Radiotherapy of brain gliomas. Diagnostic and therapeutic aspects.

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When deep X-ray therapy became available it soon was used in the treatment of supratentorial gliomas. Presumably because surgery alone was a disappointing venture in high grade gliomas as the glioma rapidly recurred even after major resections and because of the general notion of the effectivity of X-ray therapy in the treatment of cancer. At the end of the 1970s and the beginning of the 1980s a number of important advances took place which form the basis of modern radiotherapy for gliomas. In the first place the technological improvement in the tools of radiotherapy as the introduction of computer technology with CT scanning, high energy radiation, computerized treatment planning which allowed for accurate and reproducible treatment. Secondly, randomized trials proved the value of radiotherapy in GBM by prolonging survival. In the third place the introduction of histopathological classification systems such as the WHO system resulted in the identification of groups of gliomas with different genesis and prognosis in which the role of radiotherapy was different.
It was with basically these tools and knowledge that supratentorial gliomas were treated since the end of the 1970s up until the present day. In this thesis the impact on radiotherapy of several new developments in diagnostic and therapeutic possibilities were studied.

In **chapter 1** a large patient series (n=839) with a supratentorial glioma treated in Groningen in the period 1979 until 1999 is analysed in relation to radiotherapy. The primary feature of the analysis is the histopathologic grading and classification. It provides the most important prognostic factor for survival and it determines the indication and outcome of radiotherapy. However other prognostic factors also influence treatment decisions regarding radiotherapy such as age and performance of the patient . To some extent these prognostic factors are related to each other. In general low grade gliomas occur in younger patients and the high grade gliomas in older patients. Therefore after histopathologic grading age has lost some of its prognostic value. In the literature of the glioma the role of radiotherapy is separately analysed for each histopathologic category. Therefore the same approach was followed in this analysis.
In GBM the place of radiotherapy is best documented. It doubles the MST with an optimum dose of 60 Gy in 6 weeks local field irradiation and is the standard therapy after surgery. However radiotherapy in GBM is selectively applied in patients with the best performance. In Groningen radiotherapy was applied in the majority of GBM patients excluding those with poor performance. Recently selection factors were used involving the introduction of short fractionation schemes e.g. 15 x 3 Gy with the reduction of overall treatment time of 3 weeks for patients with less favourable prognostic factors such as age > 50 years and Karnovsky performance < 70.
Presently in A there is no evidence that radiotherapy prolongs survival. Therefore in prognostically favourable patients with young age and good performance radiotherapy is withheld. In symptomatic patients or in recurrent

gliomas radiotherapy is applied also as a consequence of lack of therapeutic alternatives besides surgery and the evidence that A may respond to radiotherapy. In Groningen radiotherapy was widely applied in A although the timing of radiotherapy was not evaluated (immediately after first surgery or after recurrence).
Technological improvements in radiotherapy did not improve survival in glioma. This probably means that technically more precise radiotherapy does not affect local control in glioma as long as the biological effectiveness of radiotherapy is unchanged. However radiotherapy dose reduction to normal brain tissue likely has reduced radiation induced brain damage which is of benefit to patients with a longer survival.
The problem in grading the astrocytic tumours is reflected in the definition of the AA and consequently also the role of radiotherapy. AA are treated as GBM although no separate data on the effectiveness of radiotherapy in AA exists. Histopathologically the AA cannot be sharply demarcated from A. This implies that some long term surviving AA patients are incorrectly graded A. In these patients the application of high dose (60 Gy) irradiation after surgery is of no proven value. The appearance of an oligodendroglial component in a glioma whether in case of a pure O or as a component in an A implies a better prognosis in comparison with the pure astrocytic tumours of corresponding grade of malignancy. Because these tumours are less frequent than A, the O tend to be included in low grade glioma studies and AO in high grade glioma studies. Therefore evidence regarding the role of radiotherapy in O is lacking. In general the same treatment approach is followed in O as in the astrocytic tumours of corresponding grade of malignancy. As O are biologically different from A with respect to chemosensitivity, genetically and possibly radiosensitivity, separate studies of O are needed in which the role of radiotherapy is defined.

Further refinements of the classification and grading system came from tumour kinetic studies. Tumour growth rate depends on the balance of cell proliferation and cell death. The proliferation rate was studied by MIB-1 which is an antibody against Ki-67 expressing non-G0 cells. This MIB-1 labelling was possible on archival paraffin embedded material allowing for large retrospective studies. The rate of cell death or apoptosis can also be measured on archival material using TUNEL labelling. In **chapter 2** both MIB-1 labelling index and TUNEL labelling index were used in a large retrospective patient series to assess their prognostic value. These indices were used within the current WHO classification system and not as a substitute for classification and grading. Proliferation and apoptosis were assessed within each histopathological category. It was found that the apoptotic rate appeared to be associated with the proliferation rate and was not an independent prognostic marker. For radiotherapy purposes the relevant distinction between A and AA is improved by using MIB-1. However from a biological point of view the distinction between A and AA is somewhat artificial as gliomas are regionally heterogeneous implying that the piece of tissue studied might not be representative for the tumour as a whole and in the course of time the low grade glioma has a tendency to progress to higher grades of malignancy.

Another aspect concerns the oligodendroglial tumours with a higher proliferation rate compared to the astrocytic tumours although they had a better prognosis. This apparent discrepancy was already known in the concept that the number of mitosis was of minor prognostic value in O. This points again to a different biology of oligodendroglial tumours and increases the uncertainty regarding the role of radiotherapy.

With immunohistochemical analysis of protein products the activity of genes involved in the regulation of proliferation and apoptosis can be investigated. In **chapter 3** some of these proteins are studied in relation to the outcome of radiotherapy. Some patients with a low grade glioma experience a long term recurrence free survival (>10 years) and it was shown that the gliomas in these cases had a low MIB-LI with low p53 protein expression possibly indicating non-mutated *TP53*. *TP53* plays a central role in regulating cell proliferation , differentiation , DNA repair and apoptosis. Mutations in *TP* 53 are strongly associated with progression of A to high grade astrocytomas. It is possible that in A low p53 immunoreactivity is associated with non-mutated *TP 53* and is accompanied by a low tendency to increase proliferation and progression rates. This is reflected by the low MIB LI and the long term progression free survival found in these tumours. These results further suggest that deregulation of proliferation is the hallmark of glioma progression. The protein expression of the genes involved in the apoptotic process did not differ between recurrent and non-recurring A. This suggests that the regulation of these apoptotic processes is not primarily involved in glioma progression.
In oligodendroglial tumours it is expected that O with detectable p53 immunoreactivity progress more rapidly to a higher grade of malignancy. However, mutations in *TP 53* are less frequent than in A. The analysis of pairs of primary and recurrent low grade gliomas showed an increase in proliferation parameters in the recurrent tumour compared to its primary. This is in accordance with the progression in malignancy of low grade gliomas. However this study suggest that radiotherapy plays no role in this process It may be hypothesized that some low grade gliomas are programmed to long term survival with or without radiotherapy and radiotherapy if applied does not increase proliferation and apoptosis in the recurrent tumour.

Radiotherapy does not "cure" glioma but radiotherapy certainly delays tumour progression by reducing tumour size and prolonging survival in GBM. To achieve this goal radiotherapy has to be directed to the central tumour mass only, without the need to encompass all areas of tumour cell invasion. New imaging modalities have been introduced. MRI with its superior soft tissue contrast and 3-dimensional depiction resulted in improved imaging of the glioma. The consequence of MRI for radiotherapy treatment planning was investigated in **chapter 4**. If the glioma showed no contrast enhancement e.g. in case of a low grade glioma, the observers had to rely on minor changes in density when using CT. This leads to greater interobserver variation than when in the use of MRI T2 weighted images for radiotherapy treatment field design. As the MRI T2 high

signal area encompasses the tumour area and because from within this region the low grade glioma will progress it follows that in case of a non-contrast enhancing glioma MRI should be used for radiotherapy treatment planning.

Other new imaging modalities include PET and MRSI. In **chapter 5** the ^{1}H spectroscopic imaging is introduced featuring some metabolic characteristics of the glioma. In the central tumour mass the NAA (parameter of functional neurones) is depressed consistent with neuronal loss and replaced by an increase of choline. Choline reflects the increased phosphocholine turnover in relation to membrane biosynthesis of proliferating cells. Lactate was also found in some gliomas reflecting increased glycolysis which is a feature of the metabolism of malignant cells. However, with the spectroscopic technique used, the lactate peaks cannot be fully discriminated from free fatty acids.

In **chapter 6** tyrosine PET is validated as a method to visualize and quantify protein synthesis of the glioma. Regardless of the malignancy grade the glioma exhibits an increased protein synthesis which can be measured with tyrosine PET. In some cases the area of increased choline depicted with MRSI seemed to correlate with the area of increased PSR both indicating the location of vital tumour tissue.

After radiotherapy the glioma will progress from within 2.5 cm of the outer rim of the contrast enhancing region in case of a GBM and from within the MRI T2 region in case of a low grade glioma. Therefore using MRI and CT is adequate for the purpose of treatment field design whereas metabolic imaging either with PET or MRSI is of no practical consequence for this purpose. On the other hand, PET and MRSI can be used for monitoring the radiotherapy treatment response as CT and MRI cannot discriminate vital tumour from necrotic tumour tissue, radiation induced brain damage or effects of surgery. In **chapter 7** proton spectroscopic imaging (MRSI) was used to monitor the response on radiotherapy of brain gliomas. To be certain that a true response of tumour metabolism was measured only biopsied gliomas were investigated. Metabolic responses found in several gliomas were the decrease of choline and lactate was accompanied by a shrinkage of tumour diameter on MRI. In case of low grade gliomas this adds to the evidence that although radiotherapy has no clear effect on survival it may induce tumour regression and tumour devitalization. The solid tumour mass of the glioma irreversibly has destroyed the existing brain parenchyma which is indicated by the irreversibly decreased NAA.
With longer follow-up studies supported by histopathological verification the value of metabolic imaging could be further improved although in the case of a brain tumour the taking of multiple biopsies in time is a difficult aim to realize. However, comparison of proton spectroscopy with histopathology has been

performed in other studies in the case of differentiating radionecrosis from recurrent brain glioma. Proton spectroscopy proved a useful tool for this purpose.

In **chapter 8** the response to radiotherapy in glioma was monitored with the use of tyrosine PET. The advantage of using tyrosine is the capacity to calculate the true PSR of the glioma before and after radiotherapy. The main results were again the response on radiotherapy of the volume of increased tyrosine uptake with no decrease of the PSR in the remaining tumour. This probably means that radiotherapy does not completely devitalize the glioma although it can induce tumour regression.

Conclusions

In this thesis the radiotherapy of patients with brain gliomas is studied from a clinical point of view. Historically, radiotherapy was used as a treatment modality for these incurable tumours even before the evidence came out of the survival benefit for GBM patients with radiotherapy and before the introduction of "computerized radiotherapy" with treatment planning, CT imaging and high energy irradiation. Using these modern techniques two decades of radiotherapy for glioma in Groningen were studied establishing the well known prognostic factors for survival with histopathologic classification and grading as the most important. Therefore the role of radiotherapy had to be studied for each histopathological category. Radiotherapy definitely improves survival in GBM although for the other histopathological categories the survival benefit is not proved. The lack of a curative opportunity with radiotherapy leads to the clinical policy of withholding radiotherapy in patients with a poor performance and in low grade glioma patients with prognostically favourable factors to avoid radiation-induced brain damage. The use of a grading system in astrocytic tumours implies an uncertainty in the discrimination between A and AA which has its consequence for the indication of radiotherapy. In this field the main advantage of the use of proliferation markers e.g. the MIB-LI has been demonstrated.
This study emphasizes the importance of the proliferation rate and not the apoptotic rate for the discrimination between high and low grade gliomas and the identification of long term recurrence free gliomas. The p53 protein has a role in the control of proliferation and apoptosis. This study suggests that long term prognosis in glioma depends on a normal functioning of *TP53* implying a controlled low proliferation rate. Apoptosis does not seem to play a role. Furthermore this study suggests that radiotherapy does not influence changes in proliferation or apoptosis in recurrent low grade gliomas. New imaging modalities changed the practice of radiotherapy but do not affect prognosis. MRI is to be preferred in the target definition of low grade gliomas. Metabolic imaging with MRSI or tyrosine PET can be used to monitor radiotherapy response of the glioma but persistently shows increased metabolism which indicates vital tumour tissue surviving radiotherapy.

Perspectives

Molecular biology and genetics will provide new prognostic factors, refinement of the classification and grading system and predictive testing for planning of treatment strategies. Some examples are the prognostic value of MIB-1 and p53 as outlined in this thesis. Another example is the chromosomal aberration in 1p and 19q predicting the response to chemotherapy in O.

New conformal radiotherapy techniques with intensity modulated radiotherapy (IMRT), stereotactic conformal radiotherapy (SCRT) or proton therapy may be used in reduction of radiotherapy dose to certain eloquent brain areas possibly leading to a decrease in radiation induced brain damage. An example is the avoidance of the brain areas containing proliferating and apoptosis sensitive stem cells e.g. the forebrain subependyma and the dentate gyrus of hippocampus.

New neuronavigation techniques in neurosurgery correlate the position of neurosurgical equipment during operation to its corresponding site on brain images. This will allow a correlation between the site of tumour sampling on imaging with histopathology. This implies an integration of imaging and pathology reducing the problem of tumour heterogeneity. If this will be extended to correlate metabolic imaging with histopathology new avenues will be opened in efforts to escalate the radiotherapy dose to areas of persistent vital tumour tissue. If these areas are small enough no symptomatic radionecrosis is to be expected obviating the need of repeated surgery. The idea of dose escalation with IMRT, SCRT, proton therapy or radiosurgery is based on the concept of a spatial discrimination between the tumour and normal tissue. Although this approach will not cure the glioma because of the clonogenicity of the tumour cells widely invading into functional brain parenchyma. However, a meaningful increase in survival may be expected. To cure the glioma the therapy inevitably has to be directed to these migrating tumour cells. Recently this was attempted by using neural stem cells. In a mouse model neural stem cells were engineered to produce the therapeutic immunomodulator interleukin-4. After injection into brain these therapeutic cells may travel along the white matter and potentially target the brain invading neoplastic cells *(Benedetti S., Pirola B., Pollo B., Magrassi L., Grazia M., Rigamonti D. Gene therapy of experimental brain tumors using neural progenitor cells. Nature Med. Vol 6 april 2000 : 447-450)*. Perhaps some of these techniques can be further developed to sensitize the invading tumour cells to low dose radiotherapy.