Chapter 6

General discussion
With increasing incidence and improved survival rates, quality of life following cancer and cancer treatment has become an important area of research. Adjuvant systemic therapies, such as chemotherapy and endocrine treatment, are widely used to treat breast cancer. However, these therapies have also been associated with adverse effects, including cognitive decline and changes in brain structure and function\(^1\). Also, recent studies suggest cognitive impairment and differences in brain measures in breast cancer patients compared to no-cancer controls even before the start of treatment\(^1\).

The objective of this thesis was to prospectively study the effects of cancer and cancer treatment on cognitive function and brain structure and function. We included newly-diagnosed breast cancer patients scheduled to receive anthracycline-based chemotherapy with or without endocrine treatment (BC+SYST) and compared them to breast cancer patients not requiring systemic treatment (BC), as well as age-matched no-cancer controls (NC). Multimodal MRI, neuropsychological testing and patient-reported outcomes were collected after surgery, but before the start of adjuvant treatment. A follow-up evaluation took place at approximately six months after the last cycle of chemotherapy, or at matched intervals, to assess the effects of systemic treatment.

In this final chapter, I first summarize the aims and main results of the studies presented (Chapter 2-5). Subsequently these results will be discussed and (clinical) implications of the results will be presented. Furthermore, we will discuss methodological limitations and suggestions for future research will be provided.
SUMMARY

The first chapter of this thesis (Chapter 1) provides a general introduction to the work described in this thesis.

In Chapter 2 cognitive function and brain structure and function were studied prior to adjuvant treatment. We assessed overall cognitive performance by calculating the Mahalanobis distance, a method that assesses deviant profiles, taking into account correlations between tests and variance within the tests in the control group. In addition, cognitive domain scores were analyzed to assess whether differences in cognitive performance were global or more restricted to a specific cognitive domain. Following recommendations of the International Cancer and Cognition Task Force, we also calculated cognitive impairment compared to the no-cancer control group and subsequently tested the proportion of patients classified as impaired. Multimodal MRI was acquired to allow for assessment of regional grey matter volume, white matter integrity, brain metabolites, and brain activation. To control for potentially confounding psychosocial factors, patient-reported outcomes as well as hair cortisol were collected.

We found that breast cancer patients, regardless of required treatment (BC+SYST, n=32; BC, n=33), showed significantly worse cognitive performance compared to no-cancer controls (NC, n=38), as measured with the Mahalanobis distance. When assessing specific cognitive domains, no differences between the groups were found. Also, no significant difference in proportion of impaired patients was found. Taken together, these analyses suggest a global cognitive impairment with subtle differences across the entire battery of tests. The findings of worse overall cognitive performance were no longer significant when scores of fatigue, perceived stress or anxiety and depression were included. We found prefrontal hyperactivation with increased task load on a planning task, but not during a memory task, in both patient groups compared to no-cancer controls. These differences in brain activation were no longer significant when fatigue was included in the model, while other patient-reported outcomes did not elicit the same effect. Widespread lower white matter integrity was found in both patient groups compared to no-cancer controls. However, no differences in white matter integrity were found when fatigue was included in the model. Groups did not show differences in regional grey matter volume, brain metabolites or white matter macrostructure. This was the first study in breast cancer patients that combined multimodal MRI, neuropsychological measurements and patient-reported outcomes before administration of systemic therapy. This allowed us to additionally assess the effects of psychosocial factors on different modalities, and thereby identify the role of fatigue in the pretreatment differences between breast cancer patients and no-cancer controls.
In Chapter 6 we studied the effects of systemic treatment on cognitive function. Assessments took place at approximately six months after the last cycle of chemotherapy, or at matched intervals. Cognitive performance was analyzed using two methods: 1) differences per test at the group level at follow-up, adjusted for pretreatment scores, were analyzed; 2) multivariate normative comparisons (MNC), a method adequate for small sample sizes, were performed to identify cognitively impaired patients. MNC compares test scores against the distribution of those scores in the control group. Calculations were based on performance residual scores, the difference between an individual’s scores and her predicted scores, based on baseline performance, age and IQ. In addition, confounding effects of psychosocial and biological factors were assessed. We hypothesized lower cognitive performance in breast cancer patients who had received systemic treatment, compared to both other groups. We also expected to find a relation between follow-up performance and measures of fatigue.

No significant differences in neuropsychological performance were found at the group level. However, effect sizes (range: 0.41 - 0.71) indicated worse performance on tests of attention, verbal memory, and executive function in breast cancer patients who received systemic treatment (BC+SYST, n=31) compared to breast cancer patients who did not require systemic treatment (BC, n=24), as well as no-cancer controls (NC, n=32). Assessing overall cognitive performance using MNC, a higher proportion of the patients who received systemic treatment were classified as cognitively impaired (16%), compared to the patient group that did require systemic treatment (4%) and no-cancer controls (6%). Further investigating those patients classified as cognitively impaired and comparing them to unimpaired patients, we found that premorbid verbal IQ was lower in impaired patients. Lower premorbid IQ has been related to a higher risk of developing cognitive impairment following systemic treatment. Also, impaired patients showed worse functioning on several measures of quality of life. However, given the relatively small number of impaired patients, these observations on vulnerable patients need further exploration in larger studies. Given the strong relation between fatigue and several outcome measures prior to treatment, we studied the role of fatigue, as well as several other psychosocial and biological factors on cognitive performance. However, none of these factors affected the results of our analyses. Taken together, these results show that cognitive impairment occurs in a subset of patients receiving systemic treatment. Although differences in several clinical and psychosocial factors between impaired and unimpaired patients were apparent, the predictive value for the development and course of cognitive impairment after systemic treatment should be further investigated.

In Chapter 4 we report on the results of analyses on brain activation during cognitive task performance. While previous prospective studies administered a single cognitive task, we employed two tasks. This allowed us to draw conclusions about the effects of cancer and cancer treatment on the two most commonly affected cognitive domains, memory and executive function. Given the relation between prefrontal hyperactivation and lower levels of fatigue at baseline, we assessed the relation between patient-reported outcomes and brain activation at follow-up as well as changes in brain activation between baseline and follow-up. We hypothesized
that patients who received systemic treatment would show larger deviations in brain activation, regardless of cognitive task, compared to breast cancer patients who did not receive systemic treatment and to no-cancer controls. Given the relation between prefrontal hyperactivation and levels of fatigue before treatment, we expected to find an effect of fatigue on brain function at the follow-up assessment.

Following systemic treatment (BC+SYST, n=28), breast cancer patients showed increased activation of parietal brain regions with increasing task difficulty of an executive functioning task, compared to their pretreatment baseline. Patients who did not require systemic therapy (BC, n=24) showed a decrease in parietal activation during executive functioning compared to patients who received systemic treatment as well as to no-cancer controls (NC, n=32). These results might indicate decreased neural integrity as a result of neurotoxic side effects of systemic treatment, leading to parietal over-recruitment to maintain performance. No significant differences in brain activation or task performance during episodic memory performance were found between the groups. It could be that the task was too difficult to detect subtle differences. However, previous studies by our group did show differences in brain activation during episodic memory ten years after treatment. This suggests that it could also be that the effects of cancer and cancer treatment are specific to the task employed, and become more generalized after a longer period of time. Prior to treatment, a relation between frontal activation during executive functioning and fatigue was found. After treatment, a relation between pretreatment fatigue scores as well as their change over time and parietal activation was found in the patient group not receiving systemic treatment. No relation between psychosocial factors and brain activation was found for patients who received systemic treatment.

In Chapter 5 we describe analyses of brain white matter macro- and microstructure after systemic treatment. Prior to treatment, breast cancer patients showed widespread lower white matter integrity compared to no-cancer controls. These results were associated with higher levels of fatigue. We subsequently acquired DTI data in the same group of subjects at six months after the last cycle of chemotherapy, or at matched intervals. We hypothesized that patients receiving systemic treatment would show a stronger decrease in white matter integrity compared to BC patients not requiring systemic treatment, as well as no-cancer controls. In addition, we expected baseline measures of fatigue to be related to changes in white matter integrity in patients.

White matter macrostructure was not severely affected (according to Fazekas ratings) in any of the groups. No significant group differences were found, although a clear shift from Fazekas 0 to Fazekas 1 was apparent in the patient groups (BC+SYST, n = 26; BC, n=23), which was absent in the no-cancer control group (NC, n=30). A decline in white matter microstructure, assessed using DTI, over time was found in all groups. White matter integrity in the right superior longitudinal fasciculus and corticospinal tract showed a larger decline in patients who received systemic therapy compared to patients who did not require systemic treatment. Patients who had received systemic treatment also reported worse physical functioning, higher levels of fatigue and more cognitive complaints. We did not find an association between cognitive decline and decline in
white matter integrity. This is in contrast to a previous study by Deprez et al.\textsuperscript{2}. It could be that the cytostatic agents administered in our sample have smaller effects on white matter integrity than other agents, such as 5-fluorouracil. Another explanation could be that some recovery had already occurred at the moment of the second assessment. Also, the applied statistical methods might be less sensitive to these subtle changes.

Taken together, these results show that cognitive impairment and impaired brain function and structure may already exist prior to adjuvant treatment for breast cancer. These pretreatment differences between breast cancer patients and no-cancer controls were associated with fatigue. We also found a relation between pretreatment cognitive function and perceived stress as well as symptoms of anxiety and depression. Also, other studies have shown that other psychosocial and biological factors, such as worry\textsuperscript{3}, stress, and time since surgery\textsuperscript{4–6}, may also play a role in pretreatment cognitive and brain function. It could be that these biological and psychosocial factors represent another underlying nonspecific factor, which can be expressed in different measures. After treatment, breast cancer patients who received systemic treatment showed more cognitive impairment, changes in brain activation and stronger declines in white matter integrity than patients who did not require systemic treatment as well as no-cancer controls. This shows that systemic treatment for breast cancer has adverse effects on cognitive function as well as brain function and structure. We found differences between impaired and unimpaired patients on clinical and psychosocial factors, but given the small sample size, these findings should be further investigated.
DISCUSSION

Breast cancer patients already show cognitive impairment and differences in brain activation and white matter integrity prior to treatment, regardless of the type of treatment they are scheduled to receive. After treatment, cognitive impairment is more frequently found in patients who received systemic treatment, compared to patients who did not require systemic therapy and no-cancer controls. Also, after systemic treatment, breast cancer patients show different patterns of activation, which are dependent on the type of task that is administered, as well as task difficulty. Decline in white matter integrity is largest in patients who received systemic treatment; patients who did require systemic treatment show some recovery of white matter integrity over time. These findings suggest that differences in brain functioning and structure may underlie cognitive impairment in breast cancer patients.

Pretreatment findings

Several direct as well as indirect mechanisms have been proposed in cancer and cancer treatment-related cognitive impairment. Preclinical studies have found evidence for both types of mechanisms and associated impairments in behavior following the administration of cytostatics. MRI studies have demonstrated changes in brain function and structure to occur after chemotherapy. We found that breast cancer patients, regardless of the treatment plan, showed cognitive impairment, as well as differences in brain function and structure even before adjuvant treatment when compared to controls who did not have cancer. This suggests that other factors not related to cancer therapies may also play a role. It could be that cancer diagnosis has psychosocial effects, regardless of the disease itself, and that these psychosocial factors play a role in pretreatment cognitive impairment. Another possible explanation could be that due to the cancer, levels of pro-inflammatory cytokines are elevated and that these result in changes in brain measures as well as cognitive functioning. Higher levels of pro-inflammatory cytokines have been associated with cancer-related fatigue. Fatigue has been associated with changes in cerebral blood flow which in turn lead to changes in brain function and structure, as well as changes in cognitive function. An alternative theory is a shared vulnerability for cancer and cognitive impairment. However, no evidence has yet been provided to support this theory.

Our finding of a relation between fatigue and cognitive function prior to treatment has since been replicated in a study with a heterogeneous group of cancer patients, including patients with cancer of the breast, lungs, digestive or urological tract, and gynecological or hematological malignancies. This study showed that higher levels of fatigue as well as lower premorbid IQ were associated with pretreatment cognitive function. Other factors which have been related to pretreatment cognitive function include worry, stress, cancer stage, and comorbidities. The clinical relevance of these associations is yet unknown, as none of these factors has been proven to reliably predict posttreatment outcomes in cognitive function. Interestingly, we found that pretreatment fatigue was related to subsequent decreased white matter integrity but only in patients who did not require systemic treatment. None of the pretreatment psychosocial or...
biological factors which were included in our study were related to posttreatment outcomes in patients who had received systemic treatment. This suggests that different mechanisms, or combinations thereof, may play a role in posttreatment cognitive function, including psychosocial factors and toxicity of systemic treatment.

Posttreatment cognitive function

A large number of studies has been directed toward studying the effects of chemotherapy on cognitive function. Prospective studies have shown estimates of up to 60% of cancer patients demonstrating cognitive impairment after cancer treatment\(^1\). In our study, 16% of patients who had received systemic treatment was classified as cognitively impaired compared to 4% of patients who did not require systemic treatment and 6% of no-cancer controls. These numbers are lower than several previous studies, which might have several explanations. First, the patients participating in our study were relatively young and highly educated, factors which have both been related to better posttreatment cognitive performance\(^2\)\(^-\)\(^4\). Second, different methods have been used across studies to determine cognitive impairment. Statistical models and criteria for cognitive impairment have been shown to influence study outcomes\(^5\)\(^-\)\(^7\). We chose to apply multivariate normative comparison because it has been shown to be sensitive to deviations in the cognitive profile\(^8\), a measure which has been shown to be a significant predictor for daily functioning\(^9\). Another advantage of this method is that it assesses the complete test battery, thereby eliminating the multiple comparison problem\(^8\). Since a number of studies do not correct for the number of tests included in the analyses, frequencies of cognitive impairment in those studies might be overestimated. Several studies have found that the type of chemotherapy or endocrine treatment could have an effect on cognitive and brain measures\(^10\)\(^-\)\(^12\). However, treatment in this study is similar to most comparable studies, with similar cytostatic agents and proportions of patients who receive endocrine treatment.

We found prominent differences in psychosocial factors between patients classified as impaired and unimpaired. Interestingly, impaired patients had lower premorbid IQ’s than unimpaired patients. This is in line with previous findings that suggest that higher cognitive reserve, measured as higher IQ, is associated with less decline in cognitive function following cancer treatment\(^2\)\(^1\)\(^2\). Higher cognitive reserve indicates resilience to damage, and may be influenced by factors such as education and lifestyle. An individual with higher cognitive reserve might be better capable of coping with brain damage by employing pre-existing processes or compensatory processes\(^13\). A large number of studies has shown evidence of better outcomes with higher cognitive reserve in normal aging and Alzheimer’s disease\(^1\). Our sample had a relatively high level of education, and high IQ, which may have led to an underestimation of cognitive impairment following cancer treatment due to an overrepresentation of patients with a high cognitive reserve.
Posttreatment brain activation

To further our understanding of cancer and cancer treatment-related cognitive impairment, functional MRI (fMRI) is a frequently used tool to assess brain function during cognitive performance. Thus far, tasks of executive functioning and memory have been most frequently employed to study brain activation in breast cancer patients. Taken together, studies of executive function show a robust pattern of prefrontal and parietal hypoactivation in patients treated with chemotherapy\textsuperscript{32–35}. During memory encoding, a decrease in prefrontal and medial temporal activity was found in two studies\textsuperscript{32,36}. Contrasting memory retrieval with memory encoding demonstrated hypoactivation in insula and orbitofrontal cortex in patients who had received chemotherapy\textsuperscript{4}. These studies suggest a pattern of hypoactivation in patients treated with chemotherapy.

Contradicting these findings of task-related regional hypoactivation after cancer treatment, we found increased parietal activity over time in patients who had received systemic treatment. It could be that, as an initial response to decreased neural integrity due to the effects of systemic treatment, regional hyperactivation reflects compensatory processes to maintain adequate levels of performance. This has also been demonstrated in patients diagnosed with mild cognitive impairment, where brain activation initially increased with patients being able to maintain functioning at a higher level, while activation decreased with declining cognitive functioning\textsuperscript{37,38}. However, previous fMRI studies in breast cancer have generally shown hypoactivation, regardless of time since treatment. One exception is the longitudinal study of McDonald and colleagues\textsuperscript{35} who showed a pattern of hypoactivation at one month post treatment, with activation returning to normal in some areas and hyperactivation in others at a one-year follow-up.

Breast cancer patients after systemic treatment only demonstrated hyperactivation with increasing task difficulty during executive function. No significant differences were found during memory encoding or retrieval. Interestingly, the only other study thus far applying two task paradigms in the same sample, reported parietal hypoactivation during a task of executive function as well as during memory encoding\textsuperscript{32}. This parietal involvement, irrespective of the task being performed, suggests that cognitive impairment after cancer and cancer treatment could be attention-related\textsuperscript{39,40}.

Posttreatment white matter integrity

Following preclinical studies which showed demyelination as a result of administration of cytostatics\textsuperscript{41–43}, DTI has been applied to study white matter microstructure in vivo in humans\textsuperscript{2}. To date, six studies have been published studying white matter integrity in breast cancer patients using DTI\textsuperscript{44–49}. Of these, Abraham et al.\textsuperscript{44} as well as both studies by Deprez and colleagues\textsuperscript{45,46} reported lower white matter integrity in patients at two years or 4 months after chemotherapy, respectively. The study by de Ruiter et al.\textsuperscript{47} and Stouten-Kemperman et al.\textsuperscript{49} compared breast
cancer patients exposed to high-dose chemotherapy to patients who had received standard-dose chemotherapy and patients who had only been treated with radiotherapy. They found lower white matter integrity in patients who had received high-dose chemotherapy, but not standard-dose, compared to the radiotherapy group. Twenty years after chemotherapy no differences were found in patients compared to no-cancer controls\textsuperscript{48}. These differences could be due to different chemotherapeutic regimens\textsuperscript{28,29,50}, longer time since treatment or the use of a relatively insensitive DTI sequence in the latter study\textsuperscript{48}.

To date, only one other longitudinal study has been published where white matter integrity in breast cancer patients was assessed using DTI\textsuperscript{46}. As opposed to our finding of pretreatment lower white matter integrity in breast cancer patients, Deprez et al. reported no differences in white matter integrity between breast cancer patients prior to adjuvant treatment, irrespective of treatment plan, and no-cancer controls. Four months after treatment, widespread decreases in white matter integrity were found in patients who had received chemotherapy, but not in the other groups. All patients in the Deprez study received 5-fluorouracil as part of their chemotherapeutic regimen, while only three patients in our study received 5-fluorouracil. 5-Fluorouracil is known to cross the blood-brain barrier by simple diffusion and negative effects on brain white matter and cognitive function have been reported in rodents as well as humans\textsuperscript{29,42,51–55}. Furthermore, while most patients in our study received doxorubicin, all patients in the Deprez study received epirubicin, which has been associated to have more severe neurotoxic effects on white matter than doxorubicin\textsuperscript{56}.

Another explanation for the discrepancies between our findings and those of Deprez et al. could be that our follow-up assessment was somewhat longer after chemotherapy. It might be that the neurotoxic effects of chemotherapy on white matter integrity are more severe during or shortly after treatment, with (partial) recovery after a longer period. This is also supported by an additional assessment of the patients in the Deprez study, where recovery of white matter was reported\textsuperscript{47}. Billiet et al.\textsuperscript{57} reassessed the patients from the Deprez study at three to four years after chemotherapy. Patients who had received chemotherapy showed a decline in white matter integrity from baseline to the first follow-up assessment, as reported by Deprez et al.\textsuperscript{46}. These patients showed increased white matter integrity between the second and third assessment, while no-cancer controls did not show any differences over time. Unfortunately, no data were provided on the difference between patients who did and those who did not receive chemotherapy. Billiet et al. hypothesized that recovery had occurred over time, possibly due to remyelination or axonal reorganization. Findings of white matter damage and recovery after chemotherapy could also be influenced by other factors. Although DTI is considered to be a direct measure of microstructural integrity of myelin sheaths, factors such as edema and inflammation could also affect DTI measures\textsuperscript{58}. Levels of pro-inflammatory cytokines are known to be elevated during cancer and cancer treatment and may return to normal over time. Taken together, it could be that initial measures of changes in white matter integrity are influenced by pro-inflammatory cytokines, while neurotoxic effects are expressed as accelerated aging.
Conclusion

Prior to treatment, breast cancer patients, regardless of required treatment, show worse cognitive function, differences in brain function and lower white matter integrity. These differences are associated with higher levels of fatigue. Subsequently, adjuvant systemic therapy is associated with subtle cognitive decline, changes in brain function and decreased white matter integrity. Involvement of frontal and parietal brain regions suggests impaired function of executive and attentional networks to underlie these systemic therapy-related changes. Our findings are in line with previous research, but are expressed in a smaller proportion of the patients. As previously discussed, this could be due to differences in the study sample, such as level of education and age, differences in methodology and statistical thresholding, or disease and treatment characteristics.

Clinical implications

We found pretreatment cognitive function, brain function and structure to be associated with higher levels of fatigue in patients. We were not able to identify any biological or psychosocial factors that could explain changes in cognition or brain measures after systemic treatment. Previous studies have reported varying psychosocial and biological factors related to cognitive impairment following cancer and cancer treatment. To date, none of these factors has reliably been shown to predict cognitive impairment after treatment.

Interestingly, only two clinical studies have reported a direct correlation between cognitive performance and brain measures. Deprez et al. found significant correlations between temporal and parietal white matter integrity and cognitive performance in the domains of attention and processing/psychomotor speed. A significant correlation between performance on processing speed and changes in brain activation has also been found during working memory. We did not find a relation between cognitive performance and brain activation or white matter integrity. It could be that the neuropsychological tests administered did not match the neural substrates that were assessed in these MRI scans. Another explanation could be that, given that only a small subset of patients showed cognitive impairment, we did not have enough power to detect such a relationship.

Subjective cognitive function was shown to decrease in breast cancer patients who had received systemic treatment. Although we also found that cognitive function, as measured using objective tests, also decreased in this group, no relation between subjective and objective cognitive function was found. Opposed to our findings, some studies have found a relation between subjective cognitive function and objective measures. Discrepancies between objective and subjective measures of cognitive function have been reported in multiple diseases. Self-reported cognitive function could be influenced by psychosocial factors such as depression and fatigue, suggesting cognitive complaints to reflect emotional distress.
Neuropsychological test results are usually seen as a true measure of cognitive function. However, practitioners will see those patients who have cognitive complaints, experiencing limitations in daily functioning. It is important not to dismiss these patients, but to offer treatment in a way that they can cope with their cognitive problems.

To date, treatment options are limited. Cognitive rehabilitation has been prescribed to master compensatory strategies to improve daily functioning. A number of studies have shown an improvement in both objective and subjective cognitive function as well as quality of life\textsuperscript{70–76}. Physical activity has also been investigated as an intervention for cancer-related cognitive impairment, showing improvements in cognitive performance and quality of life\textsuperscript{77,78}. However, these improvements have not been studied over a longer period of time, so it is yet to be determined if the effects are long-lasting. Preclinical studies have shown promising results for pharmacological interventions, such as fluoxetine\textsuperscript{79,80}, metformin\textsuperscript{81} and Pifithrin-\textmu\textsuperscript{82}. Erythropoietin, methylphenidate and modafinil have already been studied in breast cancer patients, demonstrating mixed results\textsuperscript{83–86}. Further understanding of the mechanisms and traits of cognitive impairment after cancer and cancer treatment might provide new targets for preventative or restorative treatment.

After ruling out alternative explanations as the most likely cause for self-perceived cognitive problems, such as depression, patients with cognitive impairment or cognitive complaints should be considered for referral to a neuropsychologist to assess severity and determine a treatment plan. Patients should be informed about the risk of cognitive problems. They should also be made aware that only a small subset are diagnosed with cognitive impairment, which is usually mild\textsuperscript{87}. However, even with mild impairments, the impact on daily functioning for a patient can be significant, especially when they are dependent on maximal cognitive performance.

**Methodological considerations**

The study presented in this thesis is the first to prospectively assess cognitive function and multimodal brain MRI in breast cancer patients. The longitudinal design allowed us to study change over time, in contrast to cross-sectional designs where only differences between groups can be determined. However, our baseline assessment took place after surgery. Although we could not find a relation between time since surgery and any of our outcome measures, others have found an effect of surgery on cognition or brain function\textsuperscript{6,68}. One recent study has assessed patients prior to surgery and found lower cognitive performance as well as lower functional network dynamics in patients with breast cancer\textsuperscript{89}. This shows that changes in cognition and brain function might already exist before surgery and anesthesia. Ideally, patients would be selected from an existing population-based cohort study, such as the Rotterdam study\textsuperscript{80}, so that pre-diagnosis data are already available to establish a true baseline. Such a study is currently performed by our group.
Preclinical studies have shown that the effects of cytostatic agents on behavior and neurobiological measures differ per agent\textsuperscript{29,50}. The different mechanisms by which the agents work may determine the neurotoxic effects. Kesler et al.\textsuperscript{28} conducted a cross-sectional study to assess the effects of anthracycline- versus non-anthracycline-based treatment. This study showed lower cognitive performance and different resting-state brain activation in patients treated with anthracyclines, suggesting anthracyclines to be more neurotoxic than other agents. We chose to include patients who were being treated with the most commonly prescribed chemotherapeutic regimens, all including anthracyclines. We can therefore not draw any conclusions about anthracycline vs. non-anthracycline-based treatment. 5-fluorouracil (5-FU) was shown to pass through the blood-brain barrier by simple diffusion\textsuperscript{29,42,51–55}, however in our study only four patients were treated with 5-FU. Also, epirubicin has been suggested to have more severe neurotoxic effects than doxorubicin\textsuperscript{56}. Since the majority of the patients had received epirubicin and only four patients received doxorubicin, we were not able to verify these findings.

Previous studies have shown that endocrine therapy may also affect cognitive function in breast cancer patients\textsuperscript{30,91–99}. At our follow-up assessment, over 70% of the patients who had received chemotherapy were prescribed endocrine treatment. All of these patients were using Tamoxifen at the second assessment. Tamoxifen has been reported to have a larger effect on cognitive function, compared to aromatase inhibitors\textsuperscript{99}. Given the small number of patients that had received chemotherapy without endocrine treatment, we were not able to identify the independent effect of endocrine treatment on cognitive function and brain measures. Since endocrine treatment is usually given for a long period of time, it is important to further investigate the independent effect of endocrine treatment, as well as the additive effect when prescribed together with chemotherapy.

The sample in the current study was relatively young and had a high level of education, both of which are associated with better cognitive outcomes after chemotherapy\textsuperscript{21}. This may have led to a smaller fraction of patients with neurotoxic effects in our study. Secondary analyses showed that breast cancer patients classified as impaired had lower IQ-scores compared to non-impaired patients\textsuperscript{100}. We also found that impaired patients reported worse physical and social functioning and more symptoms of anxiety and depression. Given the small number of impaired patients we were not able to draw firm conclusions about the characteristics of this group or the predictive value of these factors.

The statistical methods used in the current study have been selected based on sensitivity to detect subtle differences. For neurocognitive testing, we chose to apply multivariate models: Mahalanobis distance and multivariate normative comparison\textsuperscript{100,101}. These methods allowed us to assess overall cognitive function. Deviations therein could be compared to the distribution of scores in the control group. We were not able to identify a relation between objective and subjective cognitive functioning. Some recent studies have shown methods that may be better suited to detect this relation\textsuperscript{102,103}. We used voxel-based tract-based spatial statistics for analyses of our DTI scans. By analyzing skeletonized images, results were less prone to
registration misalignment, restricted to white matter, and statistical power was increased by this dimensionality reduction. However, these analyses may be less sensitive when effects are located in peripheral areas of white matter tracts. Future research should be directed towards identifying methods which are most sensitive to detecting subtle differences in brain function and structure and cognitive function. The same holds true for methods aimed at detecting relations between measures.

**Future directions**

The findings from this study should be replicated and further investigated to identify patients at risk, and to determine potential targets for treatment. By including larger samples, statistical power will be gained to allow for subgroup-analysis for factors such as chemotherapeutic regimen and endocrine treatment. Also, with larger sample sizes, differences between impaired and unimpaired patients can be further studied. We found that impaired patients had lower IQ, reported worse physical and social functioning and more symptoms of anxiety and depression. However, this group was too small to draw firm conclusions. Previous studies have reported several factors which may be related to cognitive function in cancer patients, including psychosocial factors, comorbidities, cancer stage and cytokines. These factors should be further investigated in larger samples and multivariate models should be applied to reliably predict at-risk patients. Not only identification of factors is important for the classification of at-risk patients. Different statistical methods should be investigated to determine the best predictive model. Machine learning has been proven to show promising results in predicting cognitive and brain outcomes in mild cognitive impairment.

The past decade has witnessed a surge of MRI studies that focus on network connectivity in breast cancer patients. Network connectivity can be assessed through a variety of analysis techniques and can be applied to functional as well as structural MRI. The effects of cancer and cancer treatment on cognitive function have been suggested to be diffuse, which would indicate the disruption of networks instead of specific regions of the brain. Although outside the scope of this thesis, network connectivity approaches may prove more sensitive to subtle effects of cancer and cancer treatment on brain function and structure.

Preclinical and clinical studies should further investigate the differential role of cytostatic agents. When the mechanisms by which the agents cause cognitive dysfunction are better understood, prescription of regimens can be further personalized to minimize the risk of cognitive impairment without affecting survival. The same holds for endocrine treatment, where tamoxifen was indicated to have more severe impact on cognitive function than aromatase inhibitors. Effects of therapy plans including all agents, dosage and duration should be further studied.

Also, the course of impairment during and after treatment could provide further insights in mechanisms by which impairments may develop. One study has demonstrated recovery of
grey matter volume over time. However, Koppelmans et al. showed that lower white matter integrity in patients who had received chemotherapy 20 years earlier was associated with longer time since treatment. Longitudinal studies with regular and long-lasting follow-up will prove expensive as well as a burden for patients. But they will shed light on the development of impairment of cognitive function and brain measures over time.
REFERENCES


