Summary, discussion and future perspective
Introduction

The mid- to long-term survival of glioma patients can be accompanied by a return to normal levels of functioning and well-being, but this evidently is not always the case. Patients may suffer from functional limitations and neurocognitive deficits which may have an impact on the HRQoL in the remaining survival time.

Cognitive deficits which are, in fact, quite common in glioma patients appear to be more global than would be expected from a focal lesion. It is hypothesized that the high prevalence of global cognitive deficits in brain tumor patients is caused by diffuse changes in the neural network organization of the brain.

The aims of this thesis were to explore the impact of a brain tumor on neurocognitive function and HRQoL and to understand the neurophysiological mechanisms underlying cognitive dysfunction by studying the strength and spatial structure of resting-state brain networks.

Summary

The first part of the thesis is focused on the evaluation of neurocognitive function and HRQoL in newly-diagnosed high-grade glioma patients. The two studies were part of a nationwide Dutch longitudinal study into the cognitive status and HRQoL of high- and low-grade glioma patients and their partners. In chapter 3.1, HRQoL of the high-grade glioma patient population is described. Inclusion in the study took place after surgery and before the start of radiotherapy. Self-perceived HRQoL (MOS SF-36) and brain tumor-specific symptoms (BCM-20) were assessed every 4 months up till 16 months after histological diagnosis. Out of the 68 included high-grade glioma patients, those who were still alive 2 years after initial diagnosis (long-term survivors) were selected. The results of HRQoL measurement in this group were compared with the results of those patients who died within a year after diagnosis (short-term survivors). The long-term survivors were found to show improvement in HRQoL during the course of their disease and even attain levels that are comparable to that of healthy controls, whereas short-term survivors started at a lower level and hardly showed any improvement. Furthermore, it was observed that a higher mental component score (derived from the SF-36) following surgery predicted a longer survival, although it did not independently add to the predictive value of ‘established’ patient and tumor characteristics. In the long-term survivor group, tumor recurrence interfered with HRQoL, and particularly with physical problems and feelings of future uncertainty. It was concluded that glioma patients with a long-term survival can regain a good quality of life, as long as there is no tumor progression.

In chapter 3.2, cognitive functioning of high-grade glioma patients during follow-up is described and the effect of tumor progression on cognitive function is evaluated. The included patients underwent a neuropsychological assessment every 8 months after the initial diagnosis until 16 months after inclusion. The patient group as a whole declined in cognitive function during the course of their disease. We observed that patients with tumor progression performed worse on neurocognitive tests than patients without
tumor progression. Remarkably, the deterioration in patients who suffered from tumor progression during follow-up could mainly be attributed to the use of antiepileptic drugs and not to the tumor. We therefore concluded that careful consideration when to start antiepileptic drug treatment and the choice of antiepileptic drug is important in the malignant primary brain tumor population.

**Chapter 4** contains the results of two studies concerning functional connectivity and network organization in patients with a variety of brain tumors. These studies show that there is evidence for widespread differences in network organization in the brain tumor population compared to a healthy control population. The eyes-closed resting state MEG recordings of 17 patients were compared to MEG recordings of 15 healthy controls. The data of these patients were collected from previous studies. In **chapter 4.1**, we evaluate functional connectivity by means of the synchronization likelihood in patients and controls, showing that, as hypothesized, brain tumor patients have extensive alterations in functional connectivity compared with healthy controls. Most importantly, the differences are not confined to the tumor area and are more prominent in patients with a tumor in the left hemisphere. In **chapter 4.2**, functional connectivity per frequency sub-band is further evaluated in order to determine whether differences involve connections between MEG sensors at a short- or at long-distance. A decreased functional connectivity in the long-distance connections within the higher frequency bands and an increased functional connectivity in short-distance connections within the lower frequency bands were observed. To evaluate whether differences in strength and spatial organization are evident, we used graph analysis as a mathematical instrument and found differences in the network configuration in brain tumor patients compared to the healthy controls. Compared with healthy control subjects, brain tumor patients showed a more random network organization.

Since it is expected that these network alterations are related to compromised neurocognitive function, we subsequently studied a more homogeneous group of glioma patients. For this study, 17 LGG patients who were clinically stable and 17 age-, sex-, and education matched healthy controls were included. In **chapter 5.1**, the results of a study in which we correlated cognitive function to resting state oscillatory brain activity in patients and healthy controls are presented. Indeed, a diffuse slowing of MEG background activity in LGG patients was found, which was associated with poorer executive functioning, information processing and working memory. In **chapter 5.2**, the synchronization likelihood (SL) as a measure of statistical interdependencies between MEG time series of the same patient population and their matched healthy controls was assessed. Evidence for a strong increase in the delta, theta and lower gamma band for long-distance functional connectivity was found. A decrease in the interhemispheric activity was found in the delta and lower alpha band. We observed a pathologically increased synchronization to be correlated with a more disturbed neurocognitive function in the delta, theta, and lower and upper gamma band. Up till now, it remained unclear whether changes in the mean level of coupling as we found in chapter 5.2 are also associated with changes in the global organization of functional
networks. In chapter 5.3, the phase lag index (PLI), (which is less sensitive to the effect of volume conduction than SL) is used to assess functional connectivity. The aim was to assess the functional connectivity and to evaluate the strength and spatial organization of the network. An increase in synchronization in the theta frequency band of the patient population was observed. Furthermore, differences between patients and healthy controls in the network organization were observed when the analysis made use of graph theoretical measures. We observed that the local clustering was significantly higher in the theta band in patients (more “small world organization”), whereas the opposite was true for the beta band (more random organization). The path length remained fairly stable across the different frequency bands. We furthermore evaluated whether the network organization was correlated to neurocognitive performance in the LGG patient population. Within the delta frequency band, an increased path length was associated with poorer executive functioning and attentional task performance. In the lower alpha band, an increased path length was associated with decreased verbal memory and an increased local clustering was associated with a poorer verbal memory. Within both the delta and lower alpha band, a lower degree correlation was associated with diminished attentional functioning and verbal memory.
HRQoL in brain tumor patients

Patients diagnosed with a brain tumor do not only have to cope with an incurable disease but are also confronted with cognitive dysfunction. For many years, studies evaluating new treatment protocols for cancer patients mainly focused on improvement of overall survival (OS) and progression free survival (PFS). It is nowadays recognized, however, that the choice of treatment should also entail careful consideration of its effects on health-related quality of life (HRQoL) during the remaining survival time.

Another development in studies evaluating treatment of patients with primary brain tumors is a focus on genetic and epigenetic markers in order to find a feasible and robust clinical diagnostic tool to decide which treatment option is appropriate for the specific patient. It is therefore possible that patients with a specific marker composition will be more responsive to new treatment protocols and therefore show a longer survival time. Especially for those patients it is of major importance to be informed on the influence of the treatment on the HRQoL in their remaining survival time.

We evaluated a group of newly diagnosed high-grade glioma patients to estimate the HRQoL at the start of radiotherapy and observed that patients with a long-term survival (e.g. more than 2 years) attained levels of the HRQoL which are comparable to healthy controls. Our results imply that it is worthwhile to try to prolong survival time and aim at maximal treatment results. We showed that patients appreciated their quality of life during and after tumor. Therefore, it is important that new aggressive treatment protocols not only lead to prolonging survival or progression-free survival time but also lead to quality time. For this reason, it is important to evaluate HRQoL during the course of the disease.

Are the current tools appropriate for the assessment of HRQOL in glioma patients?

Both the SF-36 and BCM-20 questionnaires contain questions on various aspects of the impact of having a tumor and the effect of tumor treatment on HRQoL, but they lack emphasis on several important aspects such as fatigue, mood disorders, and seizures.

Fatigue is a devastating symptom in cancer patients which is especially prominent in glioma patients.\textsuperscript{1,2} Fatigue can be a direct result of tumor treatment but is also frequently observed in long-term survivors. In the study of Struijk et al., nearly 40% of low grade glioma patients who were diagnosed and treated more than 8 years before and showed no clinical or radiological signs of tumor recurrence, complained of fatigue.\textsuperscript{1}

None of the 20 items of the BCM-20 refers to fatigue, whereas the generic QoL questionnaire we used, contains three questions directly concerning fatigue. Fatigue can be characterized by patients in various different terms, for instance as an overall lack of energy, sleeping problems, lack of concentration, mood disturbances and might therefore have an impact on physical, emotional, and cognitive domains of any HRQoL questionnaire.

It is important to evaluate fatigue separately as a multidimensional construct in order to
determine its effect on HRQoL. The challenge will be to further investigate the impact of fatigue and the recognition of mechanisms and treatment modalities leading to fatigue. This should eventually result in studies on the treatment of fatigue in primary brain tumor patients either pharmacologically or non-pharmacologically.

Patients frequently complain of fatigue, confusion and cognitive impairment. These symptoms are often associated with distressed mood. Depression as a neuropsychiatric symptom among brain tumor patients is quite common and varies between 15-38%. It remains difficult to differentiate between symptoms which are directly caused by the tumor and treatment itself and the reaction of patients who are confronted with the diagnosis of a brain tumor. Depressive symptomatology can negatively influence the self-perceived HRQoL. Pelletier et al., showed a high burden of depressive symptoms as measured by the Beck Depression Inventory-II (21-item self report questionnaire for measuring the severity of depressive symptoms) scoring system with 38% of 60 primary brain tumor patients scoring in the clinically depressive range. Comparatively, 15-30% of patients with a major medical condition will develop a depressive disorder somewhere in the course of their disease and an estimated 6-15% of cancer patients do. The number of patients in this study and the correlation with the HRQoL shows that a depressive disorder is a major clinical issue in brain tumor patients.

Another important issue, is the effect of epileptic seizures and the use of anti-epileptic drugs on HRQoL. Klein et al., showed that a higher epilepsy burden was negatively associated with neurocognitive functioning and HRQoL. Incomplete seizure control, despite the use of antiepileptic drugs, does have an impact on the self perceived HRQoL. The BCM-20 only contains a single item concerning epilepsy (whether the epilepsy exists or not and to what extent). Since the above mentioned study showed the dualistic effect of epilepsy burden on both neurocognitive functioning and HRQoL, it is important to obtain information on both the severity of epilepsy and the use of antiepileptic drugs.

Is it possible to replace the patient’s questionnaire by the proxies questionnaire?

A problem in the assessment of HRQoL in brain tumor patients is the relatively small sample size of previous studies including our study. Another difficulty is the assessment in those patients who are not able to fill out the questionnaire because of aphasia or physical limitations or due to the lack of energy. For this reason, the assessment of HRQoL can be biased by the exclusion of subsets of patients. Sneeuw et al. evaluated the response agreement between brain tumor patients and their spouse or close companion. They used the QLQ-C30 and found relatively high levels of agreement for symptoms like nausea/vomiting, dyspnea, constipation, weakness of both legs, and for physical and role functioning. Relatively low levels of agreement were noted for social functioning, fatigue, and future uncertainty. Compared to the brain cancer
patients, the proxies rated the patients as having more disabilities than the patients themselves and the disagreement was more extensive in mentally confused patients. Brown et al., in a study on the HRQoL in HGG patients, showed a high agreement between patients and proxies which was weaker in patients with cognitive dysfunction (MMSE score of 26 out of 30 or lower). From this study it can be concluded that if the patient assessment is the gold standard, there is some disagreement between patients and proxies, which is higher in patients who are mentally confused. It is expected that the completion rate of QoL measures in especially the mentally confused patients can be poor, since it would be more difficult for them to answer all the questions. Replacement of the questionnaire by the proxies in those brain tumor patients with cognitive dysfunctions is not recommended.

**Neurocognitive function in brain tumor patients**

Whether or to what extent changes in neurocognitive function occur in the course of the disease is important for both patients and caregivers. We observed a decline in neurocognitive function in the course of the disease of HGG patients, which was more prominent in those patients who suffered from tumor recurrence during the study.

In brain tumor patients, neurocognitive functioning can be affected by the tumor and by its treatment, by epileptic seizures and by the use of antiepileptic drugs. Since neurocognitive function has a significant impact on HRQoL, it is of major importance to obtain information on these functions in the course of the disease. It is worthwhile to try to prevent or delay neurocognitive decline in the course of the disease by effective tumor treatment, adequate treatment of epilepsy, and choosing antiepileptic drugs with minimal impact on neurocognitive function.

**Does neurocognitive impairment influence HRQoL of brain tumor patients?**

There are only a few studies that directly correlated the neurocognitive performance (by using a cognitive test battery) with a self-perceived HRQoL. One study evaluated neurocognitive performance and HRQoL in primary brain tumor patients before evidence of radiological tumor progression. In this study, it was shown that neurocognitive function began to worsen before the MRI showed evidence of tumor recurrence, whereas the HRQoL (different self-perceived questionnaires were used in this study) showed a decline only after evidence of the tumor progression. Klein et al. showed that newly diagnosed HGG patients who were more impaired on neuropsychological tests, reported more neurocognitive dysfunction. The self-reported cognitive dysfunction was associated with both the mental health and social functioning subscale of the SF-36. Self-reported mental health was also associated with information processing capacity. Also Giovagnoli et al., showed an association between the neurocognitive function and HRQoL in glioma patients with recurrent disease. One study directly evaluated the association of HRQoL and neurocognitive function in patients with brain metastases of breast and lung cancer. In this study, neurocognitive performance was strongly
correlated with HRQoL at baseline and remained correlated after whole brain radiotherapy. Also in this study, neurocognitive decline preceded the decline in HRQoL. From these studies we can conclude that neurocognitive function is both associated with HRQoL and is a sensitive tool in predicting changes in HRQoL.

**Which antiepileptic drug can be prescribed in brain tumor patients with seizures?**

Having epilepsy with incomplete seizure control may have a negative impact on HRQoL, whereas the use of antiepileptic drugs for seizure control may interfere negatively with neurocognitive function. Antiepileptic drugs, such as valproic acid, carbamazepine, and phenytoin have been used frequently for seizure control. Besides the aforementioned impact on neurocognitive function, these antiepileptic drugs interfere with each other as well as with other drug types, such as dexamethasone and chemotherapy. The reason for interference is that these drugs are all metabolized by the same pathway (CYP450 system in the liver). In recent years, new antiepileptic drugs (e.g. levetiracetam, gabapentin and lamotrigine) have been developed, which are not metabolized by this pathway and therefore do not interfere with other drugs. It is also suggested that these newer antiepileptic drugs have less impact on neurocognitive functioning although studies showing these effects are still lacking. Future studies evaluating these effects in brain tumor patients will be important.

**Understanding neurophysiological mechanisms of neurocognitive function in brain tumor patients: local destruction or global disruption?**

Brain tumor patients suffer from reduced attentional functioning, working memory, psychomotor speed, and executive functioning. As indicated above, these neurocognitive problems are likely to interfere with the quality of life of the patients. The origin of these neurocognitive deficits in glioma patients is not completely understood. One might expect that neurocognitive deficits simply reflect the specialized functions of the local brain regions that are affected by the disease ('local destruction'). However, it appears that the neurocognitive deficits in brain tumor patients are more diffuse than one would expect on the basis of the localized lesion. This is in line with the hypothesis that adequate neurocognition depends on large-scale dynamical neural systems. It is expected that the high prevalence of neurocognitive deficits in brain tumor patients is caused by changes in synchronization patterns and network organization induced by the tumor, by tumor-related epilepsy and by tumor and epilepsy treatment ('global disruption'). These dynamic neural systems can be studied through magnetoencephalography (MEG).
Why do primary brain tumor patients demonstrate altered functional connectivity and neural network organization?

By measuring resting-state functional connectivity we observed differences between patients and healthy controls. In our first studies in patients with a variety of brain tumors, we observed an increase in synchronization in the lower frequency bands and a decrease in long-distance synchronization in higher frequency bands. Using the SL in low-grade glioma patients, we observed an increase in both the lower and higher frequency bands, whereas only an increase in the theta band was observed by using the PLI.

Evidence of pathologically increased synchronization has also been found in other patient groups, including those with Alzheimer’s disease (AD). Locatelli et al., showed an increased coherence in the delta and theta band. Another study showed an increase in the theta, beta and gamma band in AD patients by using the SL, although a study using the PLI observed a decrease in alpha and beta band functional connectivity in the AD patients. In Parkinson’s disease (PD) patients, increased functional connectivity was observed in different frequency bands depending on their disease stage. Increased synchronization in the theta frequency band, as we have shown by using the PLI, has been observed before in other patient groups, such as autism spectrum disorders (ASD) and major depression.

In one recent study, the PLI was used to compare functional connectivity in brain tumor patients before and after resective surgery. A significant decrease in theta band functional connectivity was found after surgery, which was hypothesized to be a result of a normalization due to the resection of the lesion. Moreover, those patients displaying a major decrease in synchronization were more often free of epilepsy after surgery compared to the patients with just a small decrease in synchronization.

The increased functional connectivity patterns in primary brain tumor patients could be due to compensatory mechanisms. It is possible that LGG patients have to increase functional connectivity in order to compensate for the poorer neurocognitive performance, whereas the healthy controls do not have to compensate. If this is true, this mechanism is failing since we observed such an increased synchronization still to be correlated with a worsened neurocognitive performance. A study exploring the correlation between EEG synchronization and verbal memory in patients with mild cognitive impairments showed that during resting state, patients’ verbal memory scores correlated negatively with the synchronization likelihood in the alpha frequency band. These authors also proposed a compensational mechanism in patients with mild cognitive impairment: the increased synchronization in the lower alpha band could mean that the brain tries to adjust to the deleterious effect of synchronization on cognition, which is then also failing.

Bookheimer et al. evaluated patterns of brain activation during functional MRI scanning in healthy subjects, half of them being carriers of the APOE e4 allele, which has a dose-related effect on risk and the age of onset of late-onset familial Alzheimer’s disease. They found a greater increase in signal intensity in brain regions necessary for tasks requiring memory among carriers of this allele compared with non-carriers. They suggest that in persons at risk for Alzheimer’s disease, such increased brain activity may
effectively serve as a compensatory mechanism, wherein subjects use additional cognitive resources to bring memory-related performance to a normal level.\textsuperscript{20}

By analyzing the spatial configuration of brain networks in the LGG patients compared to healthy controls we observed that for both patients and controls, the clustering coefficient was twice as high as the value of the clustering in random networks whereas the path length was just slightly higher than in random networks. Therefore, brain networks show a small world configuration in both patients and controls. Local clustering was increased in LGG patients in the lower frequency band (consistent with a more regular network) and decreased (indicating a more random network) in the higher frequency bands. The path length remained fairly stable over all frequency bands compared to healthy controls.

In our study, an increased path length (more regular network) was associated with poorer executive functioning and attentional task performance in the delta band, and was associated with decreasing verbal memory within the lower alpha band within the LGG patients. Increased local clustering (more regular network) was also associated with poorer verbal memory in the lower alpha band.

Recently, van den Heuvel showed a negative association between path length and intelligence quotient (IQ), using resting-state fMRI data.\textsuperscript{22} Another study observed a lower score on the Mini Mental State Examination (MMSE) to be correlated with a longer pathlength in Alzheimer's disease AD patients by using EEG data sets.\textsuperscript{23} Li et al. correlated intelligence with structural brain organization using diffusion tensor tractography, and showed a higher global efficiency and shorter path length in the high intelligence group using both weighted and unweighted analysis.\textsuperscript{24} In another study of Stam et al., PLI-weighted connectivity networks were calculated and characterized in AD patients and non-demented control subjects showing a decreased clustering coefficient and shorter path length (closer to a random network) in the AD patients without an association with the MMSE.\textsuperscript{14}

In conclusion, differences in network organization can be observed in patients suffering from cognitive deficits due to an underlying neurological disease. AD patients, for instance, show a more random network organization irrespective of the method of analysis used. In the brain tumor population, we also observed a more random organization in the in the subset of patients with a mixture of brain tumors and in the subset of LGG patients. By using the phase lag index, a more regular organization was observed in the lower frequency band and a more random organization was observed in the higher frequency band. A more regular network organization (longer path length and higher local clustering) in the lower frequency bands, as observed in the LGG patient population, was also associated with neurocognitive deficits.

Besides the cognitive deficits in the brain tumor population, many patients are confronted with epilepsy. Ponten et al. showed an increase in synchronization during mesial temporal lobe seizure activity (increased SL in the delta band before the rapid discharges, in the delta, alpha and beta band during discharges and in all frequency bands after the rapid discharges).\textsuperscript{25} A more regularized network (higher C with a higher L) was observed during both mesial temporal lobe and absence seizures compared to the pre-ictal state.\textsuperscript{25,26} The random network organization might synchronize more easily and therefore be more vulnerable to seizures.
Our LGG population, which consisted of patients with a varying epilepsy burden showed a more random organization in the higher frequency bands. It will be interesting to evaluate the interictal network organization in a brain tumor population with epilepsy burden, to see whether their network organization is more prone to generate seizures.

**Future perspectives, using MEG in brain tumor patients**

There are several methodological as well as conceptual issues which have to be dealt with. What is the optimal way to convert functional imaging data to compute functional connectivity and to perform graph theoretical metrics in order to do further analysis? We, for instance, used two different non-linear measures to calculate functional connectivity. Are these reliable measures? We computed the C and L as a function of degree $K$, meaning that the computed graphs have a fixed average number of edges per vertex. Although the choice of $K$ is rather arbitrary, it gave us the opportunity to compare networks of different patient groups irrespective of the number of connections. It needs to be emphasized, however, that, irrespective of the method of analysis, network organization patterns in different patient populations have been identified with some consistency.27

Although a number of methodological issues still have to be considered, an interesting option for the future will be to study the dynamics of a network organization in the course of a disease. Both the influence of treatment (surgery, radiotherapy, chemotherapy) and the influence of tumor growth or tumor recurrence on network organization are important to explore. Will changes in a network organization be associated with clinical symptoms, such as cognitive deterioration or epilepsy? What will be the influence of various treatment scenarios on network properties and will this give new insight into the significance of different treatment modalities? Previous simulation studies evaluating the functional consequences of focal lesions in a computational model showed that lesions in different brain regions of the cerebral cortex have different and widespread effects on the pattern of functional connectivity.28,29

Even effects in the contralateral hemisphere were observed, which is in agreement with our own empirical studies. The pattern and extent of the dynamic effects not only depend on the localization of the lesion but also on the network properties at the lesion site and the way these regions were targeted (i.e. random attack or targeted attack of highly connected nodes ("hubs")). Incorporation of the results of the aforementioned longitudinal studies in brain tumor patients in computational models may be used to optimize these models and eventually allow us to predict the effects of e.g. cognitive function of patients with a brain tumor at a specific location undergoing treatment.

Many patients with a LGG do suffer from epilepsy, which can be refractory. It will be interesting to investigate the influence of changing network organization on the prevalence and manifestation of epileptic seizures. Are brain tumor patients due to their specific organization of the network more prone to suffer from epileptic seizures than others? Do brain tumor patients with a higher epilepsy burden have a more random network organization in contrast to patients with a lower epilepsy burden? Or do these patients more often suffer from refractory
epilepsy? Will it be possible to predict the future occurrence of epilepsy in those tumor patients who did not have seizures? These are a number of issues which possibly can be dealt with in future research. The aforementioned studies have shown that there are differences in the functional connectivity and network organization between brain tumor patients and healthy controls subjects that could be responsible for the neurocognitive deficits in patients. Future studies could possibly give us more insight in the underlying pathophysiological mechanisms underlying neurocognitive deficits in brain tumor patients and network changes in brain tumor associated epilepsy.
References