Gliomas are the most common primary brain tumors. Many of these neoplasms, especially astrocytic and oligodendrogial lesions, are characterized by diffuse infiltrative growth in the preexisting brain tissue. Gliomas, require angiogenesis for their growth. That a gliomatosis cerebri does not usually exhibit enhancement on CT or MR imaging suggests that angiogenesis may not be one of its features. In contrast with the notion that most tumors, probably also including high-grade gliomas, require angiogenesis for their growth, it seems reasonable to establish the role of neovascularization in gliomatosis cerebri, performed computerized image analyses to assess vessel density and diameter in histological sections of multiple pathological and normal areas of a brain in which a gliomatosis cerebri was present. In addition, the presence of BBB characteristics was investigated in these areas by using immunohistochemical markers (Glut-1 and Pgp). To our knowledge, this is the first quantitative study of the vasculature of gliomatosis cerebri. Analysis of our results indicates that angiogenesis was absent in the gliomatosis cerebri, suggesting that tumor growth in these neoplasms is supported by cooptation of the existing vasculature and not by the formation of new vessels.

Gliomatosis cerebri: quantitative proof of vessel recruitment by cooptation instead of angiogenesis

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Object. Gliomas are the most common primary brain tumors, many of which (especially astrocytic and oligodendrogial neoplasms) are characterized by diffuse infiltrative growth in the preexisting brain tissue. Gliomatosis cerebri is a rare glial tumor and represents an extreme example of such diffuse infiltrative growth. This growth pattern not only hampers curative treatment but also allows for vessel cooptation rather than tumor angiogenesis as a way of vessel recruitment by the tumor tissue. The goal of this study was to establish the extent to which tumor angiogenesis occurs in gliomatosis cerebri.

Methods. Computerized image analysis was performed to assess quantitatively two microvascular parameters (vessel density and diameter) in different areas of a brain harboring a gliomatosis cerebri. These regions were the cerebral white and gray matter in which there was a diffuse infiltrative tumor, cerebral white and gray matter in which there was a more compact growth pattern of tumor cells, and normal cerebral white and gray matter. In addition, the authors performed immunohistochemical stainings for blood–brain barrier (BBB) characteristics (Glut-1 and Pgp) on samples obtained in these different areas.

The results of the quantitative analysis strongly indicated that in gliomatosis cerebri tumor, angiogenesis was completely absent, a finding that is corroborated by the fact that the microvasculature in gliomatosis cerebri persists in exhibiting immunohistochemical characteristics of the BBB.

Conclusions. The results of this study may help resolve the difficulties in radiological detection and delineation of the diffuse infiltrative part of glial brain tumors and put the expectations for antiangiogenic treatment of such tumors into perspective.

Key Words • gliomatosis cerebri • vessel cooptation • angiogenesis • quantitative analysis

Abbreviations used in this paper: BBB = blood–brain barrier; CT = computerized tomography; MR = magnetic resonance; VEGF = vascular endothelial growth factor; WHO = World Health Organization.
Vessel cooptation in gliomatosis cerebri

![Image](image_url)

Fig. 1. a: Transverse CT scan demonstrating a large subcortical hypodense area in the left hemisphere on first admission to the hospital. b and c: Transverse T1-weighted Gd-enhanced and T2-weighted MR images revealing a large subcortical area in the left cerebral hemisphere that is hypointense but does not enhance after contrast administration (b), and exhibits increased signal on a T1-weighted sequence (c). d: Transverse CT scan obtained 4 months after initial admission, demonstrating increased hypodensity in not only the left but also the right cerebral hemisphere (again without contrast enhancement). Arrowheads indicate the pathological areas; R = frontal right side.

Materials and Methods

Clinical Data

This 68-year-old man presented to the hospital with a 1-month history of phatic disturbances and cognitive dysfunction. His family reported that his complaints began suddenly. His medical history was unremarkable except for a 3-year history of atrial fibrillation. Neurological examination revealed a Mini-Mental State score of 18, a regular pulse, mild right hemiparesis (including a central facial paresis), and mild disturbance in word fluency and comprehension. Transverse CT scanning revealed a large subcortical hypodense area in the left cerebral hemisphere (Fig. 1a). Transverse T1-weighted MR imaging demonstrated a large subcortical area of increased signal intensity in this hemisphere (Fig. 1b and c), but contrast enhancement was absent. Before the neurological workup could be finished, the patient was admitted to a nursing home and lost to follow up for 4 months. When he was readmitted to the hospital, the patient suffered from mutism and quadriaparesis with increased, generalized muscle rigidity and bilateral impaired reflexes. Transverse CT scanning now revealed increased hypodensity of the white matter in the left cerebral hemisphere, as well as in the right hemisphere, still without contrast enhancement (Fig. 1d). A pulmonary infection developed and the patient died a few days after readmission. Autopsy examination (including the brain) indicated that pneumonia was the cause of death.

Neuropathological Findings

The brain weight (1410 g) was within normal range. Attached to the dura mater, a small parasagittal meningioma (maximal diameter 1.5 cm) was found in the parietooccipital region. The brain showed some signs of swelling (moderate flattening of cerebral gyri, as well as moderate prominence of parahippocampal gyri and cerebellar tonsils), but no evidence of herniation. The brain swelling might have been caused by hypoxic damage in an agonal phase rather than by the brain tumor, especially because CT scans obtained several days prior to death revealed no brain swelling. After formalin fixation, the brain was cut into 1-cm-thick slices. On the cut surface, the white matter in the left frontal, parietal, and occipital lobes showed widespread, poorly demarcated softening with dispersed rarefaction. In the basal cortex of the left occipital lobe, we observed a more circumscribed occipital lobe lesion consisted of densely packed, relatively small, elongated cells with oval or elongated nuclei, moderate nuclear pleomorphism, little eosinophilic cytoplasm, and focally high mitotic activity. In the periphery of this lesion, perivascular cuffs of a highly cellular tumor were found. In the left frontal, parietal, and occipital lobes, as well as in the right half of the corpus callosum, extremely extensive, diffuse infiltrative growth of tumor cells was found in both gray and white matter, accompanied by rarefaction of the preexisting white matter, some reactive astrocytes, and occasional macrophages. Necrosis and florid microvascular proliferation were absent. In line with the definition of the WHO-2000 classification,1 the tumor in this case was diagnosed as a gliomatosis cerebri.

Quantitative Analysis

Quantitative analysis of the microvasculature in histologically normal and pathological brain tissue obtained in this patient was performed as previously described.34 In short, histological sections of paraffin-embedded tissue samples were immunohistochemically stained with a monoclonal anti–collagen IV antibody (Sigma Chemical Co., St. Louis, MO), resulting in clear-cut demarcation of the intracerebral microvasculature. Computerized quantitative analysis was performed using the VidasPlus system (Kontron GmbH, Eching, Germany). Microscopic fields measuring 400 × 425 μm were selected from the following areas of the cerebrum for further microvasculature quantitative analysis: normal neocortex (number of analyzed microscopic fields, 15); normal white matter (number of microscopic fields, 30); neocortex with diffuse infiltrative tumor from the left frontal lobe (convex and basal areas) and from the basal part of the occipital lobe (number of microscopic fields, 43); white matter with compact tumor from the frontal and occipital lobes (number of microscopic fields, 28); and white matter with compact tumor from the basal area of the left occipital lobe (number of microscopic fields, 87). In each microscopic field the number of separate vascular profiles was assessed. Additionally, the diameter of each individual vascular profile was measured. Statistical evaluation was performed using SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL.). A one-way analysis of variance was used to study the differences among the aforementioned areas. Because the Levene test showed an inhomogeneity of variances (p < 0.001), Tamhane post hoc tests were used in the analysis of variance. Finally, histological sections of paraffin-embedded tissue samples were immunohistochemically stained for BBB markers with the monoclonal antibodies.
Glut-1 and PgP (Dako, Glostrup, Denmark) after standard antigen retrieval (citrate buffer and microwave) as previously described.

Results
The vessel number in tumor-infiltrated white matter and cortex was equal to or significantly lower than in their normal brain counterparts (Fig. 2). The vessel diameter in tumor-infiltrated areas tended to be somewhat higher than in normal brain tissue, but this difference did not reach statistical significance. The results of the quantitative analysis are depicted in Fig. 2. Examples of the microvascular parameters in different tumor areas are shown in Fig. 3. In both normal and tumor-infiltrated cerebral cortex and white matter, almost all microvessels stained positively for the BBB markers Glut-1 and PgP (Fig. 4).

Discussion
The role of angiogenesis in the growth and malignant progression of tumors in general, and in malignant gliomas in particular, has gained considerable attention in the last decade. Endothelial/microvascular proliferation and contrast enhancement as signs of neovascularization, however, are rarely mentioned in conjunction with gliomatosis cerebri. Gliomatosis cerebri is an entity known for its extremely diffuse infiltration of neoplastic cells in the brain tissue, often with minimal destruction of preexisting structures. In fact, such diffuse infiltrative growth may very well allow the tumor to recruit vessels by cooption rather than angiogenesis. The main purpose of our examination was to evaluate the extent of angiogenesis in gliomatosis cerebri. To our knowledge, this is the first report in which a systematic study of the vascularization in gliomatosis cerebri is presented.

Our quantitative vascular analysis confirmed that, in contrast to other high-grade gliomas, in our patient with gliomatosis cerebri, neovascularization was not a characteristic feature. This finding indicates that tumor progression was not facilitated by the development of a neovascular bed; rather, the mechanism of migration and infiltration into the preexisting brain parenchyma allowed tumor growth. This phenomenon has recently been described as “cooptation” of the preexisting vascular bed. Interestingly, it was not only described in the brain parenchyma but also in the skin, lung, and liver.

The presence of vessel cooption, the absence of neovas-

[Fig. 2. Box plots representing median (transverse line in box), 75th, and 25th percentile (upper and lower end of boxes) and highest and lowest values (upper and lower end of lines) for vessel number and diameter in the following cerebral areas: normal white matter (WM), white matter with diffuse infiltrative tumor (WMdt), white matter with compact tumor (WMct), normal neocortex (CX), neocortex with diffuse infiltrative tumor (CXdt), and neocortex with compact tumor (CXct). Note that in both white matter and cortex, the presence of diffuse or compact tumor is associated with a decrease, rather than increase, in vessel number, whereas the vessel diameter tends to be somewhat higher in tumor-infiltrated areas.

[Fig. 3. Photomicrographs providing examples of quantitative analysis of microvascular parameters in cerebral white matter and cortex with diffuse infiltrative or more compact tumor growth. The images show the immunohistochemical staining for collagen Type IV, which highlights the basement membrane of the microvasculature and which was used for computerized image analysis. Original magnification × 100.]
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Fig. 4. Photomicrographs showing the BBB markers PgP (a) and Glut-1 (b) in cerebral white matter with diffuse infiltrative (a) and cortex with perivascular, more compact tumor (b). Note the staining of the microvessels (arrowheads), strongly indicating that these are preexisting, incorporated vessels rather than newly formed vessels. Original magnification × 100 (a) and × 200 (b).

cularization, and the apparently limited changes to the preexisting, incorporated vessels in gliomatosis cerebri explain why these lesions do not enhance on MR images after contrast administration.33 Because both clinical symptoms and neuroradiological findings are often nonspecific, histological examination is generally required to establish the nature of this condition.12 Often considered are demyelinating diseases, inflammatory or infectious processes (for example, progressive multifocal leukencephalopathy), and ischemic changes or leukodystrophia.24

Vascular leakage and neovascularization are VEGF-dependent processes.7 In a mouse model with intracerebral human melanoma xenografts, we recently showed that even man melanoma metastases, we also recently demonstrated that VEGF blockade leads to a cooptative phenotype and makes their MR imaging detection difficult.19

To date, the treatment of gliomatosis cerebri usually consists of external radiotherapy and corticosteroid therapy.12 Radiation therapy may be useful in some patients with gliomatosis cerebri, although the duration of effects is limited.2 Recently, Sanson and colleagues27 reported an effect after temozolomide and procarbazine-lomustine vincristine chemotherapy, especially in patients with slow-growing gliomatosis cerebri.

Conclusions

Based on our present and previous findings we conclude that, although MR imaging–documented contrast enhancement of parts of brain tumors may be used as an argument for responsiveness to antiangiogenic therapy, gliomatosis cerebri and the nonenhancing portion of other diffuse infiltrative gliomas are very unlikely to respond to such therapy.

References


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