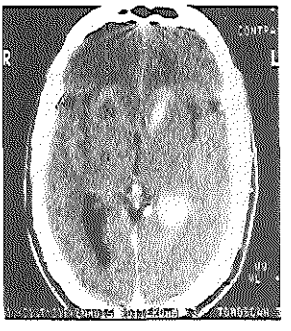

Figure 2E. An example of a CT scan of a small lesion with homogenous contrast enhancement. There is only very discrete replacement of midline structures.

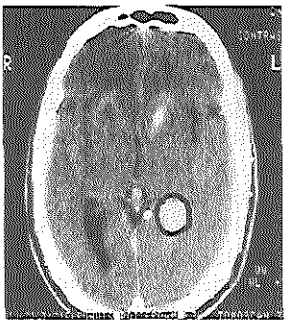

Figure 2F. The tumor can be delineated without too much difficulty, although certainly there will be tumor cell infiltrations beyond the delineation.

quantitation of tumor volumina

The CT-scans had been made by two scanner types: an EMI-scan (England) and a Tomoscan 310, later modified into 350 (Philips, The Netherlands).

Volumetric measurements were carried out by the method described by Breiman et al. in 1982 (13). CT photographs were displayed on a monitor using an interactive image analysis system (IBAS 2000). The tumor margins were delineated by a cursor, and cross sectional areas were subsequently calculated by integral software (Kontron Bildanalyse, Kontron Electronic Group, 1984, West Germany). The delineation of the tumor borders was made by inclusion of all areas of changed density, while the surrounding low attenuation in the white matter was excluded (Figure 2). Tumor volumina were calculated by summation of the cross section areas and subsequent multiplication by the cut thickness. All measurements were performed twice, and one scan was measured five times, in order to determine the intra-observer variability. Based on tumor volume three groups were defined, viz. one between 0 and 50 cm^3, one between 50 and 100 cm^3, and one consisting of tumors larger than 100 cm^3, and Analysis of Variance was used to trace differences in survival between these three groups.

statistics

The statistical tests were performed using the Statistical Package for the Social Sciences (SPSSX package). Pearson's correlation coefficient was calculated for tumor size and the survival time as well as the length of the preoperative period, and tumor size and patient age. Analysis of Variance (ANOVA) was used to detect the main effect of the grading scheme of Smith and of the tumor localization on the survival time. Furthermore ANOVA was used for detection of differences in survival times between the three groups defined by mitotic index, and between the patients who underwent radiation therapy and those who were not treated by radiation. A posteriori testing was done by use of the Student-Newman-Keuls test using significance at the 0.05 level.

RESULTS

The volume of the oligodendrogliomas ranged from 2 to 264 cm^3. The median tumor volume was 22 cm^3, while the mean tumor volume was 64.5 cm^3 (s.e.m. 60.7 cm^3). None of the tumors was multifocal. The intra-observer error with respect to the volumetric determinations was less than 20%. Some CT characteristics of the oligodendrogliomas are listed in Table 2. In almost 50% of the cases the margins were not sharply demarkated. About 60% of the oligodendrogliomas were hyperdense at CT-scan, while 40% appeared as an hypo- or isodense lesion. In 17 cases a post-contrast scan was available. Various degrees of enhancement were seen. In 5 cases the tumors were found to be more extensive after contrast (Fig. 2), while at the other 12 post-contrast scans the tumors appeared to be smaller. These differences remained below 20% of the tumor volume. In only 40% of the tumors calcium was visible on the CT-scan. No relation between the presence of calcium and the size of the tumor was found. In more than 30% of the tumors peritumoral edema was present. Edema was found around smaller as well as around larger oligodendrogliomas (Table 2).

Histopathologically, six tumors were graded A, 21 graded B (including 9 tumors grade B+, i.e. grade B with the presence of necrosis), 3 graded C and 13 tumors were

TABLE 2
CT scan characteristics of the oligodendrogliomas

	Margins		Density		Calcium		Peritumoral edema	
	sharp	unsharp	hypo/iso	hyper	present	absent	present	absent
Tumor size								
0 - 50 cm^3	28%	21%	14%	35%	12%	37%	28%	21%
50 - 100 cm^3	14%	12%	12%	14%	14%	12%	19%	7%
100 cm^3	7%	19%	16%	9%	14%	12%	21%	5%
	49%	51%	42%	58%	40	60%	67%	33%
Tumor grade								
A	3%	13%						
B/B+	23%	26%						
C	5%	5%						
D	18%	8%						
	49%	51%						

graded D. The lower grade oligodendrogliomas tended to show vague margins, while the majority of the higher grades had sharp margins at CT (Table 2). Although the survival rate decreased with increasing tumor grade, only the difference in survival time between grade D and the other grades reached statistical significance ($p < 0.001$) (Fig. 3).

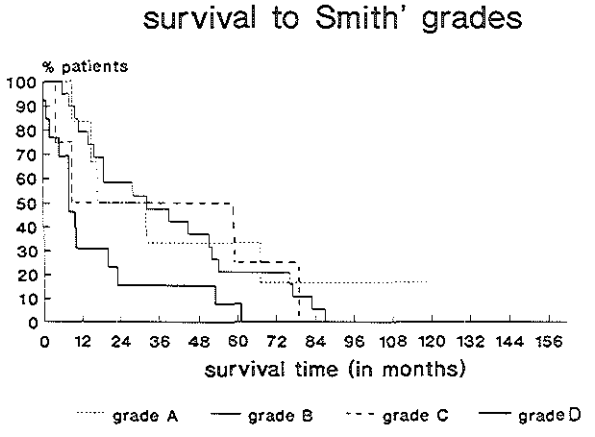

Figure 3. Survival to tumor grade. The number of patients in the respective groups are too small to yield significant differences in survival times; only grade D differed significantly from the other grades.

Anova did not reveal significant differences between the three groups defined by tumor size ($p = 0.5$). No correlation was found between tumor size and survival time for the whole group of patients ($r = -0.1363$; $p = 0.225$) (Fig. 4). Similarly, in the seventeen patients who had only undergone a diagnostic biopsy without decompression by surgical tumor debulking not even a correlative trend between tumor size and survival was found (Fig. 5). Only in the group of patients with a grade D tumor the negative correlation between tumor size and survival came close to significance ($r = -0.5534$; $p = 0.077$). Pearson's correlation coefficient showed no significant correlation between the duration of the preoperative period and the tumor size ($r = -0.074$; $p = 0.34$), or between age at first

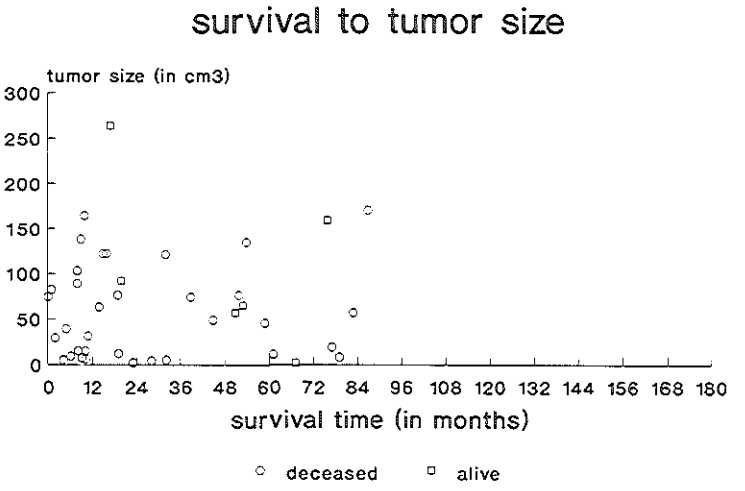

Figure 4. Relationship between tumor size (volumes) and survival time (n = 43). No correlation between tumor size and survival was found.

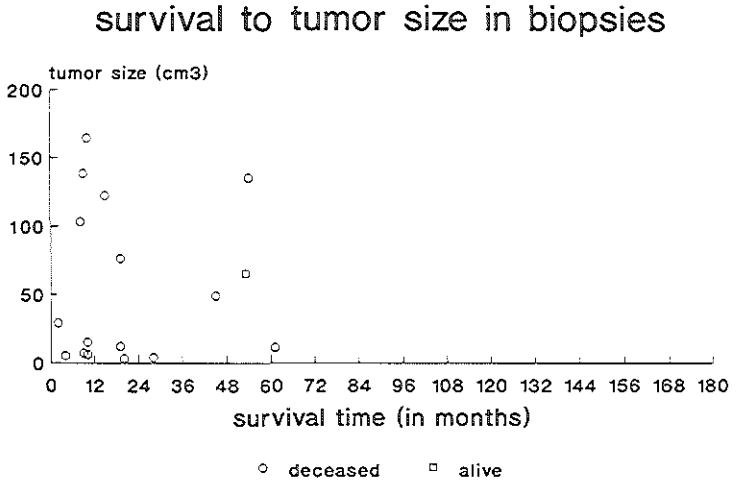

Figure 5. Relationship between tumor size (volumes and survival time for the patients who underwent only a diagnostic biopsy without substantial removal of tumor tissue (n = 17). Also in this subgroup no correlation between tumor size and survival exists.

operation and tumor size ($r = -0.16$; $p = 0.16$).

The mean survival time for patients with frontal tumors (n = 17) was 65.54 months (s.e.m. 64.46), while the mean survival times for the non-frontal cortical regions (n = 20) and the deeper regions (n = 6) were 20.07 (s.e.m. 19.61) and 7.30 (s.e.m. 2.89) respectively. Analysis of variance (ANOVA) revealed significant differences in the survival times of the three groups for localization ($p < 0.0001$) (Fig. 6). A posteriori

testing (Student-Newman-Keuls) yielded significant differences between the group with frontal tumors and the other two groups. Tumor size did not differ significantly between the three localizations ($p = 0.3870$), and no significant correlation between tumor size and survival time within the groups for localization was found.

No significant correlation between tumor size and age of the patient was found ($r = 0.16$; $p = 0.16$). No significant difference in survival time of the patients who had and those who had not undergone any form of radiation therapy was found.

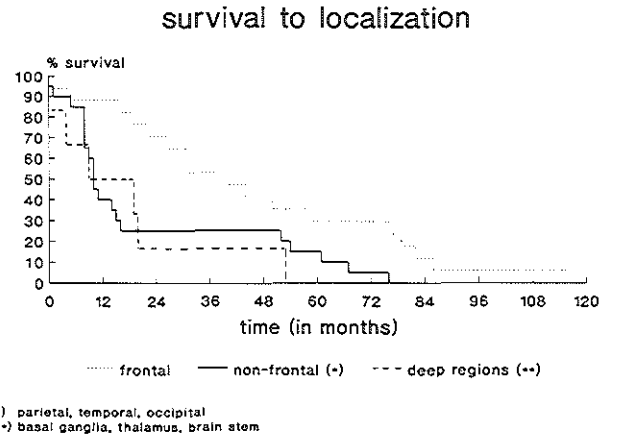

Figure 6. Relationship between tumor localization and survival time (n = 43). The patients with a frontal tumor had a significant longer survival time than patients with a tumor in a non-frontal region.

DISCUSSION

A variety of studies addresses the volumetric measurement of structures or tumors that are visualized on CT scans[9,13-18]. Some studies particularly deal with the volume of intracranial structures or tumors[9,19-24]. The method of converting surface to volume by multiplying surface area with single CT slice thickness was found to be the most practical and most reproducible means of volume determination[13,15,21,22]. This method yields a minor coefficient of variance and is superior to geometric computation based on the product of maximum diameter and perpendicular diameter, as well as the rectangular prism or ellipsoid model[13,14,18,24]. However, measurements are less accurate for lesions with a small volume[17,18,24]. In the present study the intra-observer error remained below 20%. Subsequent measurement of the volume and density of intracerebral tumors by CT-scans has been attempted in order to plan or evaluate therapeutic modalities[10,11,25-29]. Chisholm found an intra-observer variability of more than 10% of the real tumor volume, and a highly significant systematic inter-observer difference in mean value[3,30]. Furthermore, the minimal change in tumor volume that could reliably be detected appeared to be 25% of the original volume[30,31]. Therefore, growth rate estimations based on CT imaging are limited to cases with a substantial change in volume.

Often, tumor borders seen at CT-scan differ from real borders as verified by

stereotactic biopsy[32,33]. Nevertheless, the length of border trajectories vary between different gliomas and so within the same tumor. Gliomas of lower malignancy grades generally show a more diffuse growth pattern than neoplasms of higher degrees. Consequently the lower graded tumors are more difficult to delineate at CT-scan[34]. On the contrary, high grade gliomas and glioblastomas often grow as a circumscribed mass, although a narrow rim of infiltrative tumor cells is always present[35,36]. In the present study low graded oligodendrogliomas tended to be vaguely delineated, whereas indeed high grade tumors had a sharp demarcation (Table 2 and Figure 2). This finding is in agreement with Lee's results in a study of 35 oligodendroglial tumors[37]. In contrast to the clear boundaries at postcontrast CT-scan, the infiltrating malignant glioma cells in the rim of edema remain unnoticed at CT-scan[34]. The known tendency of oligodendrogliomas to infiltrate peritumoral edema makes all adjacent areas at CT potentially tumor-bearing[28].

In order to reduce sampling errors and to improve the representativity of biopsy material comparisons between CT-scan findings and histopathology have been made[1,2,38-41]. Some authors correlated the scan images with the histological findings in autopsy brains[34,40,42,43]. Good correlation between image and histopathology has been obtained for magnetic resonance but not for CT-scans[28,44]. Among some histopathologic features vascularity and necrosis were best predictable from the CT-scan pictures in various studies[35,38,45]. Contrast enhancement is mainly a reflection of damage to the blood-brain barrier caused by tumor tissue viz. neovascularization[39]. Since vascular and endothelial proliferation are features of malignancy in glial tumors[5,8], it would be a logical consequence that contrast enhancement at CT-scan corresponds to histopathological malignancy. In a study of Butler all astrocytomas of high grades showed moderate or marked contrast enhancement indeed[38]. In the study of Levin on 61 malignant gliomas the volume of enhancing tumor in combination with the absence of a peritumor low-density area were found to be indicative for a shorter tumor progression time and thus for a worse prognosis[23]. In another study on 21 oligodendrogliomas contrast enhancement was linked to malignant histopathology, although there was a considerable range in recurrences of the tumors, varying from 9 months till 8 years, and histopathological criteria of malignancy were not outlined[46]. Also in Lee's study on 35 oligodendrogliomas increased contrast enhancement was associated with higher grade tumors[37]. In a study of Schall, using radionuclide scans of oligodendrogliomas, the presence of endothelial and vascular proliferation was the main criterion for a positive scan. Nevertheless, no clear correlation with the clinical course was found[47]. In only seventeen cases of the present study postcontrast scans were available, and in this limited series no relation between increased contrast enhancement and tumor grade or tumor size became clear (Table 2).

A major reservation with respect to the present results is the uncertainty about the length of the preoperative period, in which the tumor has been present before being seen on the CT-scan for the first time, and increasing malignant histopathology with time[48]. The growth rate of the tumor could very well be an important prognostic factor. Therefore the expansion should be estimated. As mentioned before, however, changes in volume of the tumors cannot easily be established on CT-scans[30,31]. Early postoperative scans may also be misleading in the evaluation of residual tumor mass because of trauma to the blood-brain barrier during operation[25]. Determination of the mitotic index on histological slides would be an alternative method of assessing the tumor growth rate. Indeed, the mitotic count was found to be a significant prognosticator in the present patient group, and in an extended group including the same patient population[49].

The main result of the present study is the lack of correlation between tumor size and survival. This was true for the whole group of 43 cases, as well as for the group of

patients who had undergone only a diagnostic biopsy. Neither was a correlation between tumor size and histopathologic grade found. The absence of either correlation, i.e. between tumor size and survival and between tumor size and grade possibly indicate a much more complex role of tumor size in the clinical courses of patients with oligodendroglioma. Since frontal tumors show significantly longer survival, only localization would be a relevant factor in clinical staging of the oligodendrogliomas.

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PAPER 2

"OLIGODENDROGLIOMA: A COMPARISON OF TWO GRADING SYSTEMS"

J.M. Kros, D. Troost, C.G. van Eden, A.J.M. van der Werf, H.B.M. Uylings.

Cancer 61: 2251-2259 (1988)

Reprinted from CANCER, Vol. 61, No. 11, June 1, 1988.

Oligodendroglioma

A Comparison of Two Grading Systems

J. M. KROS, MD,* D. TROOST, MD,† C. G. VAN EDEN, PHD,* A. J. M. VAN DER WERF, MD, PHD,‡ AND H. B. M. UYLINGS, PHD*

In order to compare the grading system for oligodendrogliomas described by M.T. Smith (1983) with the conventional grading system according to Kernohan (1938), specimens from 72 patients were graded according to both systems, and survival times of the patients were compared. Survival rates decline in older patients. No interaction between the age of the patient and the degree of the tumor was found. No influence of localization of the tumor on survival was found. Similar to the system of Kernohan, the grading system of Smith distinguishes between only three groups of patients with significantly different survival times. In Smith's Grade A and Kernohan's Grade 1 the longest survivals are found; while in Smith's Grade D and Kernohan's Grade 4 the shortest survivals are found. Smith's Grades B and C as well as Kernohan's Grades 2 and 3 were intermediate with respect to the survival times of the patients and did not significantly differ from each other. With the independently significant features (cell density, pleomorphism, and necrosis) evaluated according to simple on-off scoring, and with the reduction from four grades to three, the grading system according to Smith would provide a simple and good, concise grading system for oligodendrogliomas of the brain.

Cancer 61:2251–2259, 1988.

SINCE THE 1920S when oligodendrogliomas were discovered and recognized,[1] many studies have been performed to detail the characteristics of patients with this disease or of the tumors in order to predict the clinical course of patients.[2–8] The old idea that the oligodendroglioma is a rather slowly growing and relatively benign tumor has recently come into dispute. Among the many parameters investigated with respect to therapeutic decisions and prognostic statements are age and sex of the patient, as well as the localization and histopathologic characteristics of the tumors. The histopathologic properties of the tumor traditionally are considered to be highly important with respect to the prognosis of the patients. However, grading of oligodendrogliomas appears to be complicated. Modifications of the grading system as described in 1949 by Kernohan for the gliomas[9–14] have been used for the oligodendrogliomas in several medical centers. Nevertheless, many studies in which these grading schemes were applied failed to correlate the histopathologic picture with prognosis. The absence of a reliable statistical base, for example, not accounting for the possible dependence between histopathologic characteristics[4,10] and drawing conclusions from too small groups of patients,[5,7,15–19] might be responsible for this failure. The need for statistical reliability was noted in a study of Schröder.[20] In 1983 Smith introduced an alternative grading system for oligodendrogliomas.[21] Not more than five histopathologic features were to be evaluated in classifying an oligodendroglioma into one of the four grades of this system. Because the histopathologic characteristics of his grading system were scored in a simple on-off schedule, Smith claimed that his system excluded most of the subjectivity among pathologists examining the tumor. Furthermore, Smith stated that these histopathologic characteristics were easily recognized, and that they were among the basic characteristics of malignancy. However, in this same study, only pleomorphism was identified as being significantly correlated with survival. Smith did not consider the localization of the tumor as a factor of prognostic significance, nor did he correct the

From the *Netherlands Institute for Brain Research, Amsterdam, The Netherlands, the †Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands, and the ‡Department of Neurosurgery, Academic Medical Center, Amsterdam, The Netherlands.

The authors thank Dr. J. van Pelt for fruitful discussion, Prof. Dr. J. A. denHartogJager and Prof. Dr. A. E. Becker for kindly providing materials for this study. We thank Dr. R. E. Baker for correcting the English, and Mr. R. Sersansie, Mrs. M. A. H. Matthijssen, Mr. W. P. Meun, Mr. K. C. Hoetmer, and Mr. J. V. Maas for their technical assistence.

Address for reprints: J.M. Kros, MD, Academical Hospital Rotterdam-Dijkzigt, Dpt. of Clinical Pathology/Neuropathology, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

Accepted for publication November 16, 1987.

survival times against the age of the patient when an operation was performed.

It seemed appropriate, therefore, to evaluate the correlation of Smith's grading system with the prognosis of the patients. Moreover, we wanted to compare this system with the more traditional system based on the criteria proposed by Kernohan in order to determine which one offered the most reliable prediction in the prognosis of the patient. The purpose of this retrospective study was to compare both systems by applying them to the same group of oligodendrogliomas and to correlate the respective grades with the survival rates. Since the age of the patients and the localizations of the tumors could very well influence the prognosis, the effects of these factors were also studied.

Materials and Methods

Clinical Data

The clinical records and the histologic material from 101 patients diagnosed as having oligodendroglioma of the brain were acquired from the files of the Department of Neurosurgery at the Academic Medical Center of the University of Amsterdam. Patients had been admitted to the hospital between 1958 and 1984. We were able to sample adequate clinical data and to verify the diagnosis in 72 of the 101 patients. Male and female patients were equally represented. At least one operation had been performed on the tumors of these patients. Fourteen patients had a second operation, while three patients had a third. All operations had been performed to debulk the tumor or to decompress the brain. In 38 cases of the disease the patient had also been treated with radiotherapy, but we were unable to obtain detailed information about the methods and dosages used. The period of time between the first operation and death was considered as the survival time. The preoperative period was estimated on the basis of the time when the first symptoms had appeared up to the date of the first operation. At the end of this study 15 of 72 patients were still alive, 52 had died, and we were unsure of the case records of five patients. These patients were considered to be alive since they could be traced for at least more than 5 years after the operation. Six patients who died within a month after the operation were excluded from this study since it was impossible to distinguish between operative mortality and mortality as a result of the tumor. All of these patients had only one operation. For the remaining group of 66 patients, Figure 1 shows the distribution of ages at the first operation. In order to explore the effects of age on survival the patients were divided into three comparable groups: patients younger than 35 years (31%), patients between 35 and 55 years (48%), and patients older than 55 years (21%). The effect of localization of the tumor was studied by distinguishing between tumors confined to the hemispheres (83%) and tumors located in more basal regions—suprasellar, cerebellar, and in the pons (17%). Tumors in the hemispheric group were divided into a frontal (56%) and a nonfrontal (27%) group.

Histopathologic Material and Grading

The original histopathologic material was reexamined or when necessary, new slides were processed for hematoxylin and eosin staining. A tumor was accepted as an oligodendroglioma when it was composed of more than 50% cells with the phenotype of oligodendrocyts. In conventionally stained sections these cells are recognized as round cells with perinuclear halos. Two neuropathologists who were unaware of the corresponding clinical data, as well as each other's scores, applied both grading systems in different sessions to the material in random order.

The Kernohan grading system adapted for oligodendrogliomas consists of the parameters, cell density and amount of neuropil (cell appearance), pleomorphism, hyperchromasia, vascular and endothelial proliferation, mitotic rate, necrosis, microcysts, and calcium. Table 1 outlines these features and the composition of the respective grades.

The Smith grading system is composed of four features, which are already used in Kernohan's grading system—endothelial proliferation, necrosis, cell density, and pleomorphism. The feature of nuclear-cytoplasmic ratio completes the grading system of Smith. The features should only be recognized as present or absent; high or low. Grade A is defined as all features low or absent; Grade B as the presence of pleomorphism and/or cell density high and nuclear-cytoplasmic ratio high; Grade C as tumors showing pleomorphism, endothelial proliferation, high nuclear-cytoplasmic ratio, and high cell density; and Grade D is defined as all five features being high or present.

Figures 2A–2C show some of the histopathologic variables of both grading systems. Table 3 shows that Smith uses one combination of histopathologic features to define his Grades A, C, and D, and that he lists three possible combinations to define Grade B. Twenty-four percent of the tumors showed a histopathologic picture that was not defined by Smith. Since the survival rates in this group were not significantly different from the survival rates in the group with Grade B oligodendroglioma (see Results), these patients were added to the B group.

Statistics

All tests were performed by means of the Statistical Package for the Social Sciences (SPSSX package). The

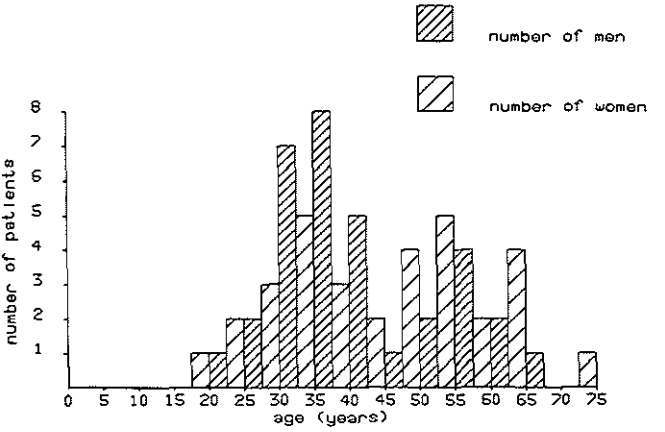


FIG. 1. Age at first operation. Number of patients distributed according to the age when the first operation on the tumor, to debulk or to decompress, was performed. Men and women are, in this consecutive order, represented. The number of patients in each age group is too small to identify the shape of the curves as significantly biphasic.

Analysis of Variance (ANOVA) was used for main effects of the grading systems, the effects of patient age, and localization of the tumor. Testing *a posteriori* (Student-Newman-Keuls test) was performed to demonstrate the differences between the particular grades. Since the frequency distribution of the survival times of the patients is an exponential one, these data were log-e transformed before the application of ANOVA. The correlation between the survival rates of the Kernohan and the Smith grading systems is indicated with Pearson correlation coefficients.

Results

When the Kernohan grading system was applied, 7% of the tumors in the total group received Grade 1; 32%, Grade 2; 30%, Grade 3; and 32%, Grade 4. When graded according to Smith, 6% of the tumors were graded as A, 24% as B, and in addition—although not strictly defined by Smith (see the previous section on histopathologic grading)—another 24% were graded as B, 11% as C, and 35% as D (Table 3). The discrepancy between the two neuropathologists in their judgments of the histopathologic features was less than 5% using the Smith grading system and up to 8% according to the Kernohan-derived system. A matrix shows the percentages of tumors as graded according to both grading systems (Table 4). Fifty-seven percent of the tumors received grades of the same order in both systems. In 28% of the patients the tumor was graded as more malignant by the system of Kernohan than by the system of Smith, while in 15% of

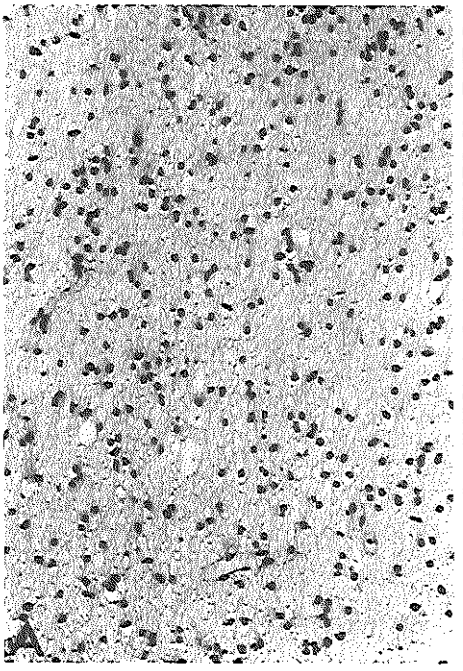
TABLE 1. Features and Grading According to Kernohan's Grading System for Gliomas

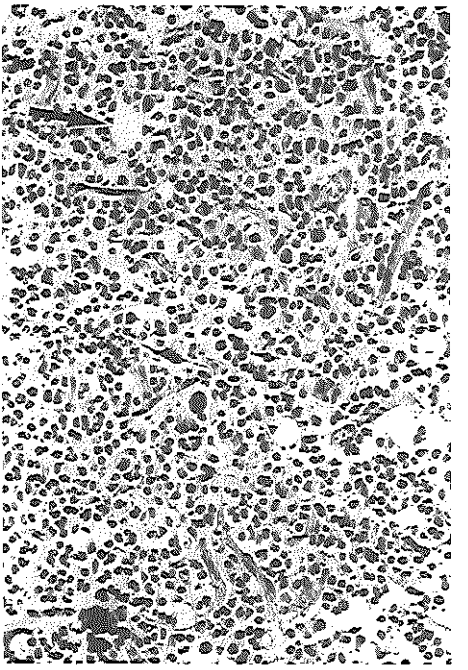
Features	Grade*			
	1	2	3	4
Cell density	+†	++	++	++
Neuropil	Less than normal	−‡	−	−
Cell appearance	Normal	Honeycomb		Atypical
Pleomorphism	−	−	+‡	++‡
Hyperchromasia	−	−	+	+
Vascular and endothelial proliferation	−	−	−	+‡
Mitotic rate	−	−	+‡	++‡
Necrosis	−	−	−	+‡
Cysts	−	−	Often	Often
Calcium	+	+	Less	Less

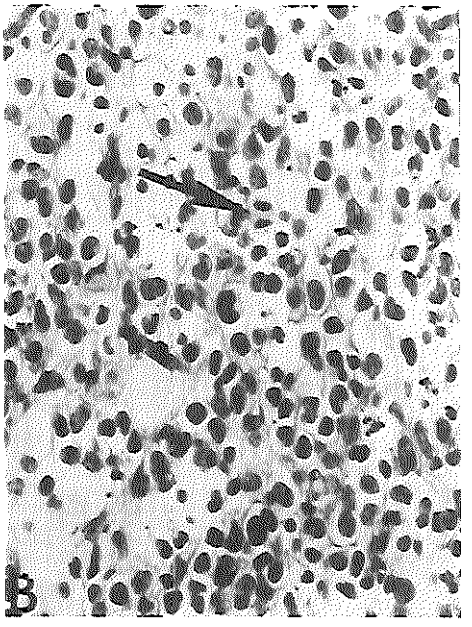
* The most important difference between Grade 1 and Grade 2 is the absence of neuropil in the latter. Therefore, Grade 2 shows the classic picture of an oligodendroglioma with the characteristic honeycomb structure. The most important differences between Grades 2 and 3 is the presence of mitoses and of pleomorphic cells in Grade 3. Grade 4 is distinguished from Grade 3 by the existence of necrosis, vascular and endothelial proliferation, more mitoses than in Grade 3, and more pleomorphism.

† Symbols: −: absent or low; +: present or high; ++: abundant or very high.

‡ Indicates the main difference(s) between the actual grade and the preceeding degree.







FIGS. 2A–2C. (A) Oligodendroglioma in which all five features from the grading system according to Smith are absent or low, and thus graded A. Since the features hyperchromasia, mitoses, and cysts were also absent, it was graded as 1 in the Kernohan system (H & E, ×230). (B) Tumor graded B in Smith's system (no necrosis, no endothelial proliferation); however, at least graded 3 in the system of Kernohan because of the presence of mitoses (arrow), hyperchromasia, and marked pleomorphism (H & E, ×350). (C) This tumor shows endothelial proliferation and pleomorphism rather a high cell density and a high nuclear-cytoplasm ratio. Since necrosis was found elsewhere in this tumor, it was graded D in the Smith system. The arrow indicates one of the microcysts, which are not considered for this grading (H & E, ×230).

TABLE 2. Features and Grading According to Smith

Features	Grade* A	B	C	D
Endothelial proliferation	−†	− − − − − − − + − + − +	+	+
Necrosis	−	− − − − − − + + + + + +	−	+
Nuclear-cytoplasm ratio	−	− + + − − + + − − + + −	+	+
Cell density	−	− + + + + − + + + + + +	+	+
Pleomorphism	−	+ − + − + + − − + − + +	+	+

* Grades A, C, and D are defined by only one combination of the five features. Grade B is represented by three combinations as defined by Smith and nine combinations as found in the present study, three without and six with the feature of necrosis.

† −: absent or low; +: present or high.

the patients the opposite was true. The most extreme differences in grading were found in the 5% of the Grade 4 tumors in the Kernohan system and Grade B in the Smith system, and in 3% of the Grade 2 tumors in the Kernohan system and Grade D in Smith's system.

Table 5 lists mean survival times. Patients with Kernohan Grade 1 tumors and Smith Grade A tumors had the longest mean survival rates as compared with the other grades. Patients with Kernohan Grade 4 tumors and Smith Grade D tumors both had the shortest survival rates. In both grading systems the survival of the second group (Grade 2 and B) was shorter than the survival of the third group (Grade 3 and C). The analysis of variance showed that the Kernohan system ($P < 0.01$) and the Smith system ($P < 0.001$) distinguished between groups of patients with different survival times. The Student-Newman-Keuls test, using significance at 0.05, showed that in the Kernohan system Grade 4 differs significantly from Grades 2 and 3, and that in the Smith system Grade D was significantly different from Grades B and C. No significant differences in survival rates could be demonstrated between Grade 2 and 3 patients and between grade B and C Patients. Since there were not enough patients in the two lowest degrees of both grading systems, the survival rates of these grades could not be tested. Figures 3A and 3B show the survival percentages within each grade of both grading systems.

The length of the preoperative period appeared to be highly variable, seemingly showing no relation to tumor degree. However, the ANOVA showed that the Kernohan grades had a significant effect on the variable length of preoperative symptoms, but only Grades 3 and 4 differed significantly from each other. Thus, in the Kernohan grading system the period of time between the onset of symptoms and the operation is shorter for patients with Grade 4 tumors than for patients with Grade 3 tumors.

The trend of decline in survival rate with increasing age is shown in Figure 4. *A posteriori* testing with a significance level of 0.05 showed that all three age groups were different from each other in their mean survival time. The Pearson correlation coefficient calculated between the ages at first operation and the survival rates showed a significantly negative correlation (-0.4722; $P < 0.001$). An interaction between age and one of the two grading systems as investigated in two-way ANOVAs was not found ($P > 0.1$). The three groups of patients that were defined on the basis of the localization of the tumor did not have significantly different survival rates as tested with ANOVA, nor did ANOVA demonstrate a difference in survival rates according to sex. No significant difference between the survival rates of patients who were treated with adjuvant radiotherapy and patients who were treated only by surgery could be shown.

Discussion

The age distribution shown in Figure 1 confirms most of the distributions reported in the literature.[4,5,10,16,18,22–24] A biphasic curve in the incidence of the oligodendrogliomas is also a common finding, although it is here not found to be of significance. The peak in frontal localization is also found in all larger studies. In this study a majority of tumors were located in the right hemisphere. A clear lateralization of the oligodendrogliomas was also noticed by Ravens, Adamkiewicz, and

TABLE 3. Percentages of the Total Group in the Respective Grades of the Grading Systems

Kernohan	Smith	Smith's Report, 1983
1 7%	A 6%	A 23%
2 32%	B 24% + 24%	B 49%
3 30%	C 11%	C 22%
4 32%	D 35%	D 6%

Note: The first two columns show percentages in each grade of the group in our study; the last column shows the percentages as found by Smith *et al.* Grade B of the Smith grading system in our group shows 24% tumors, which have a histopathologic picture that is the same as one of the three pictures strictly defined by Smith for this grade; the other 24% have pictures that were attributed to this grade by us (see text).

TABLE 4. Matrix of Percentages of Patients as Graded According to the G Systems of Kernohan and Smith

		Total no. of patients = 66			
	4	0%	5%	3%	23%
Kernohan	3	0%	18%	4%	8%
	2	2%	25%	2%	3%
	1	5%	2%	0%	0%
		A	B	C	D
			Smith		

Groff[17] and by Roberts and German.[5] However, these authors reported a distinct left side predominance. The influence of localization on survival rates could not be found in our study. Ravens *et al.* mentioned a relationship between the histology of the tumor and its localization in the brain,[17] but this relationship has not been established in later publications. Despite earlier conclusions,[25] it recently was demonstrated that there is a beneficial effect of radiotherapy on the survival of the patients whose neoplasms have been subtotally resected.[26] However, the survival rates of patients who received adjuvant radiotherapy in our study did not differ from those of the patients who only experienced an operation.

By applying ANOVA, a significant effect of age on survival was found, all three age groups having significantly different mean survival rates. The mean survival of the older patients was shorter than that of the younger patients. From Mørk's analysis in which the survival rates were corrected for life expectancy based on the age of the patient, thus eliminating age as significant factor, it is likely that the effect of age is mainly based on this natural life expectancy.[24,27] Ludwig and Smith found a relation between the age of the patient and tumor degree: older patients had tumors of a higher degree than younger patients.[28] Since an interaction between these two factors was absent in our study, we are unable to affirm these results. Neither did we find support for the results of Burger suggesting a correlation between patient age and the number of mitoses in the tumor.[29]

The difference in the distribution of patients among the grades in our study and Smith's own report is noticeable. We classified 6% of our tumors as Grade A, while Smith classified 23% of his tumors in this group. We graded 11% of the tumors as C, while Smith graded 22% as C; we also graded 35% of the tumors as D, while Smith classified only 6% of his tumors as Grade D (Table 3). A possible explanation for the discrepancy in numbers of tumors in the lowest and in the highest degrees might be that the patients in Smith's study were operated on more readily, and, therefore, his material could consist of tumors of a lower degree. However, the suggestion by Muller[18] that the histopathologic picture of an oligodendroglioma would develop from a low degree into a higher degree has never been proven.

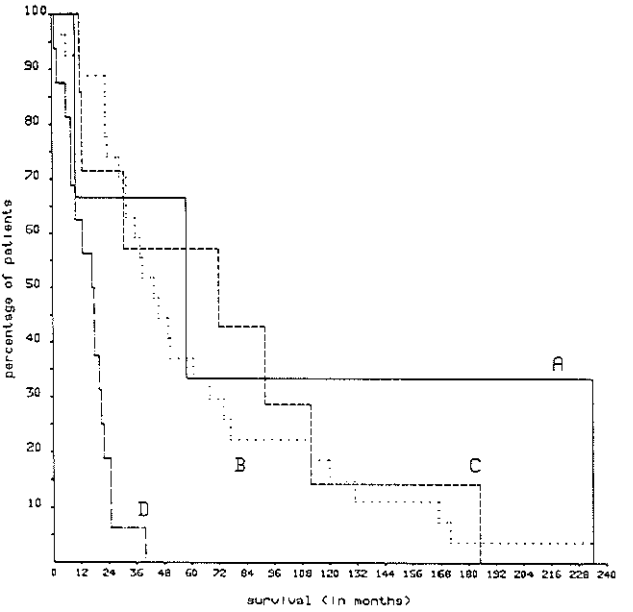
TABLE 5. Mean Survival Rates in Months for Both Grading Systems

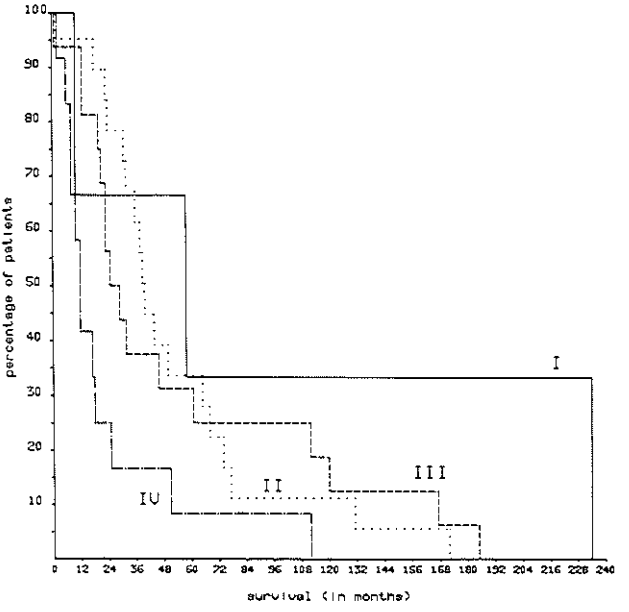
	Kernohan			Smith	
Grade	Mean*	SEM		Mean	SEM
1.	122.0	111.7	A.	112.5	102.5
2.	66.9	14.0	B.	77.0	13.3
3.	71.1	21.5	C.	82.8	32.6
4.	34.7	16.8	D.	15.7	2.5

SEM: standard error of the mean.
* Mean survival rates are given from the time of the first operation.

No significant difference in survival rates between the original Grade B group as defined by Smith and the group that was added to the Grade B tumors in the present study was found. This finding justified adding these nine combinations of features to the original three combinations that were defined as Grade B by Smith. Nevertheless, the total group of Grade B patients was about the same size as the B group in Smith's study—containing 48% and 49% of the tumors, respectively. Many different histopathologic pictures emerged from these Grade B tumors, and a distinct delineation from the C group could not easily be made. Furthermore, our finding of an absence of a significant difference in survival rates between patients with Grade B tumors and patients with Grade C tumors is remarkably similar to Smith's report.[21] Therefore, the idea of adding the one combination defined as Grade C to the Grade B group with its many combinations of features could be defended.

In recent studies Mørk and Burger identified necrosis as an independent, significant feature in prognosis.[27,29] Nelson concluded from his research on astrocytomas that necrosis was a clear, significant factor in prognosis for this type of tumor.[23] In the grading systems of Smith and Kernohan the feature of necrosis separates the third degree from the highest degree. In addition, we found that the survival rates of patients with a Grade D tumor were significantly worse than the survival rates of patients with Grade A, B, or C oligodendrogliomas. The same was found with respect to the Kernohan grading system. Necrosis is one of the features in six of the nine combinations of features that were classified in Grade B (Table 2). The Student-Newman-Keuls test was applied in our search for a difference in survival rates between patients with these necrosis-positive tumors in the Grade B group and tumors in the Grade D group. A significant difference between the survival rates of these two groups was found. It may be concluded that these particular tumors from our Grade B group are not to be added to the highest degree only because of this presence of necrosis. Together with the Grade B tumors in which no necrosis was found, they form a distinct grade in which many diverse histopathologic pictures can be found.





FIGS. 3A AND 3B. Survival percentages for grades in systems of Smith and Kernohan. (A) In Smith's system each line represents the total percentage of patients who are still alive with a tumor classified according to the particular grade as marked. As shown by ANOVA, Grades B and C cannot be distinguished in terms of survival rates. The curve of Grade A represents only three patients. The ANOVA did not show a distinction in survival rates between Grade A and the other grades of this system. (B) In the Kernohan system each line represents the total percentage of patients still alive with a tumor classified according to the particular grade as marked. The ANOVA could not disclose a significant difference between the survival rates of the patients with tumors in the two middle grades. Since Grade I consists of only three patients, the absence of a significant difference between the survival rates of patients with tumors of this grade and those of other grades is also clear in this system.

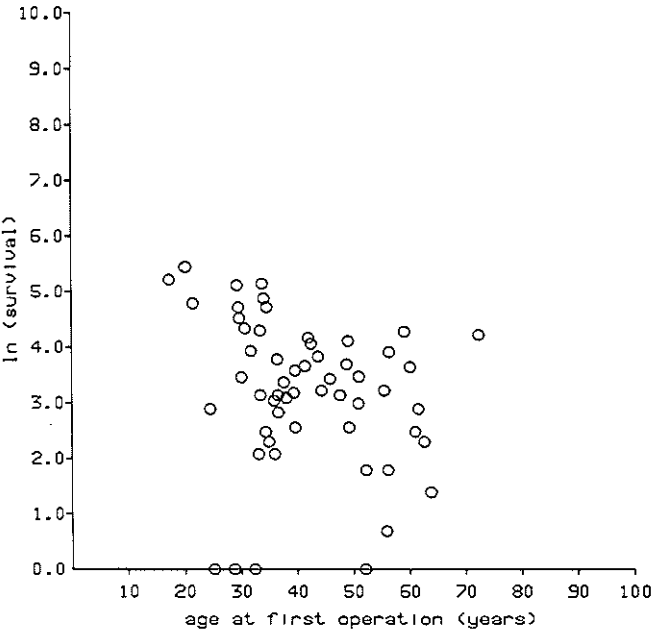


FIG. 4. Survival rates to age. Each patient is represented by a circle. The tendency of shorter survival with increasing age is clear. In the present study this trend was found to be independent of tumor degree and localization ($P < 0.001$).

In the large study by Mørk *et al.*[24,27] 20 histopathologic features of 208 oligodendrogliomas were analyzed, and by applying a multivariate analysis, cell density, necrosis, microcysts, and possible subpial infiltration were shown to have independent prognostic significance. Pleomorphism is the only feature identified by Smith as being significant in terms of prognosis.[21] In the recent report by Burger *et al.*[29] a group of 71 patients with an oligodendroglioma of the brain was studied using a univariate analysis. Here, the features of mitoses, necrosis, nuclear atypia, and vascular hypertrophy and vascular proliferation were mentioned in order of decreasing importance with respect to prognosis; the authors concluded that necrosis and the number of mitoses contain all of the prognostically useful information. If these histopathologic features that are independently significant are considered in the grading systems of Smith and Kernohan, both systems use necrosis, cell density, and pleomorphism. The Kernohan-derived system also lists (micro)cysts, which is a significant feature according to Mørk,[27] as well as the controversial feature of number of mitoses. This feature, however, although identified as a significant factor in Burger's paper, was not found as being significant in Mørk's study. In addition to the microscopic histopathology, Mørk lists the following five clinical features of a patient that are significant in terms of survival: preoperative clinical status, ABO blood group, gross necrosis and hypervascularity as seen by the neurosurgeon, and calcification as seen on radiographs of the skull.[24,27] Despite the last macroscopic feature, Mørk does not mention the microscopic presence of microcalcifications as being among the significant factors. It was not possible to trace all these clinical data in the records of the patients in our study.

The ultimate test of the validity of a grading system is a true correlation of grade with prognosis.[21] It is concluded from our statistic analysis that both grading systems contribute in predicting the survival time of a patient. With further analysis it was found that neither the grading system of Kernohan nor the newer system of Smith can discern more than three groups with different survival rates. The degree of discrimination in the preoperative period as achieved by the Kernohan grading system is not very impressive because the data on the point at which first symptoms begin are not very reliable. The advantage of Smith's grading system is that it reduces the histopathologic features. Recent literature makes legitimate four of the eight features used in the Kernohan-derived system, and three of the five features used in the Smith grading system. A second advantage of Smith's system is that it simplifies grading by scoring the features in a simple on-off schedule. The four grades

of Smith's system could be reduced to three by combining the features of Grade C with those of Grade B. The presence of necrosis alone does not justify the highest degree for a particular tumor. Grade A should be reserved for a tumor in which all features are found to be low or absent, but a significant distinction of this grade from the higher grades could not be shown in our study. The age of a patient does significantly affect survival, but probably no more so than the effect of natural life expectancy. A relation between age and tumor degree was not found. Localization was not found to be significant in prognosis in our study, and it did not interact with the histopathologic picture of the tumor as expressed in the grading systems.

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PAPER 3

"PROGNOSTIC RELEVANCE OF DNA FLOW CYTOMETRY IN THE OLIDOGENDROGLIOMA".

J.M. Kros, C.G. van Eden, C.J. Vissers, A.H. Mulder.

Cancer 69: 1791-1798 (1992)

Prognostic Relevance of DNA Flow Cytometry in the Oligodendroglioma

J. M. Kros, MD, C. G. van Eden, MD, PhD,† C. J. Vissers,* A. H. Mulder, MD, PhD,* and Th. H. van der Kwast, MD, PhD**

In a retrospective study of 85 cases, the prognostic value of DNA flow cytometry in oligodendrogliomas was evaluated. Paraffin-embedded material was processed for flow cytometry, and the survival rates of the patients with DNA diploid, aneuploid, and tetraploid tumors were compared using analysis of variance. In addition, the mitotic index was correlated with the results of flow cytometry. Finally, the results of flow cytometry, histopathologic grading, and counting mitoses were tested for dependency.

Thirty-one percent of the tumors were diploid, 39% were tetraploid, and 31% were aneuploid. The results of the DNA flow cytometry did not correlate with the survival times ($P = 0.798$) or with tumor degree. In contrast, the number of mitoses ($P < 0.05$), and the grades of the grading system of Smith ($P < 0.003$) had relevance for the prognosis. No correlation between flow cytometry, histopathologic grading, and mitotic index was found.

It is concluded that flow cytometry has no value in predicting the biologic behavior of oligodendrogliomas, whereas the number of mitoses is a valuable prognostic parameter and thus is considered to be incorporated into the grading system for oligodendrogliomas. *Cancer* 1992; 69:1791–1798.

Oligodendrogliomas represent a subgroup of glial tumors in which histopathologic grading has been a matter of controversy. The original grading system for the gliomas developed by Kernohan and coworkers yielded poor correlation with the survival times of the patients.[1–3] Therefore, histopathologic features with independent prognostic significance have been sought,[4–6] and a new grading system exclusively for oligodendrogliomas was developed by Smith *et al.*[6]

In a variety of tumors, DNA flow cytometry (DNA FCM) has proven to be a useful and objective parameter in addition to histopathology for the assessment of tumor progression or survival of patients.[7–11] The aim of the current study was to explore the value of DNA FCM for prognostic predictions in oligodendrogliomas. In gliomas, the results of DNA FCM in predicting survival times of patients have been controversial.[12,13] The largest survey comparing DNA content with biologic behavior specifically dealing with oligodendroglial tumors was done on a series of no more than 11 tumors.[14] Obviously, the results were inconclusive.

In the current retrospective study of 85 oligodendroglial tumors, DNA FCM was performed on paraffin-embedded material and the results were correlated with the survival rates of the patients. In addition, the oligodendrogliomas were graded according to the grading system of Smith, and the tumor grades were correlated with the results of FCM. The mitotic index was morphometrically determined, and the results were correlated with the survival times and with the results of FCM.

Material and Methods

Clinical Records

The clinical data and the paraffin-embedded histologic material of a group of 137 patients with cerebral oligodendroglioma were obtained from the files of the Academic Hospital Rotterdam-Dijkzigt. The patients had been admitted between 1972 and 1986. Tumors were included in the study if they were composed of more than 50% of cells with an oligodendroglial phenotype. A verified histopathologic diagnosis, adequate follow-up, and a sufficient amount of paraffin-embedded ma-

From the *Academic Hospital Rotterdam-Dijkzigt, Department of Clinical Pathology, Rotterdam; and the †Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

The authors thank Dr. S. Z. Stefanko for providing histopathologic material for this study; Dr. C. J. J. Avezaat for access to the neurosurgical records; Mr. R. W. H. Verwer (Netherlands Institute for Brain Research) for advice in statistics; Ms. Waayer-Van Batenburg for technical assistance; Ms. P. Delfos for photography; and the staff of the medical archives of the Academic Hospital Rotterdam-Dijkzigt for its services.

Address for reprints: Johan M. Kros, MD, Academic Hospital Rotterdam, Dijkzigt, Department of Clinical Pathology/Neuropathology, PO Box 1738, 3000 AD Rotterdam, The Netherlands.

Accepted for publication July 2, 1991.

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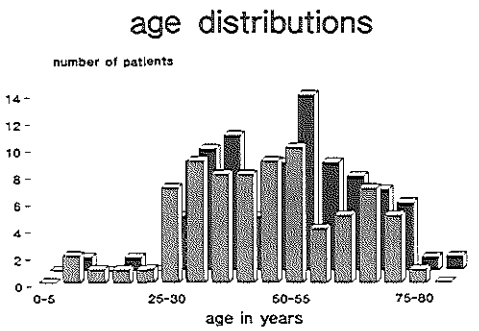

Figure 1. Age distributions at the time of the first operation (shaded bars) and the time of death (black bars).

terial to perform flow cytometry could be retrieved in 85 cases. Seventy-eight patients were dead at the time of analysis. All patients died as the result of tumor progression. The survival times were calculated as the time interval between first operation and death. The mean age at time of first biopsy was 45 years, and at time of death was 49 years. The distribution of age at first operation and age of death are shown in Figure 1. In 16 cases, the material of a second biopsy was processed. All patients underwent excisional biopsies for decompressing or debulking purposes. The localizations of the tumors are listed in Table 1. No information about the volume of surgically removed tissue could be obtained. Stereotactic biopsies were not included. Fifty-six percent of the patients received postoperative radiation therapy. The radiation doses and modes of application differed considerably because the current study covers a period of more than 14 years. None of the patients had been treated by chemotherapy.

Preparation of Nuclear Suspensions and Measurement of DNA Content

Three or four 50-μm sections of representative blocks of paraffin-embedded tumor tissue were cut. Areas with excessive necrosis or small fractions of tumor were avoided. Gray and white matter areas of non-neoplastic brain tissue were used as controls. Two additional 5-μm sections were made for light microscopic evaluation. From the 50-μm sections, suspensions of nuclei were processed by the method of Hedley *et al.*[15,16] The slides were deparaffinized and rehydrated. After rehydration, the tissue was transferred to a test tube containing 1 ml of 0.5% pepsin (0.9% saline adjusted to pH = 1.9 with 0.02% azide) and incubated for 1 hour at 37°C with repeated vortexing. The tubes were centrifuged (2000 rpm) during 10 minutes and resuspended in Hank's balanced salt solution containing 50 μg/ml ethidium bromide. The samples were filtered through a 40-μm nylon mesh filter. The stained samples were measured on an FACS Analyzer (Becton Dickinson, Sunnyvale, CA). Histograms were generated from 10,000 nuclei and displayed as linear fluorescence.

Evaluation of DNA Content

The ploidy of the tumor sample was estimated by DNA index as being the ratio between the modal channel numbers of the first and the subsequent peaks in the sample. A sample was defined as DNA diploid if there was a single G0/G1 peak, and the DNA index had a value of between 0.90 and 1.10, whereas the second peak (G2M fraction) contained less than 5% of the nuclei measured. A sample was defined as DNA tetraploid if the second peak had a DNA index of between 1.90 and 2.10 and the fraction contained more than 10% of the nuclei. Samples with a DNA index of the second peak of more than 2.10 or less than 1.90 or with a first peak with a shoulder were defined as DNA aneuploid.

Determination of the S-phase fraction of the samples was tried by using two software programs (the Polynomial Model and the Sum of Broadened Rectangles Model, Becton Dickinson DNA Cell-Cycle Analysis Software).

Grading of the Oligodendrogliomas

From the histopathologic features that were scored, five features were used for grading the oligodendrogliomas according to the grading system developed by Smith *et al.*[6] and outlined by Kros *et al.*[17] In this grading system the features of endothelial proliferation, necrosis, nuclear-cytoplasm ratio, cell density, and pleomorphism are scored in a simple on-off manner. If all features are absent or low, the tumor is Grade A, and if all are present or high, the tumor is Grade D. All of the other com-

Table 1. Localizations of the Oligodendrogliomas

	Left (%)	Median (%)	Right (%)
Frontal	18	5	18
Frontoparietal			8
Parietal	2		4
Parietotemporal	4		11
Temporal	5		5
Frontotemporal	4	1	7
Parietooccipital	2		2
Frontoparietotemporal	2		1
Frontotemporooccipital			1
Cerebellar	1		

binations of features are scored in the middle groups (B and C), whereas Grade C is defined as all features being present or high, except the feature of necrosis. Several combinations of the five histopathologic features are found in Grade B; those in which necrosis was present were defined as Grade B+.

The mitotic score was morphometrically assessed by counting the relative number of mitoses corrected for cell density per three high-power fields (hpf; objective 40×). The scores were arbitrarily grouped in three categories (*i.e.*, a category of less than one mitosis, a category of more than five mitoses, and an intermediate category).

Statistical Analysis

All tests were performed by the Statistical Package for the Social Sciences (SPSSX package). Analysis of variance (ANOVA) was used for main effects of the flow cytometry groups and the tumor grades on the survival times. Testing *a posteriori* (Student–Newman–Keuls test) was done to elucidate differences between individual grades. Because the frequency distribution of the survival times of the patients was an exponential one, these data were log-e transformed before the application of ANOVA.

Testing of dependency between the results of FCM, mitotic score, and histopathologic grading was done by using Cramér's contingency coefficient procedure.

Results

Twenty-six (31%) of 85 tumors had a DNA diploid flow pattern, 33 (39%) a DNA tetraploid pattern, and 26 (31%) had a DNA aneuploid pattern. Examples of flow cytograms are given in Figure 2. The coefficient of variation of the first peak (G0/G1) had a range from 4.0% to 10.9% and a mean value of 6.6%. In Table 2 the respective mean survival times for the various ploidy classes are given, and in Figure 3 the survival curves are shown. No significant differences in survival times were found ($P = 0.798$). The mean survival times of patients with tumors with an aneuploid pattern tended to be longer than those with a diploid pattern (Fig. 3 and Table 2). Assessment of the S-phase fractions failed in the majority of the samples because of the relative high coefficient of variation values or short S-phase trajectories.

The grading results related to the survival data are listed in Table 2, and the survival curves are plotted in Figure 4. ANOVA revealed that the grading system of Smith distinguished groups of patients with significant differences ($P < 0.001$) in survival times. The *a posteriori* Student–Newman–Keuls test showed differences between Grades A and D and Grades B and D.

In 26% of the tumors no mitoses within the 3 hpf were seen, whereas in 17%, more than five mitoses were counted (Table 2). The survival rates of the three groups are shown in Figure 5. ANOVA revealed significant differences ($P < 0.0038$) between these groups. *A posteriori* testing (Student–Newman–Keuls) showed significant differences between the survival rates of the patients with less than one mitosis per 3 hpf and those in the other two groups.

No dependencies between the results of FCM and of histopathologic grading (Cramér's contingency coefficient = 0.437), FCM and mitotic count (Cramér's contingency coefficient = 0.221) or between grading and mitotic count (Cramér's contingency coefficient = 0.320) were found (Table 3). Thus, correlations between the results of FCM, histopathologic grading, and mitotic score are not shown.

No significant effect on the survival times of patients who had been and those who had not been treated by any means of radiation therapy was found by ANOVA.

Tables 4, 5, and 6 represent the results of FCM and grading of the successive biopsies of 20 patients who had a second operation. The results of FCM on the first and the second biopsy are shown in Table 5. In 33% of the cases the tumors had the same DNA flow profile in the first and the second biopsy (Table 5). Although a tendency of increasing grade in successive biopsies was obvious (Table 6), no particular trend in the successive results of DNA FCM was seen (Table 5). No DNA diploidy was found after DNA aneuploidy in the first biopsy, and no tumors displayed DNA aneuploidy after initial DNA diploidy. No consistent relation was found between the ploidy changes and the interval of time between the two biopsies (Table 4). Radiation therapy did not affect DNA FCM pattern (Table 4).

Discussion

In the current study the grading system of Smith yielded satisfactory prognostic correlations that were comparable to those found in an earlier study on a different patient population.[17] In the meantime, histopathologic features with independent prognostic significance have been revealed by multivariate analyses.[4–6] Thus far, only Burger *et al.*[4] identified the mitotic index as a relevant prognostic factor. Nevertheless, mitotic counts have not been incorporated in Smith's grading system. The results of the current study suggest that the mitotic count should be incorporated in the grading system for oligodendrogliomas.

The value of flow cytometric DNA assessment for

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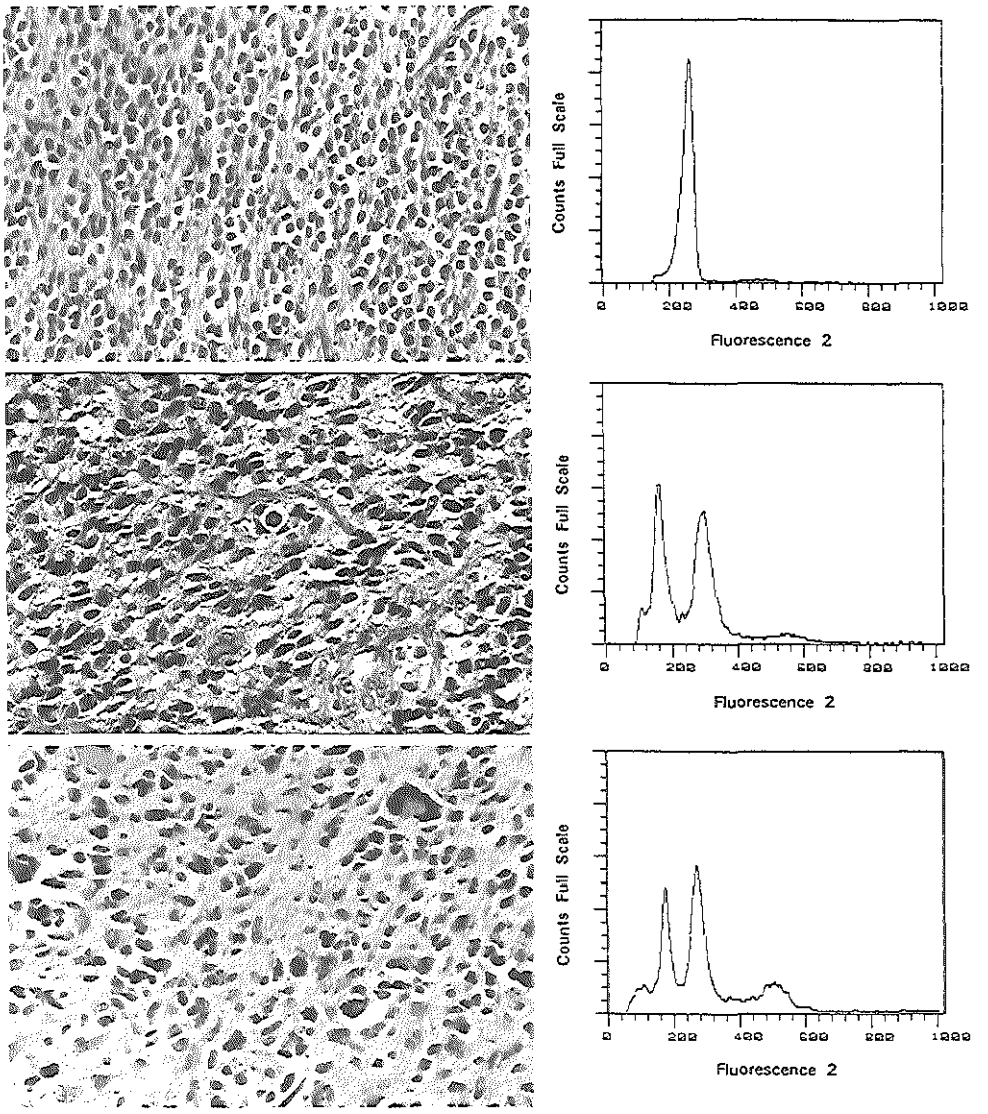

Figure 2. DNA flow cytograms in oligodendrogliomas. (Top) Oligodendroglioma Grade A with its diploid DNA flow histogram. (Middle) Oligodendroglioma Grade B (notice mitotic figure in the center) with its aneuploid DNA flow histogram. (Bottom) Oligodendroglioma Grade C (notice high pleomorphism) with its aneuploid DNA flow histogram.

Table 2. Survival Times of Patients Grouped According to Results of DNA Flow Cytometric Study, Histopathologic Grade, and Mitotic Index

	Results of DNA-FCM, grading and counting mitoses	Survival time (mo)		
		Median	*Mean ± SEM*	*Range*
Flow cytometric study				
Diploid	31%	14.5	25 ± 5.29	83
Tetraploid	39%	21.0	35 ± 5.72	104
Aneuploid	31%	21.5	46 ± 8.45	139
Histologic grade (according to Smith *et al.*[6])				
A	22%	61.0	53 ± 9.54	133
B	31%	30.5	41 ± 6.29	103
B+	20%	16.0	33 ± 8.06	100
C	8%	11.0	21 ± 11.25	79
D	19%	4.0	10 ± 3.95	61
Mitotic index				
Mitoses = 0	26%	41.0	48 ± 7.89	138
Mitoses 0–5	57%	10.0	23 ± 4.05	89
Mitoses > 5	17%	12.0	27 ± 8.92	100

predicting the clinical behavior of tumors has been explored in a variety of neoplasms.[7–11,18–21] Most studies were done on fresh tumor material, but techniques for successful application of flow cytometry on paraffin-embedded material have been developed.[15,16,22] Despite disadvantages of using paraffin-embedded material, such as decreased fluorescence intensity, a higher inter-sample variability, and the risk of missing peridiploid DNA aneuploid peaks because of increased coefficient of variation, in a variety of tumors DNA flow cytometry

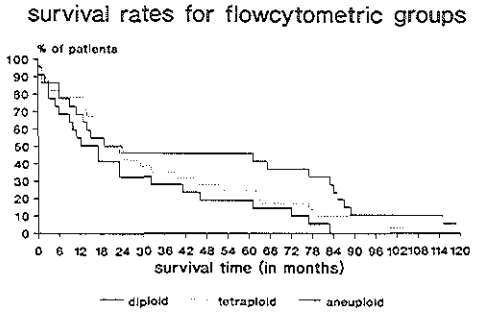

Figure 3. Survival rates for the three groups of patients according to the results of DNA flow cytometric study. No significant differences between survival times of any one of the three groups were found. The patients with aneuploid histograms tended to survive longer than those with euploid histograms.

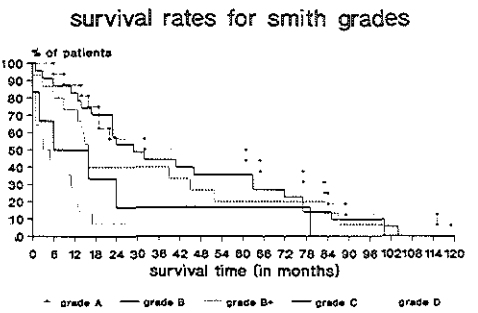

Figure 4. Survival rates for patients with tumors of different grades. Although all curves run separately, significant differences were found only between the curve of Grade D and those of the other grades.

on paraffin-embedded material proved to be a valuable tool in predicting survival rates.[22–24] In earlier studies on intracerebral tumors, and glial tumors in particular, nuclear count and nuclear area fraction seemed reliable prognostic parameters.[25–27] The rapid characterization of glial tumor cell populations by means of FCM facilitated objective quantification.[28] In a study by Coons *et al.*[12] on 32 astrocytomas, almost all patients with DNA aneuploid tumors had short survivals. Although in 67% and 71% of the cases the rapidly fatal course was predicted by histology or FCM, the combination of histopathologic grade with the flow cytogram improved the prognostic predictions.[12] In a study of 60 intracranial tumors, Lehmann and Krug[13] found a correlation between the degree of malignancy and DNA ploidy pattern.

In a study by De Reuck et al.[14] using cytophotometry on 200 cells of each sample of 11 cases of oligoden-

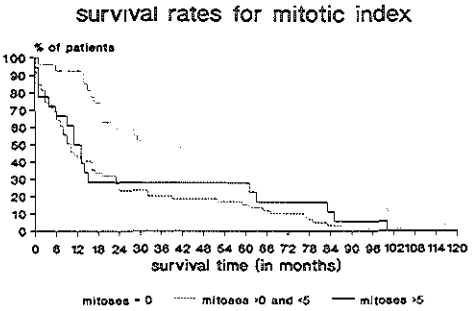

Figure 5. Survival rates of patients with tumors with different mitotic index. Patients with tumors without mitoses (per 3 high-power fields, ×40) had significantly better survival times than those in whom mitoses were found.

Table 3. Matrices Matching the Results of DNA Flow Cytometric Study, Histopathologic Grade, and Counting Mitoses

	Grades (according to Smith *et al.**)					
	A	*B*	*B+*	*C*	*D*	Total
Flow cytometric study						
Diploid	6	11	1	1	7	26
Tetraploid	3	12	11	5	2	33
Aneuploid	10	3	5	1	7	26
	19	26	17	7	16	85

	Mitoses			
	0	*1–5*	*> 5*	Total
Flow cytometric study				
Diploid	8	17	1	26
Tetraploid	8	19	6	33
Aneuploid	6	12	8	26
	22	48	15	85

	Grades (according to Smith *et al.**)					
	A	*B*	*B+*	*C*	*D*	Total
No. of mitoses						
0	12	8	2	0	0	22
1–5	7	15	7	7	12	48
> 5	0	3	8	0	4	15
	19	26	17	7	16	85

Table 4. Results of DNA Flow Cytometric Study and Grading in 20 Successive Biopsies

Sex/age (yr)	First biopsy	δT (mo)	RT	Second biopsy
F/59	T, grade B+*	4	+	D, grade D
M/53	D, grade B	12	+	D, grade D
M/6	T, grade B+	13	+	D, grade B+
M/40	T, grade B	13	+	D, grade B
F/41	A, grade A	15	+	T, grade C
M/20	D, grade A	17	+	T, grade B
M/27	T, grade B+	21	–	T, grade B
M/60	T, grade B	23	–	T, grade B+
M/45	A, grade A	24	–	T, grade B+
M/26	T, grade C	27	+	D, grade B
F/30	D, grade A	31	+	T, grade B+
F/37	A, grade A	36	+	A, grade B
F/31	D, grade B	37	–	T, grade B+
F/32	D, grade B	37	+	D, grade B
M/37	T, grade B	45	+	T, grade B
M/47	D, grade B	54	+	T, grade B
M/50	A, grade A	72	–	T, grade A
M/32	T, grade B	55	+	A, grade B
F/43	A, grade B+	79	+	T, grade B
M/33	D, grade B+	82	+	T, grade B+

δT: time interval between first and second operation; RT: radiation therapy; T: tetraploid DNA flow cytogram; +: treated with radiation therapy in addition to surgery; D: diploid DNA flow cytogram; A: aneuploid DNA flow cytogram; –: not treated with radiation therapy.

* Results of grading according to Smith *et al.**.

drogliomas, the DNA contents were correlated with the histopathology and follow-up. Among six anaplastic oligodendrogliomas only three were DNA aneuploid, whereas the other three had diploid patterns. Despite the small numbers, it was concluded that aneuploidy corresponded with a bad prognosis.[14] Because the DNA flow patterns of two oligodendrogliomas were homologous with those of benign intracranial tumors, whereas some anaplastic oligodendrogliomas showed an aneuploid pattern similar to that seen in glioblastomas, Frederiksen *et al.*[29] suggested a relation between the DNA flow cytograms and the histopathology of oligodendrogliomas. In addition, a correlation between the DNA histograms and histopathologic grading of 11 oligodendrogliomas was found by Spaar *et al.*[30] In the current study of 85 oligodendrogliomas, we were unable to confirm all of these results. However, the current findings are compatible with the findings of Jimenez *et al.*[31] and Mørk and Laerum,[32] although these authors also studied comparatively small numbers of oligodendroglial tumors.

In cytogenetic studies of gliomas, numerical deviations in a variety of chromosomes were found.[33,34] It was shown that some numerical chromosomal deviations in gliomas gave rise to the occurrence of DNA aneuploid (namely, near diploid or near tetraploid) DNA histograms.[33] Because in the current study of oligodendrogliomas the average survival times of patients with aneuploid tumors tended to be better than those with euploid tumors, a link between DNA aneuploidy and chromosomal aberrations, resulting in increased growth rates, is not easily established. In addition, it should be realized that near euploid patterns are easily missed in retrospective studies of paraffin-embedded material.

Although it is suggested in epithelial tumors[35] and in simulated biologic settings,[36] the concept of tumor evolution starting with a population of diploid cells from which a subclone of tetraploid cells arises, and

Table 5. Matrix Matching the Results of DNA Flow Cytometric Study of First and Second Biopsies

Flow cytometry results of second biopsy	Flow cytometry results of first biopsy		
	Diploid (%)	*Tetraploid (%)*	*Aneuploid (%)*
Diploid	8 (2 of 24)	17 (4 of 24)	0
Tetraploid	29 (7 of 24)	21 (5 of 24)	17 (4 of 24)
Aneuploid	0	4 (1 of 24)	4 (1 of 24)

Table 6. Matrix Matching the Grading Results of First and Second Biopsies

Grading results of second biopsy	Grading results of first biopsy				
	A (%)	*B (%)*	*B+ (%)*	*C (%)*	*D (%)*
A	4 (1 of 24)	0	0	0	0
B	8 (2 of 24)	29 (7 of 24)	8 (2 of 24)	4 (1 of 24)	0
B+	8 (2 of 24)	13 (3 of 24)	13 (3 of 24)	0	0
C	4 (1 of 24)	0	0	0	0
D	0	4 (1 of 24)	4 (1 of 24)	0	0

subsequently, because of chromosome loss, an aneuploid cell line emerges was not supported by our results of the DNA histograms of the first and second biopsies of the oligodendroglial tumors (Tables 4 and 5). Although sampling errors may be responsible for differences in flow cytograms of repeated biopsies,[28] it was demonstrated in the current study that most tumors have an equal or increased tumor grade in the second biopsy, whereas a decrease in grade, most likely caused by sampling errors, was seen in only 12% of the cases.

In studies in which the proliferation fraction in gliomas was assessed by means of tritiated thymidine, bromodeoxyuridine incorporation, or the monoclonal antibody Ki-67, a positive correlation between proliferating cells and the tumor grade or survival time was an invariable finding.[37–42] The results of bromodeoxyuridine incorporation studies correlated well with the estimated S-phase fractions that ranged from 2.3 to 13.7 in the DNA FCM.[40] However, S-phase fractions as low as 2.3 are below the detection level in DNA FCM studies on paraffin-embedded material, particularly in light of the considerable variation in coefficient of variation values.

From the current results it is concluded that DNA FCM has no prognostic relevance in oligodendrogliomas, so DNA FCM should not be added to, or replace, a grading system for these tumors. A mitotic index should be considered as part of the grading system.

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PAPER 4

"ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL SEGREGATION OF GEMISTOCYTIC SUBSETS

J.M. Kros, S.Z. Stefanko, A.A.W. de Jong, C.C.J. van Vroonhoven, R.O. van der Heul, Th.H. van der Kwast.

Hum. Pathol. 22: 33-40 (1991)

Ultrastructural and Immunohistochemical Segregation of Gemistocytic Subsets

JOHAN M. KROS, MD, STANISLAV Z. STEFANKO, MD, PHD,
ANTON A. W. DE JONG, CORNELIS C. J. VAN VROONHOVEN,
ROY O. VAN DER HEUL, MD, PHD, AND
THEODORUS H. VAN DER KWAST, MD, PHD

Gemistocytes are frequently encountered in cases of reactive gliosis as well as in glial tumors. Recently, miniature forms of gemistocytes (minigemistocytes) were recognized as cellular constituents of oligodendrogliomas. Antibodies specific for the intermediate filaments glial fibrillary acidic protein and vimentin are reactive with gemistocytic cells, but do not react specifically with these cells. In a study of 23 glial tumors we found the monoclonal antibody Pm43 selectively reactive with the classical gemistocytes as well as with the minigemistocytes. Nevertheless, at the ultrastructural level a striking difference in the arrangement of the glial filaments between both gemistocytic cell types was found. Immunoelectron microscopy showed that the reactivity for the newly discovered gemistocytic marker Pm43 was confined to identical intermediate filaments. Despite immunohistochemical homology, a clearly different ultrastructure divides classic gemistocytes and minigemistocytes into two subsets. HUM PATHOL 22:33–40. Copyright © 1991 by W.B. Saunders Company

In the beginning of this century the German histopathologists Franz Nissl and Alois Alzheimer described "fattened glial cells" with only a few short processes among a vast variety of pathologically changed glia.[1] They called these cells "gemästete Glia", a term which readily became "gemistocytes" in the English literature.[2] With their round or slightly oval outline, milky cytoplasm, and flattened eccentric nucleus, these cells were found in reactive processes in brain tissue as well as in glial neoplasms. Tumors almost exclusively consisting of gemistocytes were referred to as gemistocytomas.[3] Gemistocytes were never encountered in normal or developing glial tissue.

Several authors regarded gemistocytic cells as the smaller members of a family of large glial cells.[2,4-9] A division of these larger glial cells into three subtypes has been proposed based upon a morphometric analysis of the cell sizes and the sizes and shapes of the nuclei.[9] Recently, the term "minigemistocytes" was introduced for glial fibrillary acidic protein-(GFAP) positive cells resembling small gemistocytes in oligodendroglial tumors.[10,11]

Several markers, including anti-GFAP and anti-vimentin, are known to react with classical gemistocytes,[12,13] but not specifically. In preliminary studies Pm43, an antibody that reacts with a component of myelin,[14] reacted specifically with gemistocytic cells in gliomas.

Although the electron microscopic features of classical gemistocytes have been described,[15-20] the ultrastructure of the minigemistocytes has not yet been reported. Therefore, we made an immunohistochemical as well as an electron microscopic comparison between the classical gemistocytes and the newly recognized minigemistocytes. In addition, we used immunoelectron microscopy to visualize reactivity for anti-GFAP, anti-vimentin, and anti-Pm43 antibody at the ultrastructural level.

MATERIALS AND METHODS

Patients

Biopsy material from 23 patients with a glioma of the brain and from three patients with reactive gliosis due to hypoxia without tumor was processed for routine histology, immunohistochemistry, electron microscopy, or immunoelectron microscopy. The patient group consisted of 13 males and 10 females. Their ages ranged from 8 to 68 years. The mean age was 45 years. A predominantly frontal localization of the tumors was found. In six cases a relapse of the tumor after previous operation was seen. Four of these patients had been treated with irradiation as well.

Histology and Immunohistochemistry

Freshly obtained surgical specimens were routinely processed for paraffin embedding after fixation in buffered formalin, and small samples were rapidly frozen in chilled isopentane and stored in liquid nitrogen until further use. Immunohistochemistry was done on paraffin-embedded material or on acetone-fixed frozen sections of 5-μ thickness. Consecutive hematoxylin-azophloxin–stained slides were made as well.

As in the original study by Van Dijk et al,[14] Pm43 was found to be selectively reactive with the myelin of the peripheral but not the central nervous system in formalin-fixed tissues. Furthermore, only melanocytes were weakly positive, whereas the epithelial, muscular, and mesenchymal components of other tissues did not show reactivity with this antibody.[14] Primary antibodies included rabbit anti-GFAP antiserum (Dako Corporation, Copenhagen, Denmark), diluted 1:60 in phosphate-buffered saline pH 7.4 (PBS), rabbit anti-vimentin antiserum (Diagnostic Product Corporation, Witney, UK), and mouse monoclonal anti-Pm43 antibody (ascites diluted 1:2,000 or 1:4,000 in PBS). For detection of reactivity with the primary rabbit anti-

From the Department of Pathology, Academic Hospital Rotterdam-Dijkzigt, Erasmus University Rotterdam, Rotterdam, The Netherlands. Accepted for publication March 27, 1990.

Key words: gemistocytes, minigemistocytes, Pm43, immunohistochemistry, immunoelectron microscopy.

Address correspondence and reprint requests to Johan M. Kros, MD, Department of Pathology, Academic Hospital Rotterdam-Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

0046-8177/91/2201-0006$5.00/0

bodies, the two-step indirect immunoperoxidase technique was used on deparaffinized sections preincubated with 10% normal swine serum diluted in PBS. As a second step antibody swine-anti-rabbit immunoglobulin (Ig) antiserum conjugated to horse-radish peroxidase (Dako Corporation) diluted 1:50 in PBS was used. For detection of reacted monoclonal Pm43 antibody, rabbit anti-mouse Ig antiserum conjugated to horseradish peroxidase (Dako Corporation) diluted 1:100 in PBS containing 1% of nonimmune rabbit and human serum was used. Alternatively, the avidin-biotin-peroxidase complex method was used to enhance immunostaining for Pm43. Incubation with the biotinylated rabbit anti-mouse Ig (Dako Corporation) diluted 1:400 in PBS was followed, after washing, by a 30-minute incubation with avidin and biotinylated peroxidase complexes (Dako Corporation). In control slides the primary antibody was replaced by PBS. All incubations were performed at 37°C in a humidified chamber for 30 minutes. Prior to incubations, endogenous peroxidase activity was blocked by treatment with 2% hydrogen peroxide (H_2O_2) in methanol. Rinsing excess antibodies or conjugates was done by three washes of 5 minutes' duration in PBS. Final visualization was achieved by incubation with 0.02% diaminobenzidine in PBS and 0.075% H_2O_2 for 7 minutes in darkness. After washing with aquadest the slides were slightly stained with hematoxylin in order to recognize the cellular morphology.

Electron Microscopy

For electron microscopy, freshly obtained tumor material from five cases of tumor with oligodendroglial as well as astrocytic parts (mixed oligodendrogliomas-astrocytomas) was minced into 1-mm^3 cubes and immediately fixed in 1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) at 4°C. The specimens were fixed for 24 hours, transferred and stored in 0.1 mol/L phosphate buffer for 8 hours, and post-fixed in 1% OsO_4 in 0.1 mol/L phosphate buffer (pH 7.2) for 12 hours at 4°C. Subsequently, the specimens were rinsed in the same buffer, ethanol-dehydrated, and Epon-embedded for routine transmission electron microscopy. After ultrathin cutting, the sections were collected on mesh 100 copper grids and counterstained with uranyl acetate and lead citrate. Transmission micrographs were made on a Zeiss 902 transmission electron microscope at 70 kV.

Immunoelectron Microscopy

For postembedding immunoelectron microscopy, small 1-mm^3 tissue cubes of four cases of mixed oligodendrogliomas-astrocytomas containing minigemistocytes, and one tumor consisting almost entirely of minigemistocytes (Figs 1B and 2) were fixed in 0.1 mol/L phosphate buffer

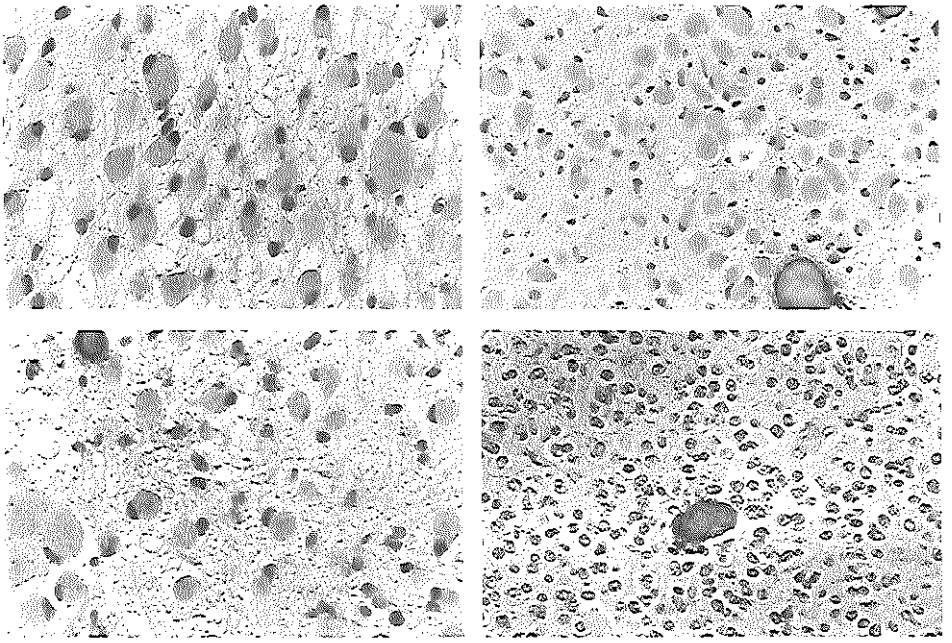

FIGURE 1. (A) Pure gemistocytoma, stained for Pm43. All parts of this glioma showed classic gemistocytes, although some smaller glial cells were seen as well. (Magnification × 500.) (B) Minigemistocytoma, stained for Pm43. This tumor consisted almost exclusively of uniform cells with the morphology of small gemistocytes. (Magnification × 500.) (C) Astrocytoma, stained for Pm43. Tumor with gemistocytes intermingled with minigemistocytes. In several tumors these two cell types were seen together. (Magnification × 500.) (D) Oligodendroglioma (with GFAP-positive oligodendroglial cells), stained for Pm43. The oligodendroglial tumor cells are, irrespective of their reactivity with anti-GFAP, not reactive with anti-Pm43. (Magnification × 500.)

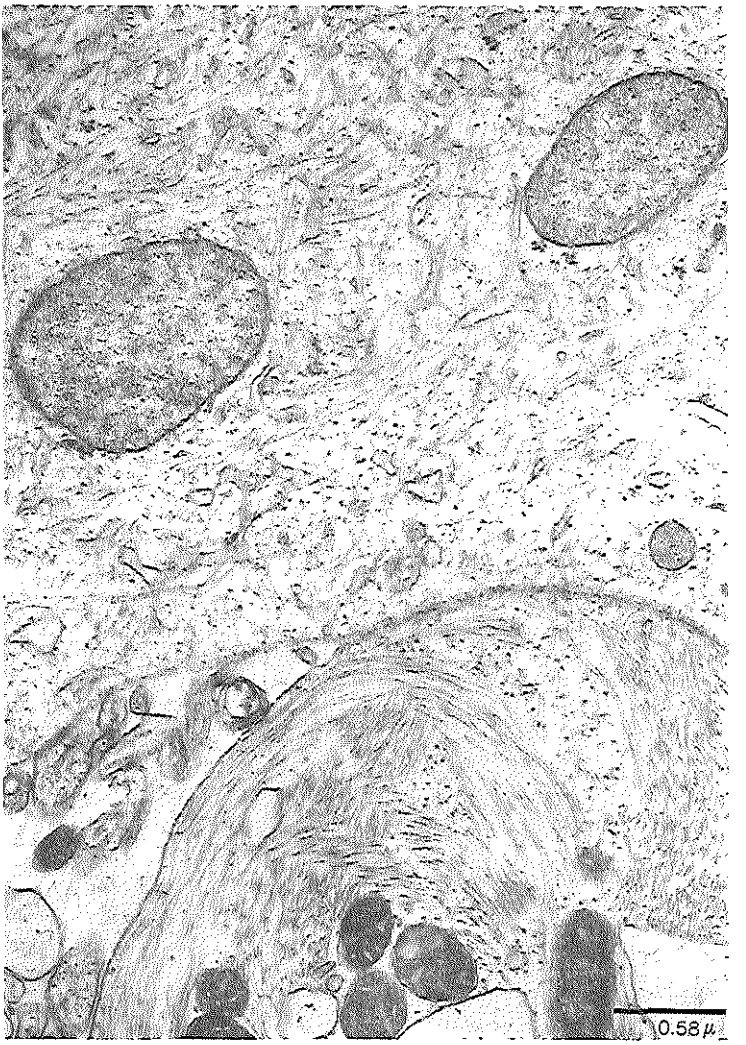

FIGURE 2. Gemistocyte and minigemistocyte in one tumor. Classic gemistocyte with interspersed filaments with adjacent minigemistocyte. The latter is readily recognized by smaller sizes and characteristic bundles of filaments, in contrast to the dispersed arrangement of the filaments in the large classic gemistocyte. (Transmission electronmicroscopy; magnification × 12,000.)

(pH 7.2) containing 1% acroleine and 0.4% glutaraldehyde at 4°C for 4 hours. Tissues were transferred and stored in a sucrose buffer of 1 mol/L sucrose in 1.1 mol/L phosphate buffer pH 7.2 with 1% paraformaldehyde at 4°C until further processing for lowicryl embedding as described previously.[22] From lowicryl-embedded material ultrathin sections were made with glass knives, and the sections were collected on carbon-coated Formvar-filmed mesh 100 copper grids. The immunologic methods for visualization of rabbit anti-GFAP, rabbit anti-vimentin, and mouse anti-Pm43 antibodies were essentially as described previously.[22,23] As a second step these reagents were used: a 10-nm colloidal gold-labeled goat anti-rabbit antiserum (GAR-10; Janssen, Beerse, Belgium) and a goat anti-mouse antiserum (GAM-10, Janssen). The incubation with colloidal gold-coupled antibodies was accelerated by microwave irradiation.[24] Control sections were incubated with PBS, normal rabbit serum diluted 1:60 in PBS, or the appropriate dilution of a similar monoclonal antibody nonreactive with glial tissue (ie, VIT-3, obtained from Dr W. Knapp, Vienna, Austria) instead of anti-GFAP or anti-vimentin, or anti-Pm43. Background staining was always negligible.

RESULTS

Histology and Immunohistochemistry

In the cases of reactive gliosis, gemistocytes were found, but GFAP-positive oligodendroglial cells (GFOCs) or minigemistocytes were absent. All gemistocytes showed expression of Pm43 as well as of GFAP and vimentin. The marker Pm43 yielded a homogenous staining of the cytoplasm.

With respect to the gliomas, seven biopsies were composed mainly of oligodendroglial tumor cells but occasionally contained GFOCs, minigemistocytes, or gemistocytes. In 11 tumors a mixture of neoplastic oligodendroglial and astroglial cells was encountered; eight of these tumors were variably mixed with GFOCs, minigemistocytes, and gemistocytes. Often seen in association with minigemistocytes were GFOCs. Five tumors were predominantly astroglial mixed with gemistocytic elements.

In all tumors gemistocytes and minigemistocytes were selectively reactive with anti-Pm43 in acetone-fixed cryostat as well as in formalin-fixed paraffin sections (Fig 1 A, B, and C, Tables 1 and 2). The marker Pm43 was seen homogenously in the cytoplasm. The GFOCs did not show reactivity with Pm43 (Fig 1D, Table 2). The few giant cells showed minimal reactivity for Pm43, but no reactivity at all was found in the monstrous* cells.

Gemistocytic cells as well as giant and monstrous cells were positive for vimentin, while minigemistocytes displayed less reactivity (Table 2). The gemistocytes as well as the minigemistocytes were positive for GFAP, whereas the giant cells showed minimal reactivity and the scarcely present monstrous cells showed no reactivity at all.

Electron Microscopy and Immunoelectron Microscopy

In transmission electron microscopy the classic gemistocytic cells were readily identified by their voluminous cell bodies, eccentric nuclei, short cytoplasmic processes, and a characteristic meshwork of short, dispersed, nonfasciculated intermediate filaments (with a diameter of approximately 10 nm) in the cytoplasm (Figs 2 and 3, A and B). However, the minigemistocytes showed filaments arranged in large, more or less parallel interwoven bundles (Figs 2, 3C, and 4), similar to the arrangement in astrocytic cells (Fig 5). No cells were found with an intermediate phenotype, ie, the arrangement of the intermediate filaments as seen in both gemistocyte subsets.

Immunoelectron microscopy with anti-vimentin and anti-GFAP antibody demonstrated intense immunogold labeling of the intermediate filaments in the gemistocytic cells (Fig 3, B and C). Furthermore, it was shown that in classic gemistocytes as well as in minigemistocytes the antigenic determinant reactive with the anti-Pm43 antibody was associated with the intermediate filaments (Figs 3A and 4, A and B). The antibody Pm43 did not stain other cellular structures or organelles.

Immunogold labeling was most intense after reaction with anti-GFAP antibody both in gemistocytes and in minigemistocytes (Fig 3, B and C).

* Monstrous cells: large cells with bizarre, irregular, and hyperchromatic multinuclei.

TABLE 1. Reactivity of the Monoclonal Antibody Pm43

Frozen Section	Formalin-fixed	Acetone-fixed
Central nervous system myelin	−	+
Peripheral nervous system myelin	+	+
Melanocytes	±	±
Gemistocytes	+	+
Minigemistocytes	+	+

Symbols: +, positive; −, negative; ±, weakly positive.

DISCUSSION

In paraffin-embedded glial neoplasms the antibody Pm43 was selectively reactive with gemistocytes and minigemistocytes, an observation that makes this antibody a valuable tool in the study of glial differentiation in gliomas. The selective expression of Pm43 both in classic gemistocytes and in minigemistocytes suggests a relationship between these cell types. The Pm43 reactive determinant in the gemistocytic cells may be one of the proteins that was isolated from the peripheral nervous system myelin in the study of Van Dijk et al (ie, a 43-kd protein and two proteins between 25 and 30 kd).[14] The expression of a Pm43-defined myelin-associated protein by minigemistocytes is in line with the suggested oligodendroglial lineage of these cells.[10,11] On the contrary, the typical structure of parallel bundles of intermediate filaments in these cells suggests an astroglial origin of these cells.

The intense immunostaining with anti-vimentin antibody in gemistocytic cells that was found in the present study affirms the results of earlier studies.[25,26] Vimentin is temporarily expressed by cells of

TABLE 2. Immunohistochemical Profile of the Different Cellular Constituents of the Gliomas

	GFAP	Pm43	Vimentin
Fibrillary astrocyte	+	−	−
Pilocytic astrocyte	+	−	−
Protoplasmic astrocyte	+	−	−
Gemistocyte	+	+	+
Giant cell*	±	−	+
Monstrous cell†	−	−	+
Minigemistocyte	+	+	±
GFOC‡	+	−	−
Oligodendrocyte	−	−	−

Symbols: +, positive; −, negative; ±, weakly positive.

* Defined as large cells with a single nucleus or multiple nuclei.

† Defined as large cells with bizarre, irregular, and hyperchromatic multinuclei.

‡ Defined as a GFAP-positive cell with the morphology of an oligodendrocyte.

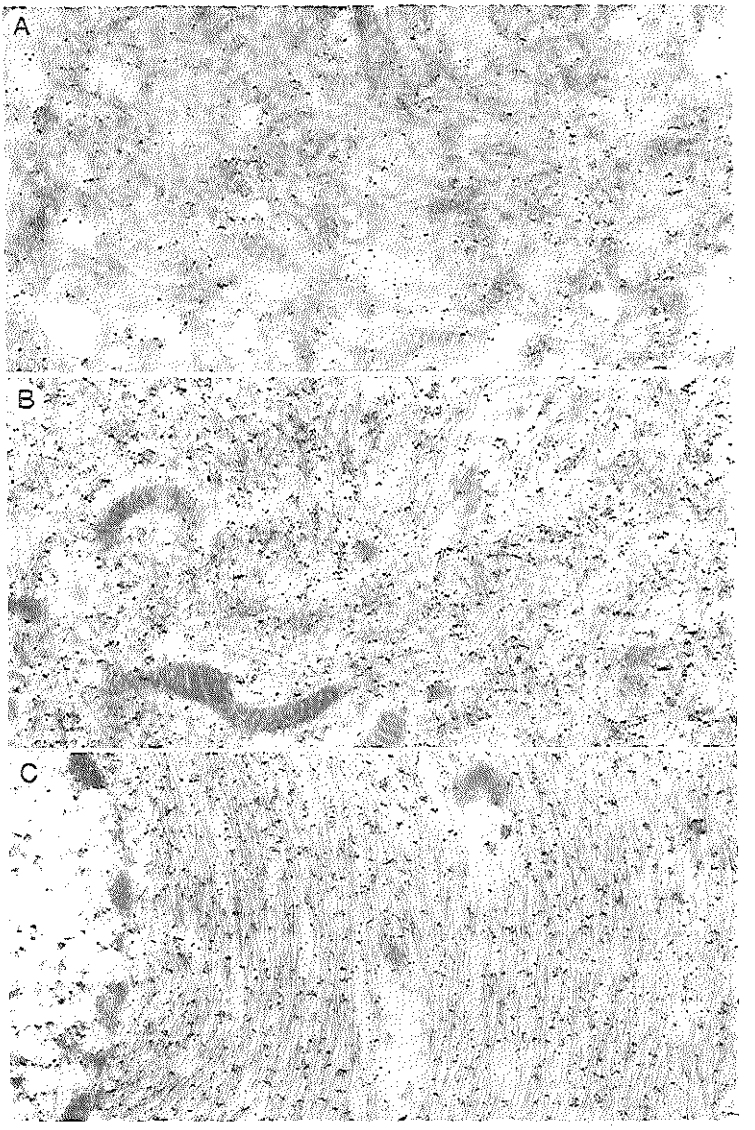

FIGURE 3. Immunoelectron microscopy of labeled filaments in a gemistocyte and a minigemistocyte. (A) Gemistocyte (detail), stained for Pm43. Characteristic dispersed arranged filaments, apparently without organization. Scarce immunogold label for Pm43 in this cell is associated with these filaments, while the organelles are free of label. Key: M, mitochondria; L, lysosome. (Magnification × 12,000.) (B) Gemistocyte (detail), stained for GFAP. Immunogold label for GFAP is associated with the filaments comparable with labeling for anti-Pm43. However, more immunogold label is seen here. (Magnification × 12,000.) (C) Minigemistocyte (detail), stained for GFAP. The characteristic parallel filaments are clearly shown. Also, in the minigemistocyte more immunogold label is found for GFAP in comparison with Pm43 (compare Fig 2). (Magnification × 12,000.)

various localizations in the developing brains of mice as well as of humans.[27,28] Later on, during human embryogenesis, the expression of vimentin disappears and expression of GFAP is found.[29] However, in some astrocytes of the adult rat brain a clear coexpression of vimentin and GFAP has been discovered.[30] In man, expression of vimentin rapidly reoccurs in normal glial cells during in vitro culture, and is found in glial tumors and cultures of neoplastic glial cells.[25,26,31-33] The abundant expression of vimentin in minigemistocytes and in the classic ballooned gemistocytic cells could very well be a consequence

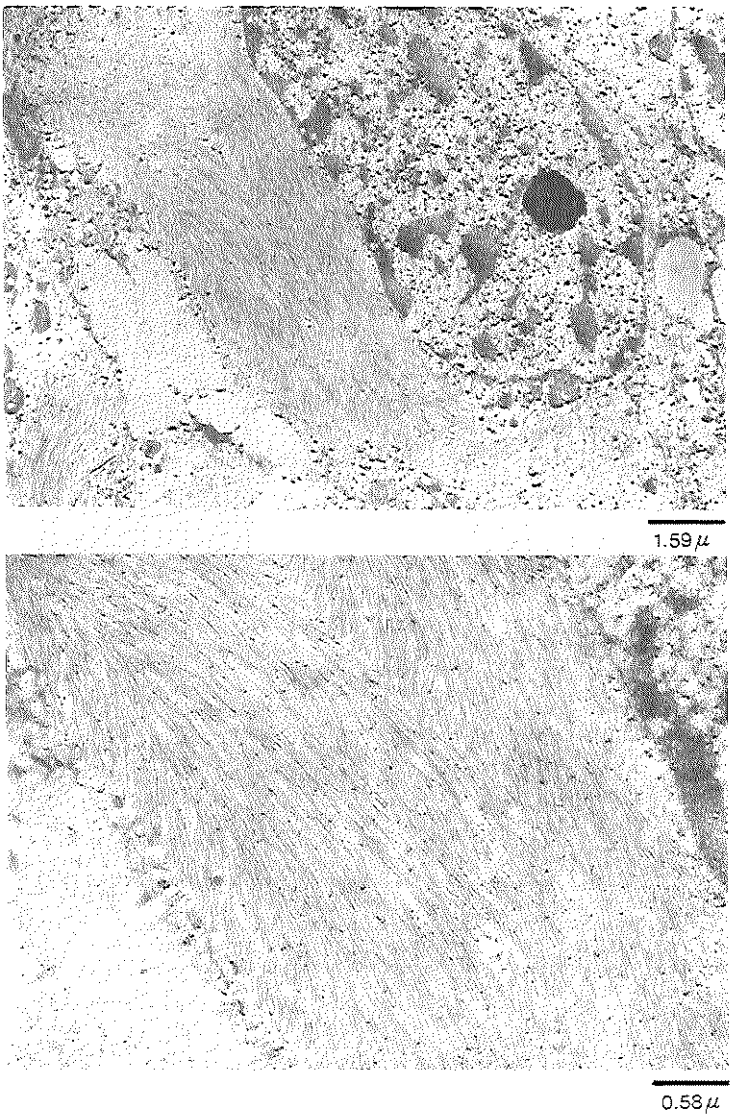

FIGURE 4. Immunoelectron microscopic picture of a minigemistocyte labeled with anti-Pm43. (A) Ultrastructure of a minigemistocyte. Note the oval eccentric nucleus and few organelles. The most prominent feature is the abundant intermediate filaments arranged in interwoven bundles. (Magnification × 4,400.) (B) Detail of A. Interwoven bundles of filaments. Immunogold label is associated with these filaments. (Magnification × 12,000.)

of adaptation to altered metabolic circumstances in vivo. The existence of heteropolymers with varying proportions of both intermediate filaments according to differentiation of the cells of origin represents an attractive hypothesis for the coexpression of GFAP and vimentin within mammalian nervous tissue as well as in human glioma cell lines.[34,35] This phenomenon of heteropolymerization offers an explanation for our immunoelectron microscopic findings of the coincidental expression of GFAP and vimentin in association with the same filaments in the gemistocytic cells.

The arrangement in parallel bundles of the glial filaments in the minigemistocytes differs essentially

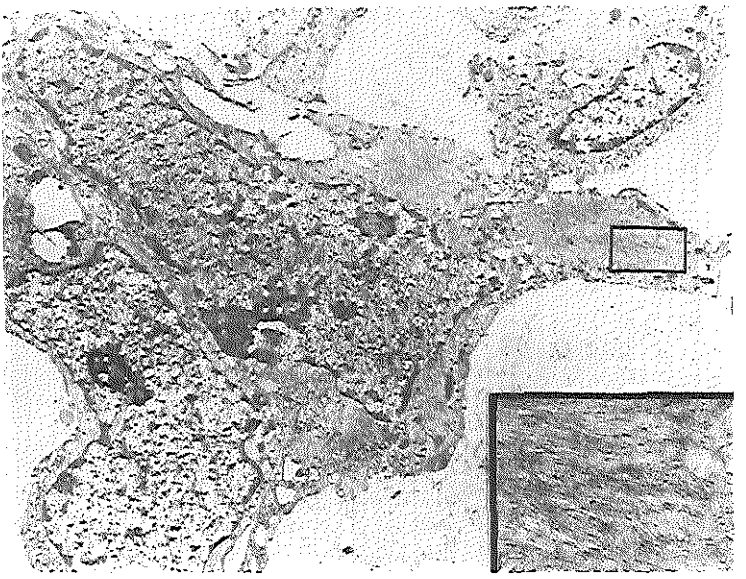

FIGURE 5. Immunoelectron microscopy of a neoplastic fibrillar astrocyte stained for Pm43. The neoplastic astrocyte is readily recognized by its plump cell processes. (Magnification × 3,000.) (Inset) Parallel bundles of intermediate filaments which are not labeled by the Pm43 antibody, except for some background immunogold label. (Magnification × 12,000.)

from the characteristic dispersed arrangement of filaments in the classic gemistocytic cells known from the literature.[15-19] Although minigemistocytes do appear together with gemistocytes within one tumor (Figs 1C and 2), suggesting a possible ontogenic relation between these two cell types, we did not observe intermediate phenotypes at the ultrastructural level (ie, cells with filaments in a dispersed pattern in the cytoplasm alternating with an arrangement in orderly bundles). In 1966, Raimondi suggested that the ballooned aspect of classic gemistocytes could be the result of realignment of cytoskeletal filaments beneath the cell membrane in astroglial cells.[6] In 1982, Meneses et al raised the possibility of a process of gradual increase in volume of the cytoplasm of neoplastic oligodendroglial cells, transforming these cells into miniature forms of gemistocytes.[10] However, the arrangement in parallel bundles of intermediate filaments in minigemistocytes resembling the ultrastructure of astrocytes (Figs 2, 4, and 5) suggests that minigemistocytes are more closely related to astroglial tumor cells than to oligodendroglial tumor cells. The mechanism of, and trigger for, a possible metamorphosis of any glial cell into a gemistocyte or into a miniature form of gemistocyte (a minigemistocyte) remain obscure. In one of the few studies focusing on gemistocytes, Hoshino et al evaluated the incorporation of tritiated thymidin (3H-T) in some high-grade astroglial tumors, and concluded that gemistocytes are not the aggressively proliferating tumor component.[7] These authors suggested that glial tumor cells take the gemistocytic phenotype after having lost the competition for nutrients with smaller tumor cells. However, this theory does not explain the existence of glial tumors consisting almost exclusively of gemistocytes: the pure gemistocytomas.

In conclusion, we found that the anti-Pm43 antibody is selectively reactive with gemistocytes and minigemistocytes. At the ultrastructural level anti-GFAP, anti-vimentin, and anti-Pm43 show an identical immunogold labeling pattern associated with intermediate filaments, although differences in staining intensity exist. No reactivity of anti-Pm43 was found in either non-gemistocytic cells or giantocellular cells. The ultrastructure of minigemistocytes differs from that of classical gemistocytes, but resembles that of astrocytic cells. Future studies should clarify the ontogeny of the different gemistocytic cells and their relevance for the biologic behavior of glial tumors.

Acknowledgment. The authors thank M. Waayer-van Batenburg for technical assistance and P. C. Delfos for assistance with the photography. The Department of Neurosurgery of the Academic Hospital Rotterdam-Dijkzigt is acknowledged for its cooperation in sampling the tumor material.

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PAPER 5

"PROGNOSTIC IMPLICATIONS OF GLIAL FIBRILLARY ACIDIC PROTEIN CONTAINING CELL TYPES IN OLIGODENDRO-GLIOMAS".

J.M. Kros, C.G. van Eden, S.Z. Stefanko, M. Waaijer-van Batenburg, Th.H. van der Kwast.

Cancer 66: 1204-1212 (1990)

Reprinted from CANCER, Vol. 66, No. 6, September 15, 1990.

Prognostic Implications of Glial Fibrillary Acidic Protein Containing Cell Types in Oligodendrogliomas

J. M. Kros, MD,* C. G. Van Eden, PhD,† S. Z. Stefanko, MD, PhD,*
M. Waayer-Van Batenburg,* and Th. H. van der Kwast, MD, PhD*

In oligodendroglial tumors the intermediate filament glial fibrillary acidic protein (GFAP) may be expressed by cells with the morphologic characteristics of typical oligodendrocytes (gliofibrillary oligodendrocytes [GFOC]) and by miniature forms of gemistocytes (minigemistocytes) as well. These latter cell types have been regarded as transitional cells that represent intermediate forms between an oligodendroglial and an astrocytic phenotype. Furthermore, in oligodendrogliomas GFAP may be expressed by intermingled classic large gemistocytes, which are not considered transitional cells. In a retrospective study of 111 oligodendrogliomas, the presence of the various GFAP-positive cell types was correlated with the survival rates of the patients. Therefore, GFAP expression was visualized with the use of an indirect conjugated peroxidase method. The survival times of the patients were recorded and statistical comparisons were made. The percentage of GFAP-positive tumor cells is increased in oligodendrogliomas of 28 patients who underwent a second biopsy (all these patients had been treated with radiation therapy as well). It was found that neither the presence of GFOC nor that of minigemistocytes is predictive of the survival. In contrast, patients with classic gemistocytes had survival lengths approximately twice as short as those of patients who did not have these cells in their tumors. No clear correlation was found between tumor grading or any of the individual histopathologic features with the presence of the various GFAP-positive cell types. The ominous sign of the presence of gemistocytes in oligodendrogliomas confirms some earlier reports about the prognostic significance of this cell type in astrocytomas. *Cancer* 66:1204–1212, 1990.

SINCE the isolation and purification of the intermediate filament protein glial fibrillary acidic protein (GFAP),[1,2] the expression of this protein has been studied extensively in a variety of normal and pathologically changed tissues.[3,4] Although the expression of GFAP has been described in some nonglial cells, GFAP is primarily found in normal and developing glia and in gliomas.[5–13] Because, on the one hand, GFAP positivity seemed to decrease with increasing malignancy grade of the astrocytomas, whereas on the other hand clear GFAP positivity was found in astrocytomas of the highest malignancy degree (*i.e.*, the glioblastomas), expression of GFAP in astrocytomas has not been considered a reliable prognostic parameter.[5,7,8,11,12]

Soon it became clear that oligodendroglial tumors also could express GFAP.[5,11,13–17] Oligodendroglioma cells with the morphologic characteristics of small gemistocytes and even classic oligodendroglial cells expressed GFAP, in addition to entrapped nonneoplastic astrocytes.[13,14,18] Abundant GFAP expression was also found in scattered classic gemistocytes. Van der Meulen *et al.* found a reverse relation between the malignancy degree of the oligodendrogliomas and the expression of GFAP: the low-grade tumors did not show any positivity, whereas GFAP was

From the *Academic Hospital Rotterdam-Dijkzigt, Department of Clinical Pathology/Neuropathology, Rotterdam; and the †Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

The authors thank Prof. Dr. C. J. J. Avezaat for providing the neurosurgical records, Mr. C. C. J. van Vroonhoven for technical assistance, and Mrs. P. Delfos for photography.

Address for reprints: J. M. Kros, MD, Academic Hospital Rotterdam-Dijkzigt, Department of Clinical Pathology/Neuropathology, Dr. Molewaterplein 40-30 15 GD Rotterdam, The Netherlands.

Accepted for publication January 16, 1990.

exclusively expressed in oligodendrogliomas with a higher malignancy grade.[13] This finding was not confirmed in later studies, however.[11,14,19]

In 1982 Meneses *et al.* termed the GFAP immunoreactive cells with morphologic characteristics of miniature gemistocytes "minigemistocytes."[18] The GFAP-positive cells with the typical morphologic characteristics of oligodendrocytes were called "gliofibrillary oligodendrocytes" (GFOC).[18] Although GFOC can only be recognized after immunostaining for GFAP, minigemistocytes are readily recognizable in hematoxylin and eosin–stained sections. Both cell types have been supposed to represent different stages of the same lineage.[18] Gliomas that consist of GFOC or minigemistocytes were called "gliofibrillary oligodendrogliomas" or "minigemistocytomas," respectively, whereas the term "transitional cell type tumor" was introduced for the gliofibrillary oligodendrogliomas as well as for the minigemistocytomas.[14]

No conclusive data with respect to the biologic behavior of the transitional tumors in comparison with that of oligodendrogliomas lacking GFAP-positive tumor cells exist in the literature. Furthermore, grading criteria of oligodendrogliomas are still a matter of dispute,[20,21] although recently some histopathologic features with independent significance for survival rates have been identified.[20,22,23]

The aim of the current study was to identify possible significance of the GFAP-positive cells for the survival rates in oligodendrogliomas and to establish their relationship to the histopathologic features. Therefore, all GFAP-positive cell types in a group of histopathologically verified oligodendrogliomas were recorded. The survival times of patients with oligodendrogliomas variably containing GFOC, minigemistocytes, or classic gemistocytes were compared with the survival times of patients with oligodendrogliomas lacking these GFAP-positive tumor cells. The tumors were graded according to a modified grading system of Kernohan *et al.*[24] Relevant histopathologic features were used to grade the tumors.[20,22,23] The presence of the various types of GFAP-positive cells was correlated with the individual histopathologic features as well as the tumor grades.

Materials and Methods

Clinical Records

The clinical data and the histologic material of 137 patients with a cerebral oligodendroglioma were acquired from the files of the Academic Hospital Rotterdam-Dijkzigt. In 111 cases the diagnosis was confirmed and accurate follow-up was obtained. All patients had died before the end of this study. The patients had been admitted to the hospital between the years 1972 and 1986. The group was composed of 69 men and 42 women. The ages of the 111 patients at first operation are given in Figure 1. The slightly biphasic age distribution is characteristic for oligodendrogliomas.[20–22] At the time of the first operation, five patients were younger than 16 years. The localization frequencies of the tumors are shown in Table 1. The pre-

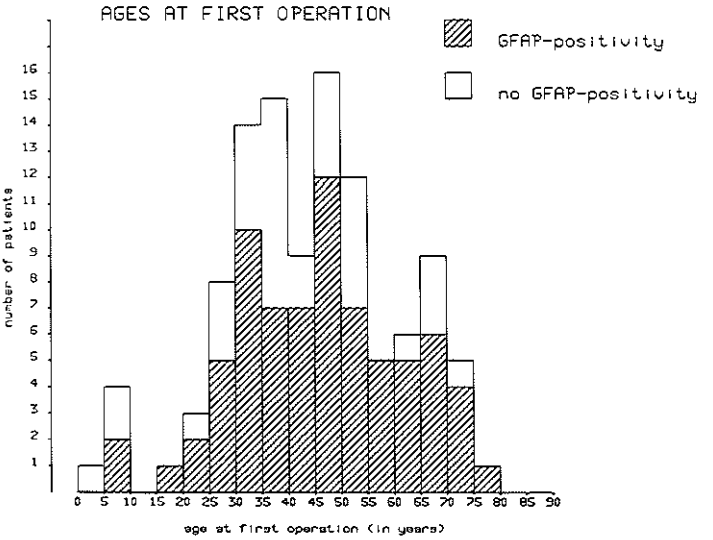


FIG. 1. Age distribution at time of first operation. Distribution for the ages at first operation. The shaded bars represent the patients with GFAP-positive oligodendrogliomas. The age distribution of these patients is congruent with that of all oligodendrogliomas together. The distribution shows a characteristic biphasic curve, with a peak incidence around the age of 35 and a second one around the age of 55.

TABLE 1. Localizations of the Oligodendrogliomas

	Left (%)	Left and right (%)	Right (%)
Frontal	14	4	14
Frontoparietal	1		6
Parietal	5		4
Parietotemporal	7		6
Temporal	5		5
Frontotemporal	5	1	6
Temporooccipital			
Occipital			
Parietooccipital	3		1
Parietotemporooccipital	3		
Frontoparietotemporal	3		3
Frontotemporooccipital			1
Third ventricle/thalamus	3		
Suprasellar		1	
Multifocal		1	

A predominance in frontal localization confirms earlier reports on this tumor.

dominant frontal localization is in line with most studies on oligodendrogliomas.[20–23] All patients had undergone craniotomy with the intention to debulk or decompress the brain. Twenty-eight patients had undergone craniotomy a second time, and four patients even a third time. In 64 cases (58%) the patients had been treated with radiation therapy subsequent to the first craniotomy. It was not possible to obtain patient selection criteria for treatment with radiation therapy, and no detailed data about application forms or quantity of radiation could be obtained.

The survival times were calculated from the date of first operation. The time from the first operation until the time of death was taken as survival time. Patients who died within 2 weeks after operation were excluded from this study (operation death).

Histopathologic Material and Immunohistochemistry

Of all paraffin-embedded material used for this study, new sections (5 μm) were made and stained with hematoxylin and eosin. The diagnosis oligodendroglioma was verified, whereas mixed oligoastrocytomas were excluded from this study because in these tumors neoplastic astrocytes are mainly responsible for GFAP expression. The histopathologic features were listed and scored, while two observers (J.M.K. and S.Z.S.) were ignorant of the relevant clinical data of the patients. Adjacent slides were processed for GFAP immunostaining. The morphologic characteristics of GFAP immunoreactive cells was not only identified by the counterstaining, but also by comparison with the adjacent hematoxylin-eosin-stained slide.

Immunohistochemistry study was done on 5-μm-thick sections of the paraffin-embedded material. Before the incubations, endogenous peroxidase activity was blocked by treatment with 2% hydrogen peroxide in methanol. The two-step indirect immunoperoxidase technique was used on deparaffinized sections preincubated with 10% normal swine serum diluted in phosphate-buffered saline (PBS). The primary antibody was rabbit anti-GFAP antiserum (DAKO, Denmark), diluted 1:60 in PBS, pH 7.4. Rinsing of the excess antibodies or conjugates was done by three 5-minutes washes in PBS. As the second step, antibody swine-anti-rabbit immunoglobulin (Ig) antiserum conjugated to horseradish peroxidase (DAKO, Denmark) diluted 1:50 in PBS was used. The incubations were performed at 37°C in a humidified chamber for 30 minutes. Final visualization was achieved by incubation with 0.02% diaminobenzidine (DAB) in PBS and 0.075% H_2O_2 for 7 minutes in darkness. Control slides, in which the primary antibody was replaced by PBS, always had negative results. The slides were slightly counterstained with hematoxylin to determine the cellular morphologic characteristics.

Grading of the Oligodendrogliomas

The oligodendrogliomas were graded according to a revised grading system modified from Kernohan's grading system for gliomas.[20,22–24] Thus, the histopathologic parameters necrosis, cell density, pleomorphism, microcysts, subpial tumor infiltration, and mitotic activity were used. Four grades were attributed according to the results of scoring the histopathologic features (Table 2).

Statistics

All tests were performed by means of the Statistical Package for the Social Sciences (SPSSX package).

Student's *t* test was applied to compare the survival times of patients with oligodendrogliomas with and without GFAP-positive cells. To trace significant differences between the survival times of the respective histopathologic grades, analysis of variance (ANOVA) was used for main effects. *A posteriori* testing was performed with the use of the Student-Newman-Keuls test, to elucidate dif-

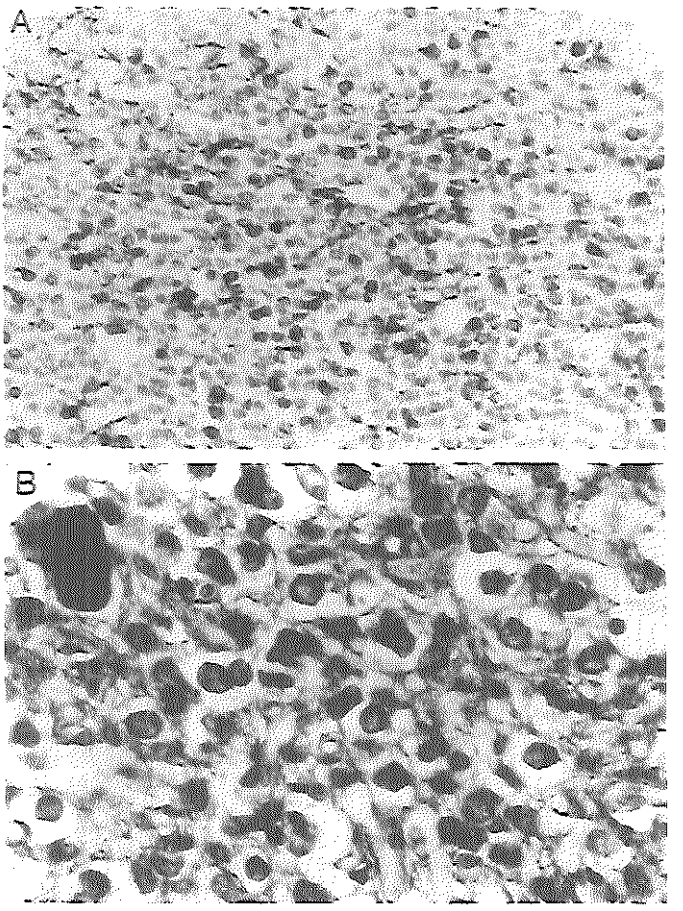
TABLE 2. Grading Scheme Modified From Kernohan's Grading Scheme for the Oligodendrogliomas

	1	2	3	4
Necrosis	–	–	–	+
Cell density	+	++	+++	+++
Pleomorphism	–	–	+	++
Microcysts	–	–	+	+
Mitoses*†	1–3	1–3	2–5	5 or more
Subpial infiltration*	–	–	–	+

A grading scheme derived from that of Kernohan for the gliomas was used. The features necrosis, cell density, pleomorphism, and microcysts were found as independent significant factors for the prognosis, whereas the presence of mitoses and subpial infiltration was of dubious significance.

* No conclusive data about significance available.

† Per 10 400× fields.



FIGS. 2A AND 2B. (A) Oligodendroglioma (Grade 2) with gliofibrillary oligodendrocytes (GFOC). The classic honeycomb appearance of an oligodendroglioma is readily recognized. The GFOC are dispersed between the GFAP-negative cells. In routine hematoxylin-eosin staining these cells are indistinguishable from their GFAP-negative counterparts (stained for GFAP, ×230). (B) Oligodendroglioma (Grade 2) with gliofibrillary oligodendrocytes (GFOC). Except for a dark ring of peroxidase positivity around the nucleus, these cells are morphologically identical to the GFAP-negative oligodendroglial tumor cells. Calcifications as seen in the upper left corner are frequently found in oligodendrogliomas (stained for GFAP, ×500).

ferences between individual grades. Because the frequency distribution of the survival times of the patients was an exponential one, a log-e transformation was done before application of the parametric tests.

To analyze the influence of the presence of either one of the GFAP-positive cells on survival, the survival times were divided into two groups containing long and short survival times. The chi-square test was applied to determine the effects of the presence of the different GFAP-positive cell types on the survival times.

Results

Glial fibrillary acidic protein positivity was found in morphologically different cells, *i.e.*, in classic oligodendrocyte-like cells (gliofibrillary oligodendrocytes or GFOC) (Figs. 2A and 2B), in cells with the morphologic characteristics of small gemistocytes (minigemistocytes) (Fig. 3), and in gemistocytic astrocytes (Fig. 4), as well as in scattered fibrillary and protoplasmic astrocytes. At first biopsy GFAP immunoreactive tumor cells were found in 68% of the tumors, whereas in the second biopsies in 86% (24 of 28 specimens) GFAP-positive cell types were found. All these 28 patients had been treated with radiation therapy as well.

Twenty-three percent of the tumors were graded as Grade 1, 27% as Grade 2, 11% as Grade 3, and 39% as Grade 4 (Table 3). Analysis of variance (ANOVA) showed significant differences in survival for the different grades

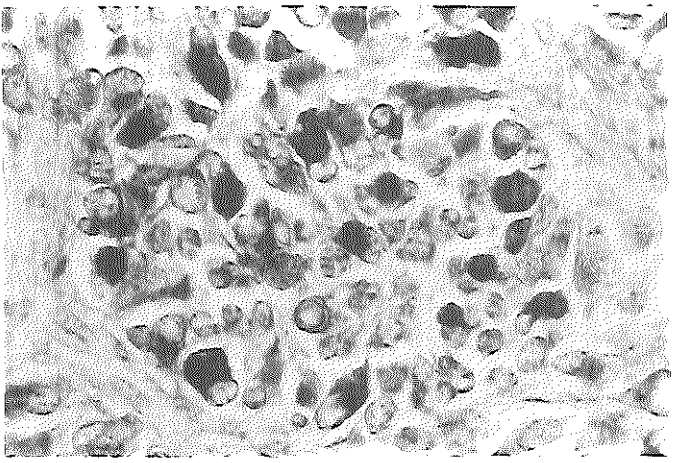


FIG. 3. Minigemistocytes in an oligodendroglial tumor. With voluptuous GFAP-positive cytoplasm without long processes and an eccentric nucleus, these cells resemble miniature gemistocytes (stained for GFAP, ×500).

of the oligodendrogliomas. The *a posteriori* Student-Newman-Keuls procedure revealed significant differences between the Grades 1 and 2 and between the Grades 1 and 3 and 1 and 4 at the 0.050 significance level. In Table 3 the percentages of tumors in which GFAP-positive cells were found are shown for the four malignancy grades (Table 3). No correlation between the presence of any of the GFAP-positive cells and any of the individual histopathologic features was found.

Figure 5 shows the GFAP-positive fractions in the tumors for the first biopsies and the second biopsies, respectively (Fig. 5). Gemistocytes, either alone or in combination with GFOC and minigemistocytes, were found in 41% of the primary biopsies; in 11% of these biopsies the gemistocytes were not accompanied by minigemistocytes or GFOC (Fig. 5). The combination of GFOC and gemistocytes without minigemistocytes was never seen. It is shown in this figure that the fraction of tumors in which the only GFAP-positive cells were gemistocytes decreased after initial biopsy followed by radiation therapy (*i.e.*, 11% to 5%), whereas the number of tumors in which the gemistocytes were found together with minigemisto-

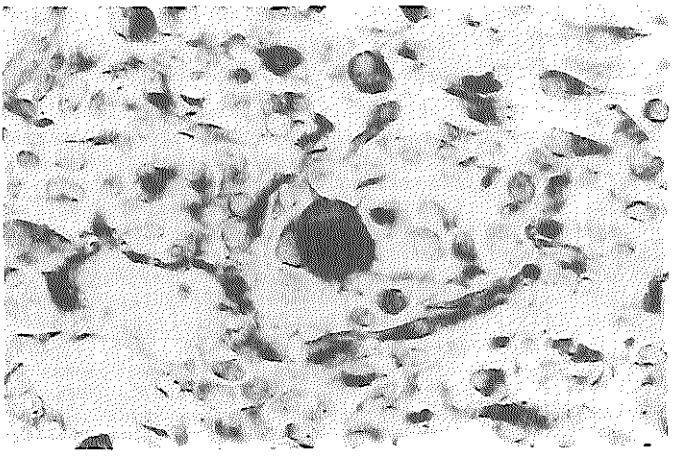


FIG. 4. Classic gemistocytic cell in an oligodendroglioma. A gemistocyte is surrounded by minigemistocytes and GFOC (stained for GFAP, ×500).

TABLE 3. Grading Results

Grade	Percentage	GFAP+ (%)	GFOC (%)	mini (%)	gem (%)
1	23	50	27	38	35
2	27	79	53	57	43
3	11	42	33	33	25
4	39	77	42	56	49

GFAP: glial fibrillary acidic protein; GFOC: gliofibrillary oligodendrocytes; mini: miniature forms of gemistocytes; gem: gemistocytes.

In the first column the percentages of patients in the respective grades are given. The percentages in the second column represent the fraction of oligodendrogliomas of each grade in which GFAP-immunoreactive cells were found. In the three columns on the right side these percentages are given for the respective GFAP-positive cell types.

cytes or GFOC increased (*i.e.*, 31% to 51%). However, these shifts in fractions did not reach statistical significance.

The *t* test did not reveal a significant difference between the survival times of patients with oligodendrogliomas with GFAP-positive cells on the one hand, and those lacking GFAP positive cells on the other hand. In Figure 6 the survival percentages of the patients of the respective two groups are shown (Fig. 6).

The chi-square test did not show significant differences in the survival times of patients with or without GFOC in the tumors, nor between those with or without minigemistocytes in the oligodendrogliomas. However, a significant ($T = 5.1$; $df = 1$; $P = 0.024$) difference was found between the survival times of patients with oligodendrogliomas in which gemistocytes were found, as compared with those without gemistocytes, irrespective of the presence of the other GFAP immunoreactive tumor cells. The mean survival time of patients without gemistocytes was about twice as long if compared with that of those with tumors in which gemistocytes were seen (*i.e.*, 41 *versus* 21 months). The respective survival rates are shown in Figure 7.

Localization of the tumors did not influence the survival rates. There was no relation between the presence of any of the GFAP-positive cells and localizations of the tumors. Effects of additional radiation therapy on the survival times of the patients were not elucidated.

Discussion

A variety of immunohistochemical markers have been explored in oligodendrogliomas.[19,25–27] Among these markers, anti-Leu-7 antibody would have value in differentiating meningeal invasion by oligodendrogliomas from oligodendroglial-like parts of syncytial meningiomas.[27] The expression of mycelin basic protein (MBP) and S-100 protein was studied in oligodendroglial tumors mainly to trace some differentiation potency in oligodendroglial tumor cells,[26,28] whereas mycelin-associated glycoprotein (MAG) and GFAP were applied mainly to identify the degree of anaplasia or the biologic aggressiveness in these tumors.[5,11,13–17,25,29] In some studies the concentration of GFAP in cell homogenates was measured irrespective of the morphologic characteristics of individual cells,[5,6,29] whereas recently the specific morphologic characteristics

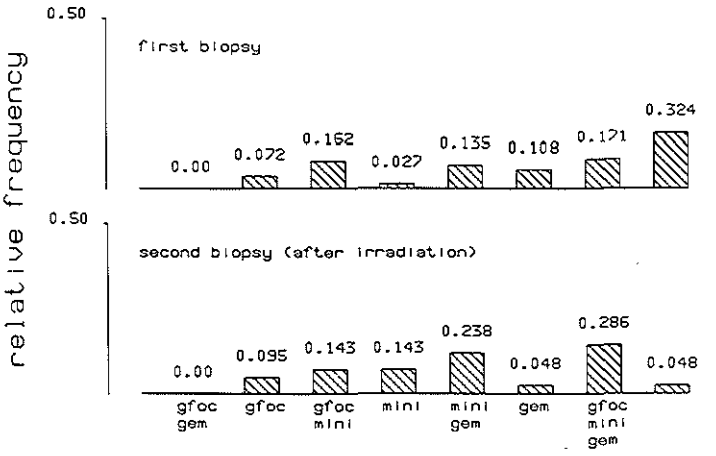


FIG. 5. Fractions of GFAP-positive cell types in the first and second biopsies. The shaded blocks on the upper line represent the proportion of tumors at first biopsy with the various GFAP-positive cell types either single or in combination. The lower line represents the GFAP-positive cells in the second excisions. The total group of patients who underwent a first operation consisted of 111 patients, whereas only 28 patients underwent a second operation. All patients who were operated on for the second time had been treated with radiation therapy as well. The respective single cells or cell combinations in a particular tumor are indicated under the second line. The last bar of each line (without connotation) represents the fraction of oligodendrogliomas without GFAP-positive cells. In the second biopsies more GFAP-positive oligodendrogliomas are found. Responsible for the increase of GFAP positivity at second biopsy are raised fractions of tumors in which minigemistocytes alone, minigemistocytes together with gemistocytes, or minigemistocytes together with gemistocytes and GFOC are seen. The fraction of tumors with gemistocytes without other GFAP-positive cells was diminished with a Factor 2 in the group of patients who were operated on a second time. The combination of GFOC and gemistocytes without minigemistocytes was not found in the first biopsies nor in the second biopsies.

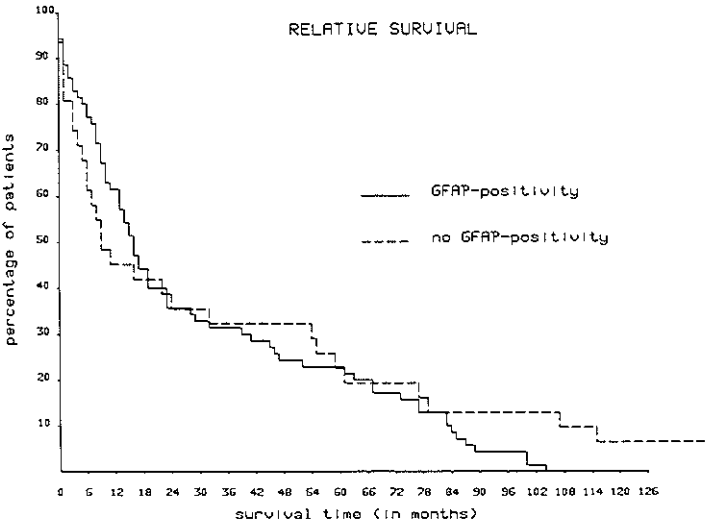


FIG. 6. Survival rates for patients with oligodendrogliomas with and without GFAP-positive cells. The two curves are intermingled, and the *t* test did not show a significant difference in survival times between the two groups.

of the GFAP immunoreactive cells were taken into closer investigation.[14,18,19] Readily it became clear that, unlike some of the results obtained in astrocytomas,[5,11,12,29] and despite the early study of Van der Meulen *et al.*, no clear-cut relationship between GFAP content and tumor grade in oligodendrogliomas existed.[11,14,19,30]

A factor responsible for controversies in prognostic implications of GFAP positivity in oligodendrogliomas may be the well-known difficulty in proper grading of these tumors. The traditional grading scheme of Kernohan for gliomas did not yield clear correlations with survival rates when applied to oligodendrogliomas, unless used in a

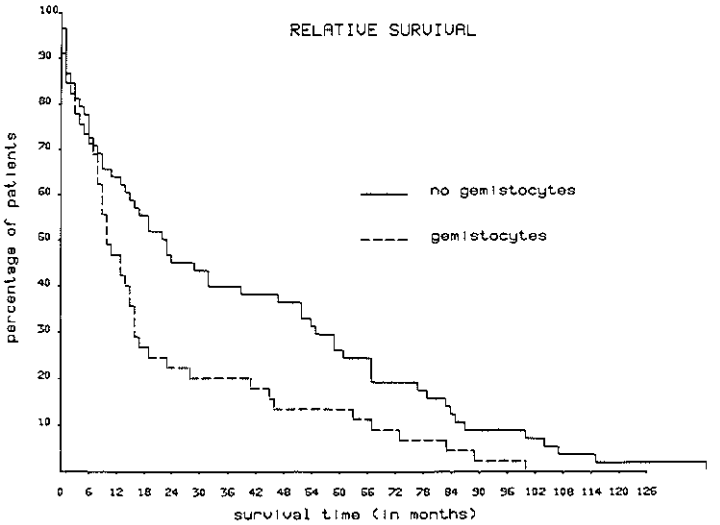


FIG. 7. Survival rates for patients with oligodendrogliomas with and without gemistocytes. The two curves are clearly separated, and the patients with oligodendrogliomas in which classic gemistocytes are found have a significantly shorter survival than patients with oligodendrogliomas without these cells.

modified version.[20,21,24] In the current study the most relevant histopathologic criteria were selected from the original grading criteria of Kernohan (Table 2). This selection was based on recent articles in which histopathologic features with independent significance for prognosis were identified.[20,22,23] Nevertheless, we did not reach significant differences in survival times for all grades. Furthermore, the respective grades of malignancy seemed to not correspond with the percentages of GFAP-positive cells. However, in Grade 1 tumors a relatively low number of GFAP-positive cells were found, whereas in Grade 4 tumors this percentage had increased (Table 3). This trend holds for all three GFAP-containing cell types but did not reach statistical significance. Although in studies on astrocytomas a positive correlation between tumor necrosis and GFAP-content was found,[5] no correlation between the occurrence of one of the three GFAP-positive cell types with necrosis or with any other histopathologic feature was found in the current study on oligodendrogliomas.

The notion of transformation of oligodendroglial tumor cells into astroglial tumor cells has been put forward by various authors in the past.[31–33] Recently it has been assumed that this transformation process eventually occurred through gemistocytic phenotypes (*i.e.*, round cells with GFAP-positive cytoplasm).[13,14,18] The arrangements of various cells in mixed oligoastrocytomas could very well be dependent on the stage of putative transformation in these gliomas.[27,34] In the current study we saw various mixtures of classic large gemistocytes with the elements of transitional tumors (*i.e.*, minigemistocytes and GFOC), but we never observed the combination of GFOC and gemistocytes without minigemistocytes (Fig. 5). This finding makes direct transformation of GFOC into large gemistocytes unlikely.

The only GFAP-positive cell type in the current series of oligodendrogliomas that was associated with a significantly shorter survival time was the classic large gemistocyte. Therefore, the presence or absence of classic gemistocytes should be taken into account when predictions on the prognosis of the patients are made. Classic gemistocytes are not considered characteristic cells for transitional cell type tumors, although transformation of a minigemistocyte into a gemistocyte has been suggested.[14]

Certainly classic gemistocytes are not specific constituents for oligodendroglial tumors because gemistocytes are often found in astrocytomas, whereas they appear in nonneoplastic processes as well. Although the relationship of a gemistocyte to any other neoplastic glial cell has not been clarified, some authors consider gemistocytes as the smallest members of a family of giant cells.[35] Scarce studies focusing on gemistocytes exist in the literature.[36] Hoshino *et al.* studied the proliferative activity of gemistocytes in malignant astrocytomas and pointed to the notion that these cells represent end-stage forms of astrocytes, which had lost the competition for nutrients to more rapidly proliferating small astrocytes.[36] Others suggested that gemistocytic astrocytomas (*i.e.*, gemistocytomas) are able to develop into the highly malignant glioblastomas.[35,37] Whatever the precise role of gemistocytes in gliomas may be, the identification of these cells as indicative for short survival expectancy in the current study is compatible with the association with malignancy of these cells in the literature.

It is known that large gemistocytic cell forms are often found after radiation therapy.[38] No data about the relation of radiation therapy and minigemistocytes or GFOC are available. In the current study an increase in the fraction of tumors in which gemistocytes were seen together with the other two GFAP-positive cell types was found in the specimens of patients who had been operated on for a second time and who had all been treated with radiation therapy in addition to the first operations. No conclusive data concerning the effect of radiation therapy on the survival rates of patients with oligodendrogliomas exist as yet because effects of selection and different application modalities invalidate retrospective nonrandomized studies.[39–41] Simply the fact of having been treated with radiation therapy did not influence the survival times in the current study.

In conclusion, we found a significant difference in survival rates of patients with and without classic large gemistocytes in oligodendrogliomas, whereas the occurrence of GFOC or minigemistocytes did not have implications for the prognosis. The presence of gemistocytes, GFOC, or minigemistocytes was not related to any of the relevant histopathologic features with known significance for survival rates in oligodendrogliomas. Whatever the relation between the classic gemistocytes and the GFAP-positive cells of transitional gliomas may be, the finding of classic gemistocytes in oligodendrogliomas is an unfavorable prognostic sign for the patients with these gliomas.

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PAPER 6

"ULTRASTRUCTURAL CHARACTERIZATION OF TRANSITIONAL CELLS IN OLIGODENDROGLIOMAS".

J.M. Kros, A.A.W. de Jong, Th.H. van der Kwast

J. Neuropathol. Exp. Neurol. 51(2): 186-193 (1992)

Journal of Neuropathology and Experimental Neurology

Vol. 51, No. 2
March, 1992
pp. 186–193

Ultrastructural Characterization of Transitional Cells in Oligodendrogliomas

J. M. Kros, M.D., A. A. W. de Jong, and
Th. H. van der Kwast, M.D., Ph.D.

Abstract. In oligodendroglial tumors the expression of glial fibrillary acidic protein (GFAP) is found in cells with an astrocytic morphology representing preexistent or neoplastic astrocytes. In addition, a proportion of the GFAP-positive cells has the morphology of miniature gemistocytes (minigemistocytes) or oligodendrocytes (gliofibrillary oligodendrocytes or GFOC). Both minigemistocytes and GFOC are considered as cells transitional between astrocytic and oligodendroglial lineage. Though minigemistocytes can readily be distinguished in routinely stained histological sections, GFAP immunostaining is obligatory for the identification of the GFOC. In the present study, the GFOC is characterized at the ultrastructural level using an immunogold-silver stain on semithin (1 μm) slides for identification of GFAP immunoreactivity and subsequent processing of the adjacent slide for immunoelectron microscopy. In analogy with the minigemistocytes, the glial filaments in the GFOC are arranged in parallel bundles. The finding of cells with ultrastructural features intermediate between those of GFOC and minigemistocytes suggests a close relationship and a possible interconvertibility between the two transitional cell types in oligodendrogliomas.

Key Words: Electron microscopy; Gliofibrillary oligodendrocyte; Glioma; Immunoelectron microscopy; Minigemistocyte; Oligodendroglioma.

INTRODUCTION

Although oligodendrogliomas consist predominantly of neoplastic oligodendrocytes, cells with obvious astrocytic differentiation have also been observed in these gliomas since the earliest microscopic investigations (1–8). Traditionally, cells with slender processes have been considered as interspersed preexisting astrocytes, while cells with coarse and irregular cytoplasm and eccentric nuclei have been interpreted as neoplastic astrocytic cells (9, 10). Various mixtures of neoplastic astrocytes and oligodendrocytes were found in mixed gliomas. The oligoastrocytomas are defined as glial tumors with separate areas (compact variant) or mixtures (diffuse variant) of two cell types (8). Whereas some authors refused to recognize mixed gliomas as a separate group (11, 12), others believed that basically all gliomas would be of a mixed character (13). The latter view was supported by the finding of mixed cell populations in experimentally induced gliomas (14, 15), although pure astrocytomas and pure oligodendrogliomas might be the result of experimental tumor induction as well (16).

Small-sized gemistocytic cells have been recognized in oligodendroglial tumors long before immunohistochemical techniques were available (7, 8, 17). These cells were considered as transitional cells that changed their oligodendroglial morphology into cells with an astrocytic appearance. It was speculated that tumor age, changes in vascularity and increase in intracranial pressure might be causal factors for this transformation (5). More recently, the application of immunohistochemistry led to the discovery of oligodendrocytes which expressed glial fibrillary acidic protein (GFAP) (18–22) and the term "gliofibrillary oligodendrocyte" (GFOC) was introduced, while the miniature forms of gemistocytes were called "minigemistocytes" (19). Most authors considered these GFAP-positive cells as true elements of oligodendroglial tumors (9, 18, 21–25), while some classified tumors with these cells as mixed gliomas (22). Oligodendrogliomas containing

From the Department of Pathology, Erasmus University and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands.

Correspondence to: Dr. J. M. Kros, Department of Pathology/Neuropathology, University Hospital Rotterdam-Dijkzigt, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

TABLE 1
Clinicopathological Data of Seven Patients

Case	Sex	Age	Localization	Diagnosis	Grade
1	Male	43	Right parietal	Oligodendroglioma	Grade A
2	Male	34	Right frontal	Oligodendroglioma	Grade C
3	Male	31	Left frontal	Oligodendroglioma	Grade B
4	Female	33	Right frontal	Oligodendroglioma	Grade A
5	Male	40	Right fronto-parietal	Oligodendroglioma	Grade B
6	Male	60	Left fronto-parietal	Oligo-astrocytoma	Grade 2
7	Female	33	Left frontal	Oligo-astrocytoma	Grade 2

The oligodendrogliomas were graded according to the modified grading system of Smith (30), while the oligo-astrocytomas were graded according to the grading system derived from Kernohan (31).

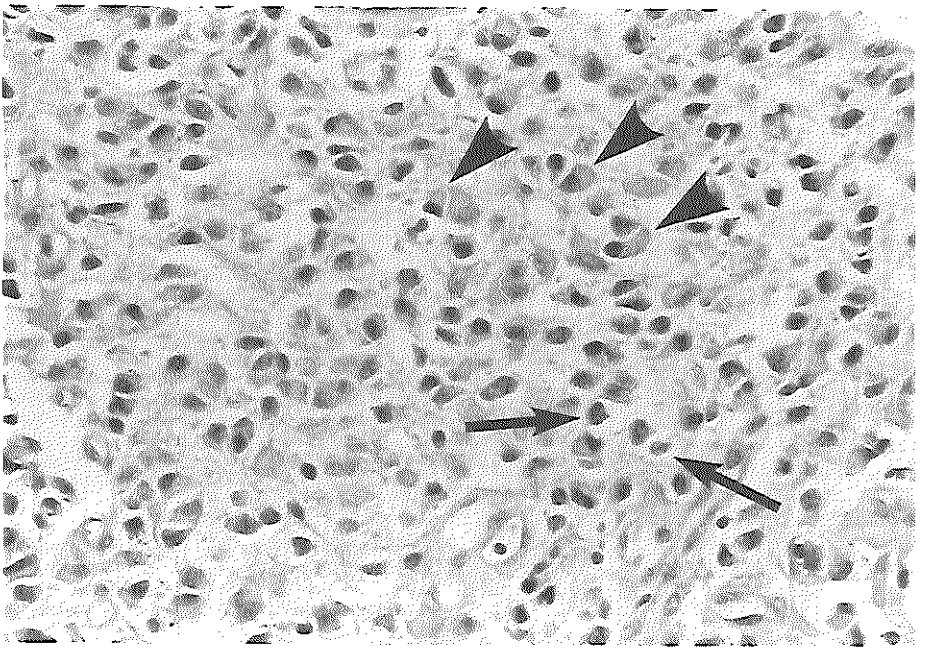

Fig. 1. Oligodendroglioma with transitional cells. In this routine hematoxylin-azophloxin stained slide the minigemistocytes are readily recognized (arrowheads) between the classic oligodendroglial cells with their perinuclear halos (arrows). For the distinction of GFOC, immunostaining for GFAP is necessary; in routine staining these cells cannot be differentiated from their GFAP-negative counterparts (arrows). (Patient 1, hematoxylin-azophloxin, ×25).

GFAP-positive cells, i.e. GFOC or minigemistocytes, were termed transitional gliomas (22).

At the ultrastructural level the minigemistocytes are readily recognized by their voluptuous cell bodies, blunt cell processes and eccentric, flattened nuclei. In a previous study we showed that minigemistocytes contain characteristic dense bundles of GFAP-positive intermediate filaments in their cytoplasm which clearly separate this cell type from the classical gemistocytes (26). As yet, no ultrastructural description of the GFOC has been established, probably because this cell type is only recognized after GFAP immunohistochemistry (15, 27–29). The aim of the present study was to describe the ultrastructure of the gliofibrillary oligodendrocytes by using a gold-silver enhanced stain for detection of GFAP immunoreactivity on semithin plastic sections, and subsequently processing the adjacent ultrathin sections for immunoelectron microscopy. Comparison of the ultrastructural features of the minigemistocytes with those of the GFOC revealed a close relationship between these two cell types.

MATERIAL AND METHODS

Patient Material

Biopsy material of seven patients with oligodendroglial viz. mixed oligo-astrocytic tumors in which GFOC or minigemistocytes were found was selected for processing for immunoelectron microscopy in addition to routine histology. The clinical data of the seven patients are summarized in Table 1.

The oligodendrogliomas were graded according to the modified grading scheme of Smith (30). The mixed oligo-astrocytomas were graded following the grading scheme derived from Kernohan (31).

Histology and Immunohistochemistry

Freshly obtained surgical specimens were processed for paraffin embedding after fixation in phosphate buffered formalin while part of the material was kept apart for immunoelectron microscopy (see below). Sections of 5 μm were made and stained with hematoxylin and eosin. Consecutive sections of the same thickness were used for immunohistochemistry. The primary antibody included rabbit anti-GFAP antiserum (DAKO Corporation, Copenhagen, Denmark) diluted 1:60 in phosphate

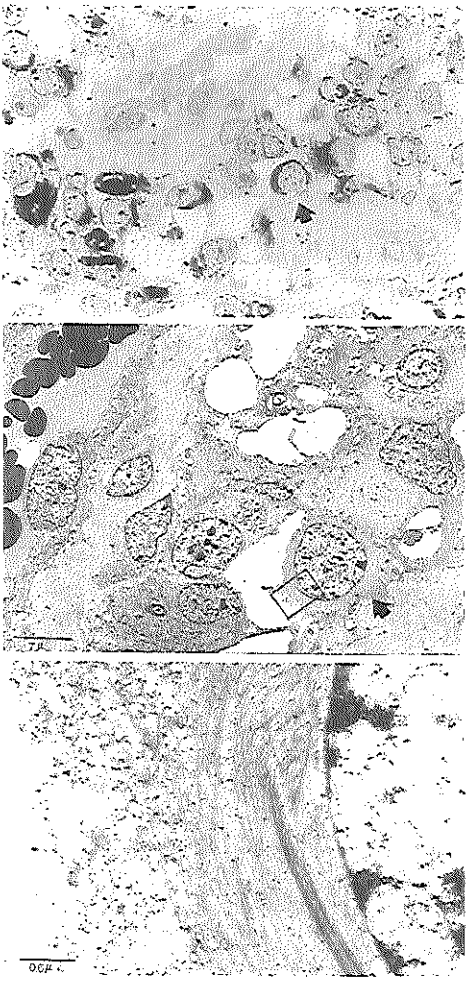

Fig. 2. Since the immunoreaction takes place on the cut surface while the tissue is within the Lowicryl, either the gold-silver stain or the cellular details are in focus in the photomicrographs. A) Semithin (1 μm) section of oligodendroglioma with GFOC and minigemistocytes. A GFOC (arrow) is tagged for ultrastructural investigation. (Patient 1, GFAP-immunostained gold-silver enhanced, counterstained with hematoxylin-azophloxin, ×100). B) Ultrathin adjacent section demonstrating the same GFOC as tagged in Figure 2A (arrow). This cell contains large bundles of filaments and lacks significant cell processes. (Patient 1, immunoelectron microscopy stained for GFAP, ×1,100). C) Detail of the cytoplasm of the GFOC from Figure 2A and B (frame). The filaments are arranged in parallel bundles. The colloidal gold particles represent immunoreactivity with GFAP which are confined to the filaments. (Patient 1, immunoelectron microscopy stained for GFAP, ×12,000).

buffered saline (PBS), pH 7.4. Endogenous peroxidase activity was blocked by treatment with 2% hydrogen peroxide in methanol. The two-step indirect immunoperoxidase technique was used on deparaffinized sections preincubated with 10% normal swine serum diluted in PBS. As the second step antibody, swine anti-rabbit immunoglobulin (Ig) antiserum conjugated to horseradish peroxidase (DAKO) diluted 1:50 in PBS was used. The incubations were performed at 37°C in a humidified chamber for 30 minutes. Final visualization was achieved by incubation with 0.02% diaminobenzidine (DAB) in PBS and 0.075% H_2O_2 for 7 minutes in darkness. Control slides in which the primary antibody was replaced by PBS always were negative. The slides were counterstained with hematoxylin.

Neoplastic oligodendrocytes were recognized by their round or polygonal cytoplasm borders, lack of cell processes, and round, centrally located nuclei. The tumor cells showed the typical honeycomb texture. The only distinction at the light microscopic level between the classic oligodendroglial cells and the gliofibrillary oligodendrocytes is the immunoreactivity for GFAP of the latter. Immunopositivity was seen as a small perinuclear rim. The transitional cells, or minigemistocytes, are readily recognized in the routine hematoxylin and eosin stain. The cytoplasm of these cells stains homogenously pink and gradually increases, leaving the nuclei in an eccentric position.

Electron Microscopy

Fresh tumor material was minced into 1 mm^3 cubes and fixed immediately in 1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) at 4°C. The specimens were fixed for 24 hours, transferred and stored in 0.1 mol/L phosphate buffer for 8 hours, and post-fixed in 1% OsO_4 in 0.1 mol/L phosphate buffer (pH 7.2) for 12 hours at 4°C. Subsequently, the specimens were rinsed in the same buffer, ethanol-dehydrated, and Epon-embedded for routine transmission electron microscopy. After ultrathin cutting, the sections were collected on mesh 100 copper grids and counterstained with uranyl acetate and lead citrate. Transmission micrographs were made on a Zeiss 902 transmission electron microscope at 80 kV.

Immunoelectron Microscopy

For postembedding immunoelectron microscopy, 1 mm^3 tissue cubes were fixed in 0.1 mol/L phosphate buffer (pH 7.2) containing 1% acroleine and 0.4% glutaraldehyde at 4°C for 4 hours. Tissues were transferred and stored in a sucrose buffer of 1 mol/L sucrose in 0.1 mol/L phosphate buffer (pH 7.2) with 1% paraformaldehyde at 4°C until further processing for Lowicryl embedding as described previously (32). From Lowicryl-embedded material semithin (1 μm) and adjacent ultrathin sections were made with glass knives. The semithin sections were processed for gold-silver enhanced immunostaining for anti-GFAP as described below. The ultrathin sections were collected on carbon-coated Formvar-filmed mesh one hole copper grids. The immunological methods for visualization of rabbit anti-

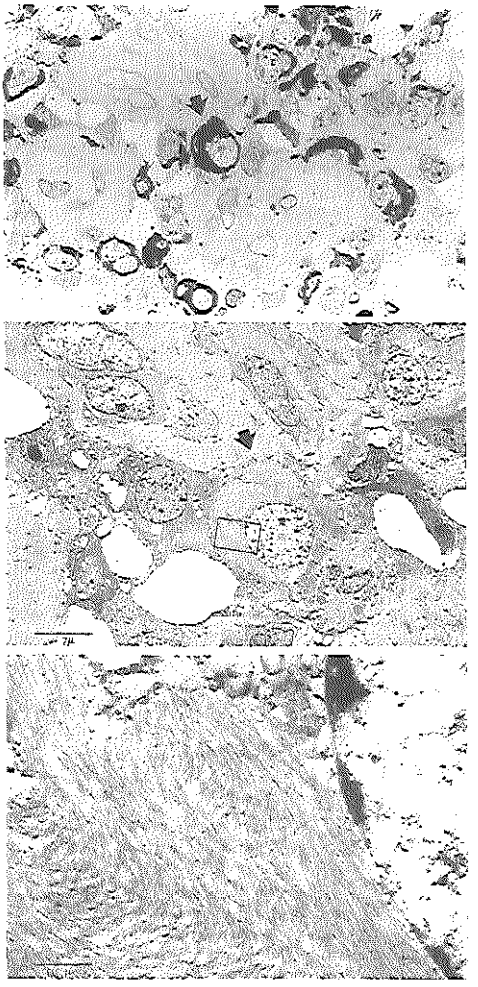

Fig. 3. A) Semithin (1 μm) section of oligodendroglioma with GFOC. A GFAP-positive oligodendroglial cell is indicated by the arrow. The volume of its cytoplasmic rim is intermediate between that of a typical GFOC and a typical minigemistocyte. (Patient 1, GFAP-immunostained gold-silver enhanced, counterstained with hematoxylin-azophloxin, ×100). B) Ultrathin adjacent section. The cytoplasm of the tagged cell contains a large amount of filaments. Besides these filaments, mitochondria are present in the cytoplasm. (Patient 1, immunoelectron microscopy stained for GFAP, ×1,100). C) Detail of GFAP-positive cell from Figure 3A and B (frame). The filaments are arranged in large parallel, intertwisted bundles. The immunogold label is associated with the filaments while the nucleus remains free of immunogold. (Patient 1, immunoelectron microscopy stained for GFAP, ×12,000).

GFAP were essentially as described previously (32). A 10 nm colloidal gold-labeled goat anti-rabbit antiserum (GAR-10, Aurion, Wageningen, The Netherlands) was used as the second step. Control sections were incubated with PBS, diluted in normal rabbit serum 1:60 in PBS, or the appropriate dilution of a similar monoclonal antibody nonreactive with glial tissue. Background staining was always negligible.

The semithin (1 μm) sections were incubated with anti-GFAP as the first step. Secondary incubation was done with Auroprobe IM GAR-5 nm Au (Aurion). The immunogold-silver enhancement was done by magnification of the gold particles by precipitation of metallic silver (Aurion R Gent Developer and Enhancer, Aurion) in darkness at room temperature. The slides were counterstained with hematoxylin-azophloxin.

RESULTS

Patients and Histopathology

The patient group consisted of five males and two females. The mean age of the patients was 39 years with a range from 31 to 60 years. Most tumors had a frontal localization. The length of preoperative symptomatic period ranged from two years to one month.

In three tumors the cell density and the nuclear-cytoplasm ratio was low, while vascular and endothelial proliferation, pleomorphism and necrosis were absent. Subsequently these tumors received Smith grade A. In the tumors of cases 3 and 5, an increased cell density and vascular and endothelial proliferation led to the attribution of grade B. Two gliomas had a considerable neoplastic astrocytic component and were subsequently diagnosed as oligo-astrocytomas (mixed gliomas). These tumors were graded according to the grading system derived from Kernohan (31). Both neoplasms received grade 2.

In the oligodendrogliomas, the GFOC and minigemistocytes were present in several areas. In Figure 1 an oligodendroglioma with transitional cells is shown stained with hematoxylin-azophloxin. In the mixed gliomas, the GFAP-positive oligodendroglial cells as well as the astrocytic cells were dispersed throughout the tumor.

Immunoelectron Microscopy

At the ultrastructural level, the oligodendroglial tumor cells were characterized by the absence of substantial numbers of filaments in the cytoplasm. Most of these cells had prominent mitochondria. The cells had round or oval nuclei. In the GFOC that were selected on semithin sections, the cytoplasm was filled with bundles of filaments in parallel arrangement (Figs. 2, 3). There were no other characteristics that separated these cells from the GFAP-negative neoplastic oligodendrocytes. The minigemisto-

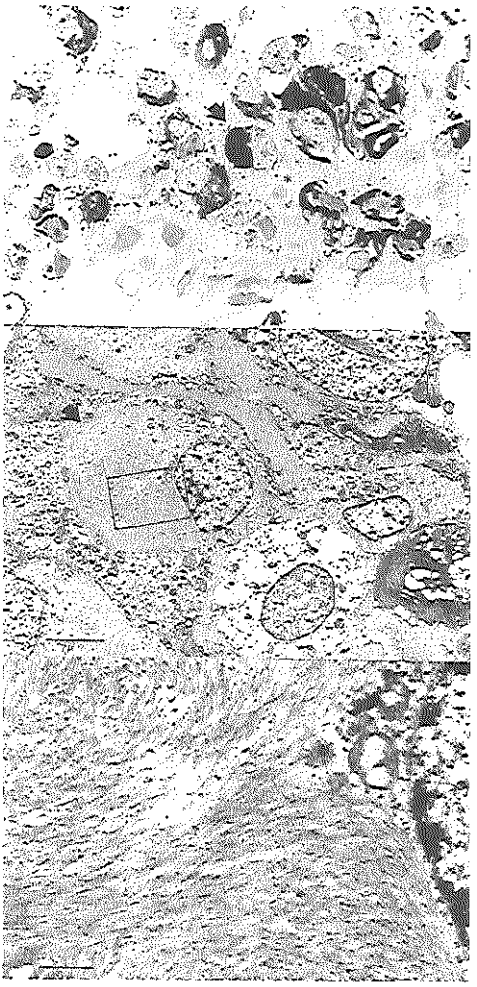

Fig. 4. A) Semithin (1 μm) section of oligodendroglioma with GFOC and minigemistocytes. A typical minigemistocyte (arrow) is seen adjacent to a typical (GFAP-negative) oligodendroglial cell (asterisk). The latter has a clear cytoplasm. In the upper left corner an interspersed astrocytic cell with prominent cell processes is strongly immunoreactive for GFAP. (Patient 1, GFAP-immunostained gold-silver enhanced, hematoxylin-azophloxin counterstained, ×100). B) Ultrathin adjacent section. The cytoplasm of the minigemistocyte (arrow) is filled with dense bundles of filaments, in contrast to the cytoplasm of the oligodendroglial cell (asterisk) which shows an electron lucent cytoplasm. In the latter cell, mitochondria represent the most prominent cell organelles. (Patient 1, immunoelectron microscopy stained for GFAP, ×1,100). C) Detail of GFAP-positive cell from Figure 4A and B (frame). The immunogold particles are associated with the intertwisted bundles of filaments. (Patient 1, immunoelectron microscopy stained for GFAP, ×12,000).

cytes were readily recognized by their more voluminous cytoplasm without substantial cell processes and eccentric nuclei (Fig. 4). In the cytoplasm large intertwisted bundles of filaments were seen (Fig. 4B, C). Regularly, cells with an intermediate phenotype between that of a GFOC and a minigemistocyte were seen (Fig. 3). These cells are characterized by a round or oval nucleus and cytoplasm densely filled with GFAP-positive bundles of filaments. In serial sections, it was confirmed that these cells indeed had a cytoplasmic volume between that of a GFOC and a minigemistocyte and, therefore, these cells did not represent superficially cut minigemistocytes.

In two mixed oligo-astrocytomas GFOC and minigemistocytes and cells with an intermediate phenotype were seen in some areas. Furthermore, large parts of these tumors consisted of GFAP-negative oligodendrocytes intermixed with (neoplastic) astrocytes (Fig. 5). The astrocytic cells had cell processes of variable lengths filled with an abundancy of glial filaments (Fig. 5B, C).

DISCUSSION

To date, ultrastructural studies have not been able to identify unequivocal lineage-specific differences between neoplastic cells of putative oligodendroglial or astroglial lineage. A variety of ultrastructural features was claimed to be preferentially present in oligodendrogliomas, but none of these seemed to be specific for oligodendroglial tumor cells (12, 27–29, 33–45). In spite of the incidental observation of the entire cytoplasmic occupation by protogliofibrils in neoplastic oligodendrocytes (34), glial filaments were not found as a prominent ultrastructural feature of neoplastic oligodendrocytes in other studies (33, 46). Ebhardt et al (44) occasionally found microtubuli and cytofilaments in typical oligodendroglial cells, but these structures are a common finding in astrocytic cells.

Kamitani et al (27, 28) also reported the presence of "glial" filaments in neoplastic oligodendrocytes, and therefore supposed that these cells belong to the astrocytic lineage. The absence of GFAP in a proportion of oligodendroglial cells was attributed to the relative scarcity of filaments (27). The presence of abundant glycogen particles and perivascular end-feet was interpreted as additional evidence for the astrocytic nature of some oligodendroglial tumor cells (27). Now, the discrepancies with regard to the finding of filaments in neoplastic oligodendrocytes by different authors can easily be explained by our observation that a subset of (light microscopical) oligodendroglial cells contains dense bundles of filaments

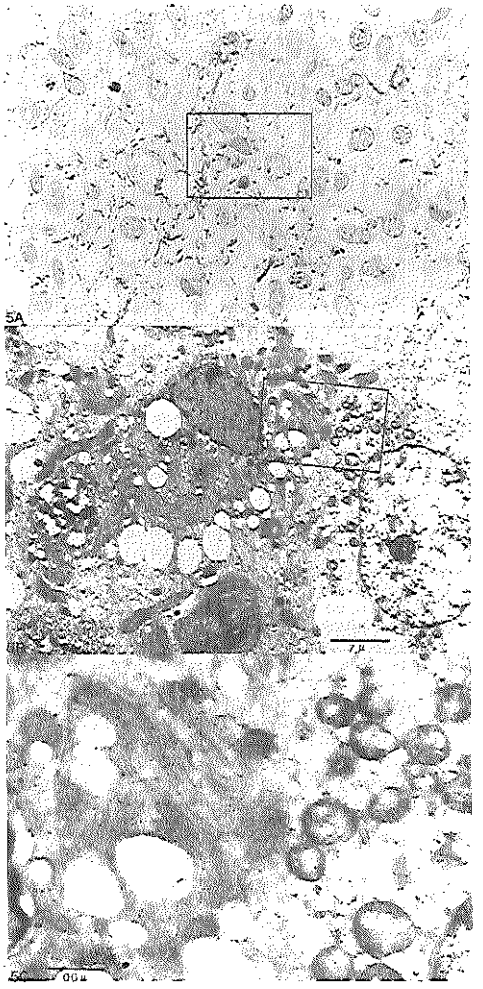

Fig. 5. A) Semithin (1 μm) section of a mixed oligo-astrocytoma. The oligodendroglial cells are free of immunostain. The interspersed glial processes, however, react strongly for anti-GFAP. A cell with the morphological features of an astrocyte with intense GFAP-positivity is framed. (Patient 6, GFAP-immunostained gold-silver enhanced, hematoxylin and eosin counterstained, ×100). B) Ultrathin adjacent section. A neoplastic astrocytic cell and neighboring oligodendroglial cell are seen (from frame, Fig. 5A). While the cytoplasm of the oligodendroglial cell lacks filaments, the cell processes of the astrocytic cell are densely filled with bundles of filaments. (Patient 6, immunoelectron microscopy stained for GFAP, ×1,100). C) Detail of GFAP-positive astrocytic cell from Figure 5A and B (from frame, Fig. 5B). The filaments are densely labeled by the gold particles. (Patient 6, immunoelectron microscopy stained for GFAP, ×12,000).

(29, 33, 42, 43). Since these gliofibrillar oligodendrocytes (GFOC) are defined both with light microscopical and immunohistochemical criteria (i.e. reactivity with anti-GFAP antibody), immunoelectron microscopy was required to identify GFOC at the ultrastructural level. Using adjacent semithin GFAP immunostained sections of the corresponding tumors, we showed that GFAP-positive tumor cells with the light microscopical morphology of GFOC were identical to the cells examined with the transmission electron microscope.

A striking similarity was found between GFOC and minigemistocytes in that both neoplastic cell types contain large bundles of GFAP-positive intermediate filaments in their cytoplasm. The presence of cells with ultrastructural features intermediate between GFOC and minigemistocytes indicates that the two cell types are closely related. In a retrospective analysis of the distribution of GFOC, minigemistocytes and large classic gemistocytes in oligodendrogliomas, with special reference to prognostic implications of these cells, GFOC were seen in combination with classic gemistocytes only if minigemistocytes were present (47). Therefore, a transformation of GFOC into classic gemistocytes via the minigemistocytic phenotype was suggested. The total number of GFAP-positive cells increased with the degree of malignancy of the tumor, and the presence of classic gemistocytes was correlated with significantly shorter survival rates, whereas the presence of transitional cell types viz. GFOC and minigemistocytes did not influence the survival times (47). These clinicopathologic observations are supportive for the hypothesis of close kinship between the GFOC and the minigemistocytes. Furthermore, an eventual transformation of a minigemistocyte into a classic gemistocyte cannot be excluded. The association between classic gemistocytes and short survival might be explained by the appearance of classic gemistocytes in a later developmental phase of the neoplasm.

No morphological counterpart for the gemistocytic cell has been described during normal development of humans or animals. In developing brain tissue, oligo- and astroglial cells are believed to derive from a common precursor cell. In tissue cultures of developing glial cells derived from the optic nerve of the fetal rat and the human fetus, it was found that a common glial precursor cell may transform into either an astrocyte or an oligodendrocyte depending on the presence of fetal calf serum in the culture medium (48, 49). The dual character of developing glial cells was illustrated by the finding of astrocytic features of glycogen particles and glial filaments

in cells with the light microscopical morphology of oligodendrocytes in the human spinal cord and in cell cultures of the mouse brain (50–53). In cell cultures derived from adult human white matter obtained at autopsy, a large increment of the GFAP-positive cell population was found along with a significant number of cells expressing both the oligodendroglial surface marker galactocerebroside (GC) as well as GFAP (54). Because none of these cells incorporated radiolabeled thymidine, a direct metamorphosis of an oligodendrocytic cell into a cell with phenotypical properties of an astrocyte was suggested. The transformation was promoted by the addition of dibutyryl cyclic AMP, which provides additional evidence for the interconvertibility of developing astrocytes and oligodendrocytes (54). These *in vitro* studies illustrate the potential for developing glial cells to express astrocytic and oligodendroglial markers simultaneously.

Recently, the glial precursor surface marker A2B5 was selectively found in oligodendrogliomas, but not in astrocytomas (55, 56). Since the oligodendroglial lineage of astrocytic tumor cells in some mixed oligo-astrocytomas was suggested by immunostainability for A2B5 (55), A2B5 cannot be used as a specific oligodendroglial tumor cell marker (10, 55, 57). Nevertheless, the immunohistochemical studies provide some circumstantial evidence for conversion potency of oligodendroglial into astrocytic lineage under neoplastic circumstances. The results of the present study suggest that in transitional tumors oligodendrocytes form a separate lineage together with the GFOC and minigemistocytes. In keeping with data from the literature, this lineage might be a reflection of a conversion of neoplastic oligodendroglial to astrocytic lineage.

ACKNOWLEDGMENTS

The authors thank the Department of Neurosurgery (head: Prof. Dr. C. J. J. Avezaat) for their kind cooperation and considerable efforts to supply fresh tumor material for this study, and Mrs. P. Delfos for her assistance with the photography.

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(Received June 7, 1991/Accepted August 12, 1991)
MS 91-43

CONCLUDING REMARKS

prognostic factors

The puzzling clinicopathological behavior of the oligodendrogliomas, and the difficulty in predicting their clinical course, is the consequence of a number of different factors. Most importantly, only part of these tumors appear in a morphologically pure form, while many oligodendrogliomas contain neoplastic astrocytic cells or transitional cell types. Whether oligo-astrocytomas are included or not, the oligodendroglial tumor group remains a minority of glial neoplasms. As a consequence, oligodendrogliomas often form a minor part of studies which mainly concern astrocytomas.

Although localization is an important prognostic factor in tumors of the brain - low grade gliomas may lead to immediate danger when localized in the brain stem, while high grade tumors of the cerebellum may show considerably protracted clinical courses - it was only after a rough division in three groups of tumor localization that we obtained significant differences in survival times (**paper 1**).

Aware of the limitations and insecurities of measuring tumor volume from CT scans we divided the volumes in three large groups in order to obtain rough differences in survival. The absence of any correlation between tumor size and survival was surprizing indeed. From a methodological point of view, assessment of tumor volume at a single point in time has an inherently ambiguous relationship to tumor growth rate. It would be more meaningful to record the increase of volume in a certain period of time, and correlating the change in tumor volume with the survival. It might very well be that slow tumor progression (increase in volume) enables surrounding brain structures to adapt to the expansion and maintain function, while rapid tumor progression will induce fatal edema. The growth rate seems to be independent of actual tumor size. In spite of the absence of correlation between tumor size and survival, good correlation between histopathological grade and survival was found in the same - relatively small - group of 43 patients.

In our comparison of the Smith grading system with the scheme derived from Kernohan (**paper 2**) correlation with survival not only of the system of Smith, but of Kernohan too, was satisfying, notwithstanding discouraging results of studies in the past, including those of Kernohan himself. Statistical analysis showed significant differences in survival between the grades of both systems. However, the reduction in inter-observer variability gained by the system of Smith is the major reason to prefer this latter scheme.

In the comparison of the grading systems, in the study on the prognostic effects of GFAP positive cells, and in the study on the effect of DNA-flow cytometry, the effect of the age of the patient on the survival was tested repeatedly. It was found that the survival was influenced by the age, but not more than one might anticipate due to of lower life expectancy of older patients. No correlation between age and tumor degree was found.

Because of the conflicting data in the literature concerning the role of mitotic count in the biological behavior of the oligodendroglioma, in addition to DNA-flow cytometry the mitotic count was determined morphometrically, and correlated with the survival. Unfortunately, we were unable to obtain reliable S-phase fraction estimations from the DNA-flow cytograms for comparison. Since the mitotic count had a significant effect on the prognosis we suggest that this parameter should be incorporated in the grading system. Multivariate analysis on the features of the grading system of Smith, viz. necrosis, pleomorphism, vascular and endothelial proliferation, cell density and mitotic count, should elucidate which factors independently influence the survival times, and

result in further minimizing the number of features necessary for grading.

The largest study correlating DNA content with biologic behavior in the oligodendrogliomas was done using a cytophotometric method on a group of no more than 11 tumors, with inconclusive results. Therefore, we engaged on a study of a much larger series, using DNA-flow cytometry (paper 3). Our failure in correlating the results of DNA-flow cytometry in oligodendrogliomas contrasts strongly with results of studies on astrocytomas and several non-cerebral tumors. No obvious reason for the absent correlation in oligodendroglial neoplasms is clear. In order to exclude the drawbacks of this technique on paraffin-embedded material, as for instance, missing small peridiploid peaks, DNA-flow cytometry should be applied to fresh tumor samples in a prospective study.

transitional tumor cell types in oligodendrogliomas

Only tumors with more than 50% of tumor cells with oligodendroglial phenotype in the biopsies were included in our retrospective studies. No samples with as much as 50% astrocytic cells were present in the series under study. Besides astrocytic cells with cell processes and classic gemistocytes, transitional cell types were encountered in a large part of the oligodendrogliomas. The latter cell types are subdivided into the gliofibrillary oligodendrocytes and the minigemistocytes. In spite of homologies in immunoreactivity between the minigemistocytes and the classic gemistocytes, the striking difference in ultrastructure provides evidence that these cell types differ in more aspects than only the volume of their cytoplasm (paper 4). Since classic gemistocytes are end-stage cells, possible transformation of these cells into minigemistocytes is unlikely. Nevertheless, we cannot rule out that minigemistocytes are able to transform into classic gemistocytes. It was shown (paper 5) that the presence of transitional cells had no influence on the survival. The presence of classic gemistocytes, however, was significantly associated with a less favorable prognosis. This finding confirms reports of classic gemistocytes often appearing in high-grade gliomas and glioblastomas, heralding a rapid tumor progression. The classic gemistocytes were shown by 3H-T studies to be non-proliferating cells, probably metabolic loosers in a competition with surrounding smaller and more aggressive cells. Therefore, if minigemistocytes transform into classic gemistocytes, one would expect an unfavorable effect of the appearance of minigemistocytes in oligodendroglial tumors as well. In order to explain the lack of prognostic significance of the minigemistocytes, one might speculate that classic gemistocytes are found in a later phase of tumor development.

Finally we have shown in an electronmicroscopic study that GFOCs and minigemistocytes essentially belong to the same lineage (paper 6), although reactivity with the newly discovered gemistocytic marker Pm43 was not found in the GFOCs. At the ultrastructural level, both cell types harbor glial filaments, and what is more, the arrangement of the filaments is alike. These findings are in agreement with our observation that GFOCs are often found together with minigemistocytes (paper 5). A gradual increase of cytoplasm content would result in the gradual metamorphosis of a GFOC into a minigemistocyte. The missing link is still an oligodendroglial cell with only few glial filaments in its cytoplasm, transitional between the GFAP negative oligodendrocyte and the GFOC.

The finding of the GFAP-positive cells might be a reflection of increased genetic instability of the oligodendroglial lineage during the progression of oligodendrogliomas. Alternatively, oligodendrogliomas may be considered as "null cell tumors" with the expression of GFAP as sign of differentiation. This option would imply that the oligodendroglial and astrocytic phenotypes are not more than subsequent phases in the develop-

ment of a glioma. An argument in favor of the theory that an oligodendroglial phase preceeds an astrocytic phase is the observation that more often astrocytic cells are seen between the cells of an oligodendroglioma, than oligodendroglial cells are found in an astrocytoma. The immunohistochemical and ultrastructural characterization of transient cells in oligodendroglial tumors has enhanced and detailed the knowledge of the group of oligodendroglial tumors. Future studies may unravel the stimuli inducing change in cellular differentiation of the neoplastic glial cells.

SUMMARY

Whereas in astrocytomas grading results yielded satisfying clinico-pathological correlations, grading procedures were without acclaim in the oligodendroglial tumor group. The reason for this might be that only small series of this uncommon neoplasm were studied, and delineation of oligodendroglial tumors from mixed gliomas or astrocytic tumors was hampered with difficulties.

In order to study the relationship between tumor size and survival, as well as tumor size and histopathologic grade, tumor volumes were calculated from the CT-scans of 43 oligodendrogliomas (**paper 1**). Although a good correlation between grade and survival was shown, no correlation between tumor size and survival or histopathologic grade was obtained.

In **paper 2** a comparison was made between the traditional grading system of Kernohan, and the recently developed grading system for oligodendrogliomas of Smith. In a retrospective study on 72 patients the grading results of both systems were related to the survival times of the patients. It was found that grading according to both systems yielded three groups of patients with significant differences in survival. Nevertheless, the grading system of Smith is preferred because of its lower inter-observer error.

The prognostic value of DNA-flow cytometry and mitotic count was tested retrospectively on the paraffin-embedded material of 85 oligodendrogliomas (**paper 3**). The results of the DNA-flow cytometry neither correlated with survival, nor with the histopathological grades. The mitotic count, however, had relevance for the prognosis. Thus, the mitotic count is a valid parameter for grading oligodendrogliomas.

In oligodendroglial tumors the intermediate filament glial fibrillary acidic protein (GFAP) is expressed in reactive and neoplastic astrocytes, in classic gemistocytes, and in transitional cell types known as gliofibrillary oligodendrocytes (GFOC) and minigemistocytes. In a small series of oligodendroglial neoplasms the gemistocytic cell types (i.e. the classic gemistocytes and the minigemistocytes) were compared immunohistochemically and ultrastructurally (**paper 4**). Both cell types reacted positively with anti-GFAP as well as with the newly developed monoclonal antibody Pm43. However, at the ultrastructural level a striking difference in the arrangement of the glial filaments was found.

The hypothesis that the presence of GFAP-positive cells in oligodendroglial tumors might have an influence on the biological behavior was tested in a retrospective study on 111 patients (**paper 5**). Whereas the presence of minigemistocytes and GFOCs did not influence the prognosis, the presence of classic gemistocytes was associated with an unfavorable clinical course. This finding is in agreement with the ominous reputation of these cells in astrocytic tumors.

In the final paper of this thesis the relationship between the two transitional cell types was established at the ultrastructural level, using an advanced method of cell targeting, with direct correlation between light- and (immuno)electron microscopy at the single cell level (**paper 6**). Both in the GFOC and the minigemistocyte the same arrangement of glial filaments in large interwoven bundles was found. The only difference between these two cell types was the larger cytoplasmic volume of the latter. The occurrence of intermediate cell types strongly suggests an interconvertibility between GFOC and minigemistocytes.

SAMENVATTING

Het in dit proefschrift beschreven onderzoek bestaat uit twee delen. In het eerste deel (publicatie 1, 2, 3) werden histopathologische parameters getoetst op hun voorspellende waarde voor het biologische gedrag van oligodendrogliomen. Het tweede gedeelte (publicatie 4, 5 en 6) behelst het onderzoek naar de verschillende celtypes en hun onderlinge relaties in oligodendrogliale tumoren.

Publicatie 1 betreft een onderzoek naar het verband tussen tumorgrootte en tumorgraad respectievelijk overlevingsduur van patienten met een oligodendroglioom. Het tumor volume werd berekend aan de hand van CT scans. Niettegenstaande een goede correlatie tussen de graad van de oligodendrogliomen en de overleving, kon - in tegenstelling tot vele andere tumoren elders in het lichaam - geen verband tussen tumorgrootte en de tumorgraad, of tussen tumorgrootte en de overleving, worden aangetoond.

In **publicatie 2** werd het traditionele graderingsschema van Kernohan vergeleken met het specifiek voor de oligodendro-gliomen ontwikkelde graderingssysteem van Smith. In deze retrospectieve studie betreffende 72 patienten werd gevonden dat beide graderingssystemen drie patientengroepen met onderling verschillende overlevingscurves onderscheiden. Het resultaat van graderen volgens Kernohan bleek beter dan verwacht op grond van literatuurgegevens, mogelijk als gevolg van de relatief grote patientengroep. Het kleinere inter-individuele beoordelingsverschil dat werd bereikt met het schema van Smith maakt echter dat dit schema prevaleert boven dat van Kernohan.

In een volgende retrospectieve studie (**publicatie 3**) werd de voorspellende waarde van DNA-flow cytometrie voor het biologische gedrag van de oligodendrogliomen onderzocht, gebruik makend van het paraffine-ingebedde materiaal van 85 oligodendrogliomen (**paper 3**). De resultaten van de DNA-flow cytometrie correleerden niet met de overlevingstijden, en evenmin met de histopathologische graad van de tumoren. Het bleek niet mogelijk de S-fase fractie te berekenen. Echter, de morfometrisch bepaalde mitose-index bleek goed te correleren met de prognose, en zou betrokken kunnen worden bij het graderen.

Het "glial fibrillary acidic protein" (GFAP) is een cytoskeleteiwit, dat in oligodendrogliale tumoren tot expressie gebracht wordt door reactieve en neoplastische astrocyten, klassieke gemistocyten, en zogenaamde transitionele cellen, d.w.z. de gliofibrillaire oligodendrocyten (GFOC) en minigemistocyten. In een kleine prospectief verzamelde serie oligodendrogliale tumoren werden de klassieke gemistocyten en de minigemistocyten zowel immunohistochemisch als ultrastructureel met elkaar vergeleken (**publicatie 4**). Beide celtypen reageerden met anti-GFAP en met de monoclonale antistof Pm43, een marker voor myeline van perifere zenuwcellen. Op ultrastructureel niveau bleken beide celtypen echter duidelijk te verschillen in de wijze waarop de intermediaire filamenten zijn gerangschikt.

In een retrospectieve studie betreffende 111 patienten (**publicatie 5**) werd de hypothese getoetst dat de aanwezigheid van GFAP-positieve celtypen het biologisch gedrag van oligodendrogliale tumoren zou beïnvloeden. Hoewel het aanwezig zijn van transitionele celtypen geen invloed op de prognose uitoefende, bleek de aanwezigheid van klassieke gemistocyten te zijn gecorreleerd met een slechtere prognose. Deze bevinding is in overeenstemming met de slechte reputatie van deze cellen in astrocytomen. De minigemistocyten werden veelal in combinatie met de GFOCs gezien, hetgeen een verwantschap tussen beide celtypen suggereert.

In **publicatie 6** werd de ultrastructuur van de transitionele celtypen met elkaar vergeleken, waarbij gebruik gemaakt werd van een elegante methode om dezelfde cellen, die met een immunohistochemische techniek gekleurd waren, ook met behulp van immunoelectronen microscopie te onderzoeken. Uit dit onderzoek bleek de nauwe (morphologische) verwantschap tussen GFOCs en minigemistocyten. Het enige verschil tussen de GFOC en de minigemistocyt bleek het volume van het cytoplasma te zijn, terwijl met name de rangschikking van de gliale filamenten een zelfde patroon van parallel verlopende bundels toonde. Deze phenotypische overeenkomst suggereert dat de GFOC en de minigemistocyt sterk gerelateerde cellen zijn.

DANKWOORD

Mijn opleiders Prof.Dr. R.O. van der Heul en Prof.Dr. F.T. Bosman worden bedankt voor de gelegenheid die zij boden om het proefschrift te bewerken.

Graag wilde ik Dr. C.G. van Eden en Prof.Dr. H.B.M. Uylings danken voor de wetenschappelijke vorming en continue steun die ik van hen ontving. Mijn co-promotor Dr. Th.H. van der Kwast, die mij in Rotterdam voortdurend in mijn wetenschappelijke arbeid steunt en stimuleert, wordt daarvoor bedankt.

Professor S.Z. Stefanko dank ik voor zijn grote geduld bij het overdragen van zijn encyclopedische kennis van de Neuropathologie.

Prof.Dr. F.C. Stam dank ik bijzonder voor zijn inbreng in de inleiding van het proefschrift.

Prof.Dr. C.J.J. Avezaat dank ik voor het kritisch lezen van het manuscript, en tevens voor de grote coöperatieve instelling die hij en zijn medewerkers tonen bij het verzamelen van neurochirurgisch materiaal.

Zonder ook maar iemand te kort te willen doen dank ik alle medewerkers van de afdeling Pathologie Rotterdam-Dijkzigt voor hun medewerking.

J.M. Kros, Amsterdam, mei 1992

CURRICULUM VITAE

Johan Marinus Kros werd geboren te Apeldoorn op 23 januari 1958. Na het behalen van de gymnasium-ß opleiding te Heerenveen begon hij in 1976 met de studie psychologie aan de Universiteit van Amsterdam. In 1977 werd het propedeutisch examen behaald, en tevens aangevangen met de studie geneeskunde aan dezelfde universiteit. Na het artsexamen in 1986 werkte hij op het Nederlands Instituut voor Hersenonderzoek, onder leiding van Dr. C.G. van Eden en Prof.Dr. H.B.M. Uylings, aan het onderwerp "the transient projections of the tractus corticospinalis in the rat". Sinds 1987 wordt hij opgeleid tot patholoog-anatoom op de afdeling Klinische Pathologie van het Academisch Ziekenhuis Rotterdam-Dijkzigt (opleiders achtereenvolgens Prof.Dr. R.O. van der Heul, Prof.Dr. F.T. Bosman), en onder leiding van Prof.Dr. S.Z. Stefanko wordt de promovendus ingewijd in het gebied van de neuropathologie.

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