Review Article

Angiogenesis in brain tumors; pathobiological and clinical aspects

Pieter Wesseling¹, Dirk J. Ruiter¹, and Peter C. Burger²
¹Department of Pathology, University Hospital Nijmegen, Nijmegen, The Netherlands; ²Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore MD, USA

Key words: brain neoplasms, neovascularization, astrocytoma, glioblastoma multiforme, meningioma

Summary

Angiogenesis is the outgrowth of new blood vessels from the preexistent vasculature. In 1971, Folkman hypothesized that solid tumors are dependent on angiogenesis for sustained growth and that anti-angiogenic treatment is a potential antineoplastic therapy. Because glioblastoma multiforme (GBM) frequently shows florid microvascular proliferation (MVP), this tumor has been considered since then as a suitable candidate for such treatment that attempts to eradicate or control a neoplasm by interfering with its blood supply. Indeed, in animal models the growth of glioma xenografts can be inhibited by targeting the angiogenic process. However, unlike many glioma xenografts, human infiltrating gliomas such as GBMs have a diffuse infiltrative growth pattern, and preexistent vessels may suffice to provide many tumor cells with much of their blood supply, particularly in the critical peripheral infiltrative margins. Thus, while attractive in concept, anti-angiogenic therapy of GBM must address the anatomic vascular realities of this neoplasm. Even if antiangiogenic therapy ultimately has a role in infiltrative neoplasms, there are a host of other intracranial neoplasms whose discrete architecture might make them attractive candidates for anti-angiogenic therapy. This review summarizes the angiogenic process in GBM and suggests other types of tumors for which the efficacy of anti-angiogenic therapy might be studied.

Introduction

Like normal cells in most human tissues, tumor cells are dependent on the vasculature for their supply of oxygen and nutrients and removal of waste products. Solid tumors need to increase their vascular supply to meet the metabolic demands of a growing population of tumor cells. The outgrowth of new blood vessels from the preexistent vasculature, 'angiogenesis', accomplishes this goal [1, 2]. In several neoplasms, angiogenesis not only seems to allow tumor growth, but also to increase the probability of tumor cells to enter the circulation and metastasize [3]. Given this critical role for vessels in the enlargement, and possibly metastatic potential, of solid tumors, the angiogenic process is a logical target for new anti-cancer therapies [4].

The most frequent and most malignant human glial neoplasm, glioblastoma multiforme (GBM), has become an especially strong candidate for such treatment [5–7] since it frequently shows a remarkable microvascular proliferation (MVP) characterized by multilayered proliferation of swollen microvascular cells in the vessel wall [8, 9]. Expectations for success were strengthened by animal studies in which the growth of gliomas could indeed be inhibited by anti-angiogenic therapy [10–13].

The aim of this review is to put the expectations about the value of anti-angiogenic therapy for human brain tumors into perspective of the anatomic realities of the vasculature in brain tumors, espe-
cially the high grade infiltrating types. Since most studies on angiogenesis in brain tumors concentrated on astrocytic neoplasms, the astrocytic tumors will be highlighted in this review, but other tumors with perhaps more favorable tumor-vasculature relationships are also discussed.

**Tumor angiogenesis**

Angiogenesis is involved in a wide range of biological and pathological processes, including embryogenesis, endometrial proliferation, wound healing, retinal neovascularization, and solid tumor growth [2, 14]. In 1945, Algire et al. suggested that an outstanding characteristic of tumor cells is their capacity to elicit continuously the growth of new capillary endothelium from the host [15]. Folkman in 1971 hypothesized that solid tumors are dependent on angiogenesis for sustained growth, and that anti-angiogenic treatment is a potential therapy for these tumors [1, 16]. In tumor biopsies, the angiogenic capacity may be reflected by the number of microvascular profiles in the area with the highest vessel number (‘angiogenic hot spot’) [3]. In several tumor types (e.g. breast, prostate, and non-small cell lung carcinoma) an increased intratumoral microvessel density correlated directly with metastasis and inversely with survival [3]. Angiogenesis thus not only seems to allow tumor growth, but also to increase the probability of malignant tumor cells to enter the circulation and metastasize.

Angiogenesis is a complex process, including multiple coordinated steps, such as production and release of angiogenic factors, directional migration and proliferation of microvascular cells, proteolytic degradation of extracellular matrix (ECM) barriers, and the composition of new vessels [17, 18]. Angiogenesis is regulated by multiple stimulatory and inhibitory factors that are able to modulate the migration and/or proliferation of microvascular cells [4, 19]. Of the angiogenic factors, Vascular Endothelial Growth Factor/Vascular Permeability Factor (VEGF/VPF) and acidic and basic Fibroblast Growth Factor (a- and b-FGF) have been studied the most intensively. The mitogenic activity of VEGF is described to be restricted to endothelial cells (ECs), while FGF is mitogenic for a spectrum of cells, including vascular smooth muscle cells (VSMCs) and ECs. The VEGF expression appears to be upregulated by hypoxia [20, 21]. As is discussed below, the latter observation is of great interest since it may account for the well known propensity for glomeruloid MVP in GBMs to occur adjacent to necrotic foci. It also suggests that this MVP might be a late event in the tumor’s evolution.

During angiogenesis new blood vessels originate mainly from capillaries and small venules [2]. By definition, the formation of functional vascular sprouts depends on directional migration of microvascular cells in the surrounding tissue. The mechanisms for migration of these cells are comparable with those involved in invasion of tumor cells [22]. Experimental data show that directional endothelial migration is controlled by the ability of these cells to exert mechanical forces on the ECM of the surrounding tissue [23, 24]. Most of the identified ECM-receptors on ECs are of the integrin type [25]. Recently, two angiogenic pathways were defined in corneal and chorioallantoic membrane models on the basis of dependency on vascular expression of the integrins αvβ3 and αvβ5, respectively [26]. For proteolytic degradation of ECM barriers, several classes of enzymes, including matrix metalloproteases, serine proteinases (plasminogen activators), and cathepsins have been implicated [27].

While most studies on the effect of angiogenic factors on microvascular cells have focussed on ECs, pericytes are extensively involved in the angiogenic process as well [28–32]. Pericytes can be considered as multipotential, microvascular representatives of VSMCs, but their role during angiogenesis is largely unknown [33, 34]. *In vitro* studies demonstrated that pericytes can inhibit proliferation of ECs by a mechanism that requires contact or close proximity between the two cell types, the inhibition being mediated by Transforming Growth Factor-β1 (TGF-β1) [35–37]. Under hypoxic conditions, however, cultured pericytes were shown to produce VEGF and thus may promote EC growth in a paracrine way [38]. The increased α-smooth muscle (α-sm) actin expression in the tumor microvasculature [30–32] suggests a contractile role of these cells [39]. In cultured human brain pericytes
the increased expression of α-sm actin has been shown to be mediated by TGF-β1 [40]. The recent demonstration of the ectoenzyme aminopeptidase A on pericytes in the tumor microvasculature indicates a role in the metabolism of biologically active oligopeptides [41].

Neuropathology of brain tumors and angiogenesis

Brain tumor growth patterns

The most common primary tumors of the human brain are gliomas, including neoplasms displaying astrocytic, oligodendrogial, and ependymal differentiation. Gliomas rarely metastasize to distant organs, even when high grade. Astrocytic tumors can be divided into the more circumscribed lesions (e.g., pilocytic astrocytomas) and the diffusely infiltrating neoplasms such as the common fibrillary astrocytic tumors. The latter include the GBM. On the basis of four histopathological criteria (nuclear atypia, mitotic activity, MVP, and necrosis) diffuse astrocytic neoplasms can be divided arbitrarily into three groups of increasing malignancy: astrocytoma (A), anaplastic astrocytoma (AA), and GBM [8, 9, 42, 43]. Other tumors that generally show diffuse infiltration in the preexistent brain tissue are oligodendrogliomas and primary central nervous system lymphomas [8, 44]. Examples of brain tumors with an expanding rather than infiltrative growth pattern are meningiomas (Figure 1), meningeal hemangiopericytomas, medulloblastomas, hemangio blastsomas,ependymomas, pilocytic astrocytomas, and metastatic carcinomas [8].

The diffuse infiltrative growth of the fibrillary astrocytic neoplasms is now well recognized [8, 45–47]. Because of this growth pattern, complete surgical resection of such astrocytic tumors is often impossible, and eradicating tumor cells by chemotherapy and/or radiotherapy without damaging the involved brain parenchyma has been difficult to achieve. The prognosis for patients with these tumors thus remains poor; the median survival of patients with GBMs is less than 1 year and of patients with AAs less than 3 years [9]. Infiltrating astrocytic tumor cells show extensive spread along white matter tracts and blood vessels, with isolated cells throughout much of the hyperintense areas on T2-weighted MRI scans [45–49]. For migration along blood vessels, interactions between integrins on tumor cells and vascular ECM components such as collagens, laminin, and fibronectin are considered to be important [50]. Within the glial compartment such ECM components are generally lacking [51], and diffuse infiltrative growth may be facilitated by other molecules like gangliosides and adhesion molecules of the CD44 family, especially since the glial ECM is rich in hyaluronic acid, a potent ligand of CD44 [44, 52, 53]. A recent study suggested that the infiltration of glioma, but not of meningioma,
Florid MVP with prominent, multilayered proliferation of swollen microvascular cells in the vessel wall is a well recognized phenomenon in some gliomas [8, 9]. The most prominent form of this MVP was called ‘glomeruloid’ MVP because of the formation of coiled, glomerulus-like capillary loops [55] (Figure 2). Glomeruloid MVP can also be present in non-glial tumors such as extracranial neural and neuroendocrine neoplasms [56], but is classically associated with GBMs. In the current histopathological grading of diffuse astrocytic neoplasms, florid MVP is an important criterion to distinguish high grade from low grade tumors [42, 57]. While in most glial tumors glomeruloid MVP is a sign of malignant progression, in pilocytic astrocytomas this MVP does not carry this prognostic significance [58]. Microscopically, other brain tumors may show a particular microvascular architecture as well. In oligodendrogliaomas the delicate capillaries are often arranged in a ‘chicken wire’ pattern. Hemangioblastomas and (meningeal) hemangiopericytomas are known for a very high number of delicate microvessels, in the latter these vessels can be present in a ‘staghorn’ configuration [8].

Florid MVP has long been considered as proliferation of ECs, as is apparent in the common designation ‘endothelial proliferation’. Recent immunohistological and immuno-electron microscopic studies, however, have demonstrated an extensive contribution of pericytes with cytoplasmic α-sm actin staining [30–32] (Figure 2). These cells are external to ECs in the malformed capillaries. Glomeruloid MVP in GBMs often forms arcs or serpiginous patterns that are oriented parallel to necrotic areas or to the tumor-brain interface [59, 60]. Because of the presence of florid MVP, it has been suggested that GBMs depend more on extensive neovascularization for continued growth than is the case for ‘endothelial-poor’ tumors. If so, then GBMs are good candidates for anti-angiogenic therapy [5–7, 10]. Unlike angiogenesis in many other tumors [61, 62], the MVP in gliomas is usually not accompanied by desmoplasia with proliferation of myofibroblasts. An interesting GBM variant combines a glioma and a spindle-cell component historically felt to be mesen-

Figure 2 a–c. Light- (a, b) and electron microscopy (c) of glomeruloid MVP in GBM. Immunohistochemistry demonstrates extensive contribution of α-sm actin-positive, abluminal cells to this MVP (b, c). a, hematoxylin- eosin staining; b, c, α-sm actin staining; a, b, serial sections, original magnification ×200; c, bar = 5 μm, asterisk = microvascular lumen.

cells in brain tissue is a reflection of the strong expression of the standard form of CD44 in glioma cells versus the weak expression in meningioma cells [54].
chymal, hence the designation ‘gliosarcoma’. Abundant vascular proliferation is common in these tumors. Based on α-sm actin staining of the sarcomatous component of gliosarcomas, it has been suggested that this part can originate from the malignant transformation of VSMCs in some of these ‘combined’ neoplasms [63]. Cytogenetic studies, however, indicate that in other gliosarcomas the sarcomatous component is, in fact, glial in origin on the basis of identical numerical chromosome aberrations and p53 tumor suppressor gene mutations in the gliomatous and sarcomatous component [64, 65].

Pathogenesis of brain tumor angiogenesis

Brain tumor angiogenesis ranges from the formation of an abundant delicate capillary network in hemangioblastomas to glomeruloid MVP in GBMs. That GBMs are a potent source of angiogenic factors has been suggested for many years [5, 59]. Among these, release of the angiogenic growth factor VEGF appears to play a pivotal role [66–70]. In situ analysis of GBMs showed that the production of VEGF is induced especially in tumor cells situated close to necrotic foci [20, 68]. This finding explains the topographical relationship between florid MVP and necrosis described above. Florid MVP in apparently unaffected neural tissue surrounding glial neoplasms may be explained by a relatively high interstitial pressure within the tumor, leading to a flow of interstitial fluid containing angiogenic factors to the tumor periphery [59, 71]. For detailed information about the role of angiogenic factors and their receptors in brain tumor angiogenesis, the reader is referred to recent reviews [66–68]. Since the mitogenic activity of VEGF appears to be restricted to ECs, other angiogenic factors must be involved in the early and extensive contribution of pericytes to florid MVP in GBMs. Potential candidates in brain tumors are FGF and Platelet-Derived Growth Factor (PDGF) [19, 30, 66, 67]. In human healing wounds and colorectal adenocarcinomas, pericytes rather than ECs were found to express PDGF-receptor [72]. The prominent phenotype of MVP in GBMs may be the result of production of unusual levels or combinations of angiogenic factors in these tumors, leading to unbalanced angiogenesis.

As seen in light microscopic and electron microscopic sections, the architecture of glomeruloid
MVP suggests that this proliferation of microvascular cells is combined with a relative lack of directional migration into the glial (tumor) tissue. The result is the formation of coiled masses of capillaries instead of delicate vascular sprouts as seen in classic neovascularization [73, 74] (Figure 3). The finding that glomeruloid MVP can be present around rather than within intracerebral metastatic carcinomas [31, 59, 75] supports the notion that there may be something about the brain’s microenvironment that inhibits the formation of vascular sprouts. Although recent studies show that microvascular cells in GBMs express ECM-binding adhesion molecules relevant for angiogenesis (especially αvβ3) [76–78], and indicate that the enzymes for proteolytic degradation of perivascular ECM-barriers are available [79–82], inadequate production and/or activation of such adhesion molecules and proteases cannot be excluded. Furthermore, since in normal human brain and in most glial neoplasms ECM proteins such as fibronectin, laminin and the different types of collagen are essentially confined to the vessel walls [51], a scaffold of complementary matrix components for directional migration of microvascular cells may be lacking in glial (tumor) tissue. If this interpretation is correct, glomeruloid MVP would represent angiogenesis that is incomplete or even ‘frustrated’ because of the lack of a supportive

Figure 4a–d. Computer-assisted analysis of microvascular parameters in GBM showing the two forms of angiogenic reaction schematically represented in Figure 3: a, b: very high density of delicate microvessels; c, d: glomeruloid MVP; a, c: digitized microscopic image (PAS-collagen IV staining, original magnification 100 ×); b, d: delineation of microvascular profiles. See [84] for image analysis procedure and definitions of microvascular parameters. Although the contribution of these different angiogenic reactions to the perfusion of the tumor tissue remains to be established, the formation of delicate microvessels may well be functionally more important for the survival of tumor tissue than the striking glomeruloid MVP illustrated in c, d and in Figure 2.
ECM in the brain parenchyma. Some areas of GBMs do show an increased number of microvessels [83–85], in such areas the migration of microvascular cells into the glial tumor tissue during angiogenesis may have been facilitated by an altered expression of ECM components by tumor cells [77, 86–89].

Pathobiological significance of brain tumor angiogenesis

Quantitative aspects

Consideration of the origin of microvessels in a tumor must recognize two different populations: (a) preexistent host vessels incorporated into the tumor tissue and (b) microvessels arising from neovascularization due to the influence of angiogenic factors [71]. Especially in tumors showing diffuse infiltrative, rather than expanding, growth, many intratumoral vessels may be incorporated preexistent vessels rather than those newly formed by angiogenesis [90]. Consequently, a high, but normal, microvessel density can be expected in a diffuse astrocytoma infiltrating in the abundantly vascularized cerebral cortex [84, 85].

While florid MVP in glial tumors suggests a highly vascularized tumor, quantitative analysis showed that in areas with florid MVP the number of separate vascular structures was often not increased. In other areas, however, a very high number of delicate microvessels can be found, indicating that here classic angiogenesis with directional growth of microvascular sprouts may have occurred [83–85] (Figure 4). Recent studies of astrocytic and other brain tumors described an inverse correlation between microvessel density and survival [91, 92] and suggested that microvessel quantitation in biopsied brain tumors may provide improved prognostic information. In a biopsy study of human cortex infiltrated by malignant gloma, an increase in vessel density was only found in some cases of markedly and completely infiltrated cortex. This angiogenic reaction to tumor infiltration was considered as late, slow, and inconstant [93].

Qualitative aspects

The morphological appearance of the tumor vasculature does not allow direct judgments concerning the tumor blood flow [71, 94], and the functional contribution of the aberrant, glomeruloid MVP in GBMs is unclear. The coexistence of glomeruloid MVP and (sometimes extensive) necrosis in GBMs indicates that this angiogenic reaction occurred as a late, secondary effect of VEGF production by hypoxic tumor tissue, and that this reaction was insufficient or too late to save (part of) the endangered tumor tissue from death [95, 96]. If the last is true, then the interdiction of glomeruloid MVP may have limited therapeutic effect. Even a high number of delicate tumor vessels does not guarantee a high nutritive flow, as is illustrated by the fact that in human gliomas xenografted in nude mice the perfused fraction of tumor vessels ranged from 20% to 85% [97].

Interestingly, angiogenesis/MVP in GBMs does not seem to substantially contribute to extracranial metastatic spread, since extracranial metastases of GBMs are very rare. The occurrence of such extracranial metastases may be related to neurosurgical procedures (craniotomy, placement of ventriculoperitoneal shunt) [98]. Various hypotheses were generated to explain the lack of extracranial dissemination of glial tumors, including short survival of patients, elimination of metastatic gloma cells because of immunogenicity, differences in extracellular environment between brain and other tissues, and lack of relevant adhesion molecules on glioma cells [53, 54, 99–101]. Furthermore, in some tumor systems production of anti-angiogenic factors by the primary tumor may keep extracranially disseminated tumor cells in a dormant state [102].

Therapeutic opportunities

Anti-angiogenic therapy

Folkman in 1971 hypothesized that anti-angiogenic treatment is a potential therapy for solid tumors [1, 16]. The proliferation rate of ECs in tumors largely exceeds that in most adult tissues, this difference of-
membrane, migration and/or proliferation of ECs, and three-dimensional organization of microvessels [104, 105]. Some anti-angiogenic agents are now being studied in phase 1 and 2 clinical trials for solid tumors like breast, colon, lung, and prostate cancer [4]. It remains to be seen whether the anticipated beneficial anti-tumor effects are accompanied by unwanted compromises in wound healing, endometrial proliferation, etc. [105].

The efficacy of anti-angiogenic tumor therapy will be determined by the amount of tumor tissue that is dependent on neovascularization for sustained growth and the level of functional neovascularization in these areas. While solid, expanding brain tumors require ingrowth of blood vessels for survival and growth, tumor cells infiltrating in the well vascularized brain tissue will encounter nutrient supplies at frequent intervals as they advance [74, 93, 106, 107]. At first, such infiltrative tumors may escape the requirements of neovascularization and be resistant to anti-angiogenic treatment. When, however, in a certain area (e.g. during malignant progression) the amount of infiltrating tumor cells surpasses a critical number or the metabolic demands of the tumor cells increase, ultimately hypoxia will occur. In this tumor area the cells will become dependent on angiogenesis for survival and growth [106, 107]. In the interim, other areas of the same tumor (e.g. less malignant or less densely populated areas in the critical tumor periphery) may not be angiogenesis-dependent.

### Brain tumors

Inhibition of tumor angiogenesis forms an exciting new approach to control tumor growth, but its applicability for infiltrating human brain tumors such as GBMs remains thus to be established and there are anatomic reasons in GBMs to be cautious in ones enthusiasm. The diffuse infiltrative growth of many glial neoplasms could very well hamper the success of anti-angiogenic therapy. Glomeruloid MVP in such tumors indicates that angiogenic activity (and hypoxia) was present, but the functional significance and thus the effect of inhibiting this aberrant MVP could be limited. The efficiency of

---

**Figure 5 a, b.** (a) Subtraction angiography of internal carotid artery demonstrating extensive vascularization of a frontal meningioma and its dependence on external vessels for its supply; part of this tumor (indicated by asterisk) was fed by vessels from the middle meningeal artery; (b) immuno-light microscopic demonstration of very high microvascular density in meningioma using an endothelial marker (CD34/QBend staining, original magnification × 200).

fers a target for anti-cancer therapy [16, 103]. Based on new insights into the mechanisms of angiogenesis, novel therapeutic approaches targeting this process have been developed [4, 104, 105]. Numerous compounds display inhibition of angiogenesis under experimental conditions. While the mechanism of action of some of these compounds is not yet established, other factors interfere with one or more of the following steps in the angiogenic process: release of angiogenic factors, binding of angiogenic factors to their receptors, degradation of basement
anti-angiogenic therapy may be determined by the extent of the formation of delicate microvascular sprouts rather than by the degree of glomeruloid MVP in these tumors. Animal studies showing that the growth of glioma xenografts can be inhibited by anti-angiogenic treatment [10–13] should be interpreted with caution. While some studies describe extensive migration of human glioma cells xenografted in the brain of laboratory animals [108, 109], these glioma models are generally expansive rather than diffusely infiltrative [52]. According to the hypothesis of Folkman [1, 16] a more solid growth pattern of glioma models implicates an increased dependency on angiogenesis compared to the original, diffuse infiltrative, human neoplasms.

The question then arises if there are not brain tumors other than GBMs where anti-angiogenic therapy might, because of either ‘cidal’ or ‘static’ effect, be more effective. Meningiomas, meningeal hemangiopericytomas, medulloblastomas, hemangioblastomas, and metastatic carcinomas are examples of such lesions. Meningiomas and hemangiopericytomas form solid and expanding masses, implicating that these tumors require ingrowth of vessels for sustained growth (Figure 5). Consequently, these tumors may be good candidates for anti-angiogenic therapy, albeit a ‘static’ form which seeks to stabilize rather than eradicate a mass. Hemangiopericytomas are notoriously difficult to cure, in this setting a ‘static’ response of a recurrent lesion or lesions could be a major therapeutic advance. Some gliomas also show an expanding, rather than infiltrative growth pattern, and local recurrence of a mass rather than diffuse infiltration is the common expression of treatment failure. Such lesions include the richly vascularized pilocytic astrocytomas (especially the chiasmatic and hypothalamic types) and ependymomas. These should in our opinion be considered as potential targets for anti-angiogenic therapy.

**Perspectives and conclusions**

The hypothesis of Folkman that solid tumors are angiogenesis-dependent has stimulated an enormous amount of research that has increased our awareness and knowledge about the angiogenic process and how it might relate to brain tumor therapy. MVP in GBMs may provide a target for interference with tumor angiogenesis and growth. However, the applicability of anti-angiogenic therapy for human brain tumors is not yet established. The results of anti-angiogenic treatment of glioma-xenografts in animal models should be interpreted with caution, because the growth of these xenografts is often dissimilar from their human counterparts. The diffuse infiltrative growth of most human gliomas could hamper the success of anti-angiogenic therapy. Brain tumors with a more solid, expanding growth pattern may be additional neoplasms which are candidates for such therapy.

**Acknowledgements**

Mr. Jeroen A.W.M. van der Laak, M.Sc., and Mr. Willem G. Witte for excellent support with the preparation of the figures; Mrs. Monique Link, M.T., and Mr. Frank J.R. Rietveld, M.T., for expert technical assistance; Lee H. Monsein, M.D., The Johns Hopkins University School of Medicine, Baltimore MD, USA, for kindly providing the angio-gram in Figure 5.

**References**

8. Burger PC, Scheithauer BW: Tumors of the central nerv-
ous system. Atlas of Tumor Pathology. 3rd series, fascicle 10, Armed Forces Institute of Pathology, Washington DC, 1994


55. Tooth HH: Some observations on the growth and survival-period of intracranial tumours, based on the records of 500 cases, with special reference to the pathology of the glioma. Brain 35: 61–108, 1912


70. Böthling T, Haiva E, Kujala M, Claesson-Welsh L, Alitalo


98. Huang P, Allam A, Taghian A, Freeman J, Duffy M, Suit HD: Growth and metastatic behavior of five human glioblastomas compared with nine other histological types of

Address for offprints: P. Wesseling, Division of Neuropathology, Department of Pathology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands