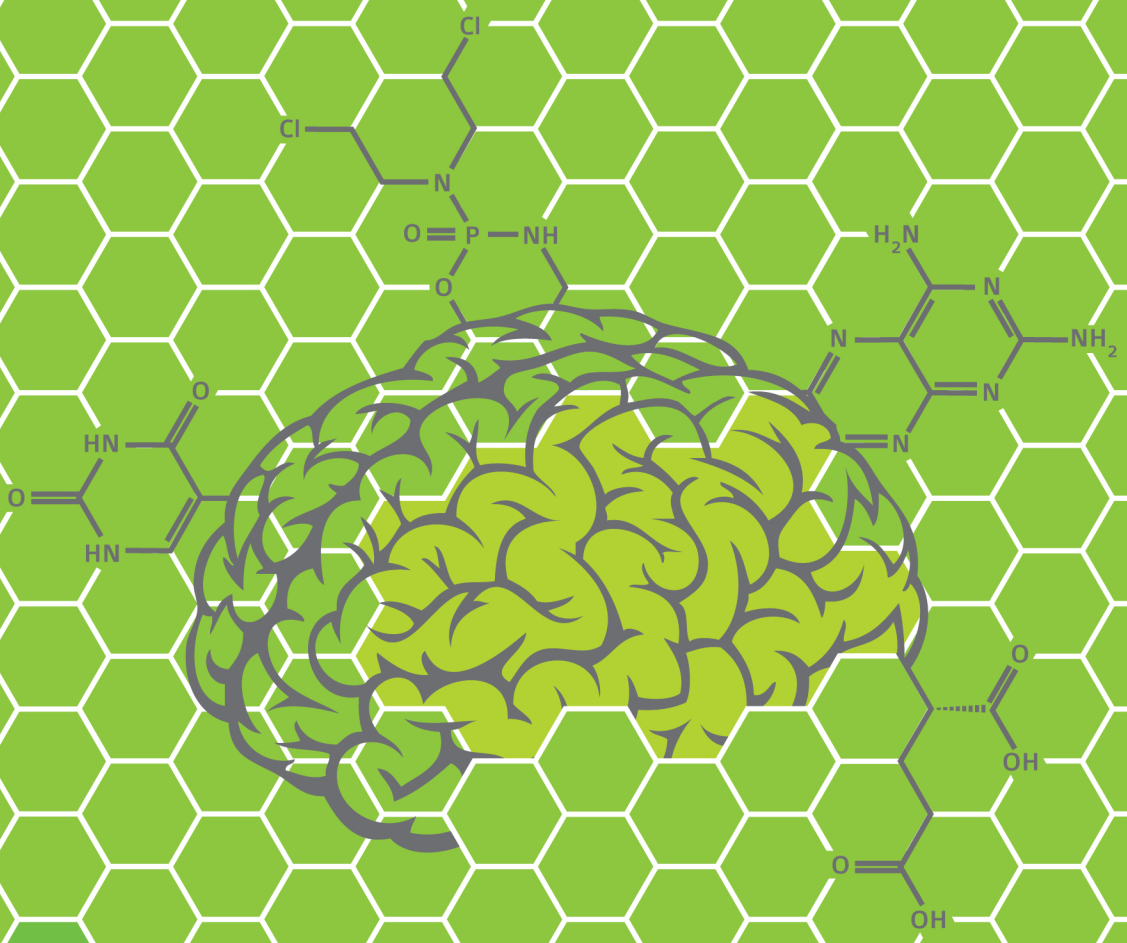
Late Effects of Adjuvant Chemotherapy on Brain Function and Structure



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Acknowledgements

Cover design: Stefanie de Blank
Thesis layout: Vincent Koppelmans
Printed by: Optima Grafische Communicatie

The work described in this thesis was conducted at the Department of Epidemiology of the Erasmus University Medical Center, Rotterdam, the Netherlands and the Division of Psychosocial Oncology and Epidemiology of the Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands.

The research described in this thesis was supported by a grant from the Dutch Cancer Society (2007-3797) to S.B. Schagen and M.M.B. Breteler. The Rotterdam Study is supported by the Erasmus University Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

Financial support for the publication of this thesis was kindly provided by the Department of Epidemiology of the Erasmus University Medical Center, Rotterdam; the Erasmus University Rotterdam, Alzheimer Nederland, Lundbeck BV, Boehringer Ingelheim BV, and the Dutch Cancer Society.

ISBN: 978-94-6050-007-7

Late Effects of Adjuvant Chemotherapy on Brain Function and Structure

Late effecten van adjuvante chemotherapie op het functioneren en de structuur van de hersenen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 20 april 2012 om 09:30 uur

door

Vincent Kopppelmans
geboren te Eindhoven

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie:

Promotor:	Prof.dr. M.M.B. Breteler
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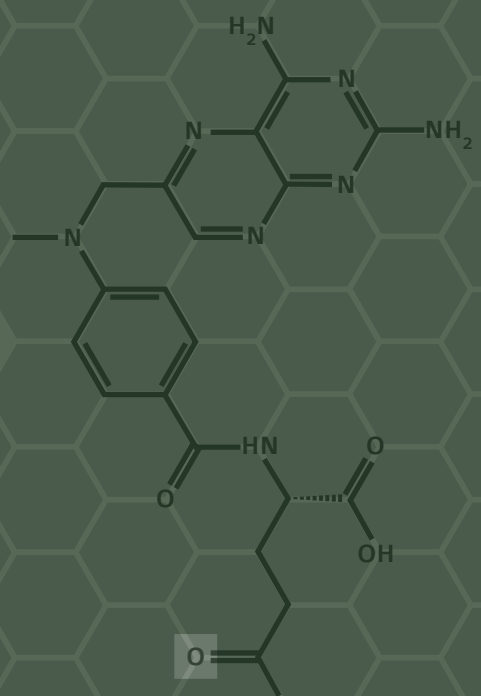
Contents

Chapter 1

General Introduction



Trends in breast cancer incidence, mortality and survival

Breast cancer is the most frequent malignancy in women in Western Europe and the United States. In 2009, 13,177 new cases of breast cancer were diagnosed within the Netherlands alone (1). The lifetime risk of breast cancer in Dutch women reached 1 in 8.3 between 2005 and 2009 (1), which is comparable to the lifetime risk of 1 in 8.2 in United States women that was observed between 2005 and 2007 (2).

The European Standardized Rates of breast cancer in the Netherlands significantly increased from 97.4 per 100,000 in 1989 to 129.6 per 100,000 in 2007 (1). This increase has been ascribed to lifestyle changes such as delayed childbearing, lower parity, reduced breast-feeding and increased body-mass index (3-6). At the same time, mortality rates have decreased slightly as a result of improved treatment and possibly as a result of population screening (7-9). Because of the increased incidence and slightly decreased mortality, the number of survivors has increased (8).

Chemotherapy and cognitive functioning

Adjuvant chemotherapy for breast cancer is given in addition to the primary loco-regional treatment and aims to eliminate occult micro-metastases. Nowadays, 60% of the newly diagnosed breast cancer patients younger than 65 years of age receive chemotherapy, although only about half of them benefit from it (10). To identify those patients that are likely to profit from cytotoxic treatment, the patients risk profile of metastasis is determined on the basis of tumor size and grade, age, number of positive lymph nodes and tumor receptor status (11). In addition, the use of gene-expression profiles can be used to distinguish between patients with a favorable and an unfavorable prognosis and to predict cytotoxic treatment response (11). This is important as chemotherapy can induce various adverse reactions such as nausea, hair loss and fatigue. A less known side effect of chemotherapy that has been increasingly studied over the last decade is cognitive dysfunction (12).

Initial studies on the potential effect of chemotherapy on cognitive functioning were conducted in the early nineties following patients complaints of impaired cognition after conventional cytotoxic treatment (13). Since then, cognitive problems after chemotherapy have been recognized by patients who often refer to them as "chemobrain". Besides the work reported in this thesis, 41 studies have investigated the effects of chemotherapy on cognitive functioning in breast cancer patients (13-53). Of these studies, 19 were cross-sectional (see **Figure 1**) and 23 had prospective designs (see **Figure 2**). Of the 41 studies, 27 (66%) reported significant adverse effects of chemotherapy on cognitive functioning, mainly in the domains of information processing speed and memory (12). The remaining 13 studies reported no significant adverse effect or no effect of cytotoxic treatment. No studies reported a positive effect of chemotherapy on cognitive functioning. The adverse cognitive effects of chemotherapy have been observed during, shortly and up till 16 years after various conventional and high-dose cytotoxic regimens.

A meta-analysis revealed that effect sizes of studies on chemotherapy-induced cognitive dysfunction varied from small to moderate (-0.07 to -0.50) (54). Chemotherapy-induced cognitive dysfunction has been almost exclusively been studied in Caucasian samples, except for a few studies in Japanese women (25, 31). Therefore, not much is known about whether this problem is similar in size and in other populations. Some prospective studies with pre-chemotherapy assessments reported that breast cancer patients already performed worse on neuropsychological tests before cytotoxic treatment than non-cancer controls (21, 40). Follow-up measurements however showed deterioration of cognitive functioning after chemotherapy, indicating that the adverse cognitive effect observed in chemotherapy-exposed patients is at

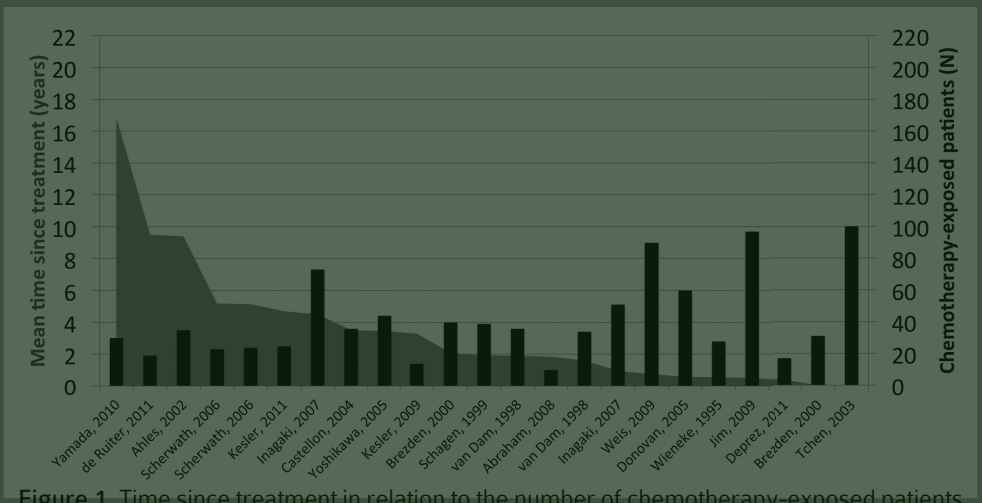


Figure 1. Time since treatment in relation to the number of chemotherapy-exposed patients in cross-sectional studies on the effects of chemotherapy on cognitive functioning.

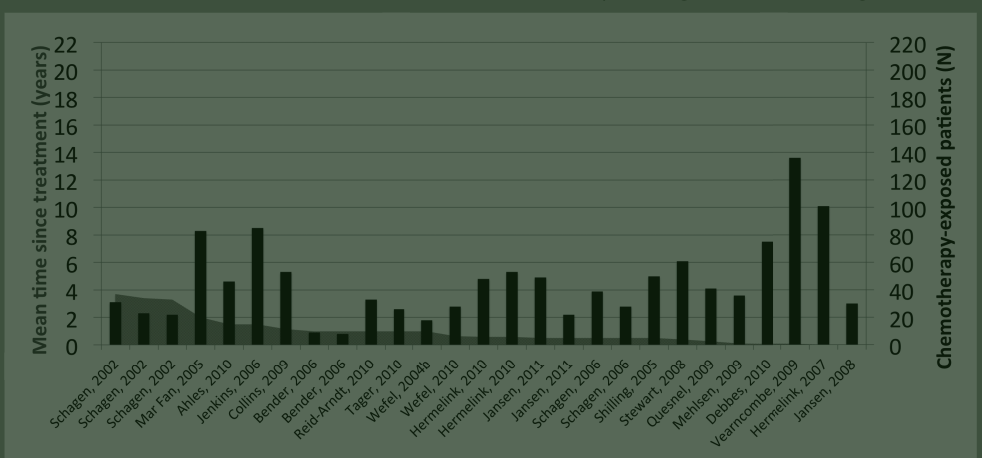


Figure 2. Time since treatment in relation to the number of chemotherapy-exposed patients in prospective studies on the effects of chemotherapy on cognitive functioning.

least partly due to the cytotoxic treatment and not only a result of the possible effect of cancer itself (48). Various studies have investigated whether cancer related mood disorders and fatigue are associated with cognitive dysfunction, but no strong evidence has been observed for such a relationship (55). Fatigue and mood disorders after cancer diagnosis and treatment have been related to subjective cognitive complaints, which also do not predict objective cognitive functioning (12, 56). Animal models have offered a good opportunity to investigate the effects of single cytotoxic agents and avoid the potential cancer and treatment related psychological effects on cognition (57). These studies showed that single common agents such as cyclophosphamide and methotrexate can induce memory problems in rodents (58-60). Of important notice is that not all patients who receive chemotherapy encounter cognitive side effects of chemotherapy. Across studies, the proportion of patients with cognitive dysfunction following chemotherapy ranges from 13-70% (56). Putative explanations for the large differences in proportion between studies include the different cytotoxic regimens under investigation, differences in the mean time since treatment, the diverse neuropsychological test batteries, the various definitions of cognitive impairment and change cognitive functioning, and the use of different reference groups or reference data that are used.

Besides our research, only four other studies have investigated these adverse effects five or more years post-treatment (17, 29, 49, 50). Although the results of these studies suggest that chemotherapy is associated with long-term cognitive functioning, they all had cross-sectional designs and small sample sizes. Therefore the long-term relationship between chemotherapy and cognitive functioning remains largely unknown.

Mechanisms for chemotherapy-induced cognitive dysfunction

Little is known about the mechanisms that underlie chemotherapy-induced cognitive dysfunction (61). Chemotherapy may have a direct neurotoxic effect on central nervous system cells. Depending on the frequency of administration, prescribed dosage, administration route, and whether or not given in combination with radiotherapy, certain cytotoxic agents are able to cross the blood brain barrier in substantial amounts (62). Examples of such agents that have long been prescribed and are still applied in the adjuvant treatment for breast cancer include methotrexate and 5-fluorouracil (63, 64). By entering the brain parenchyma, cytotoxic agents may cause seizures, cerebellar dysfunction, extrapyramidal disorders and cognitive dysfunction (62). Animal studies support these findings by showing that several single agents such as methotrexate and cyclophosphamide that are administered peripherally in clinically relevant dosages are associated with reduced neurogenesis, apoptosis, oxidative stress, (delayed) white matter damage, and learning disabilities (57, 65, 66).

Furthermore, chemotherapy has been associated with DNA damage, increased rate of telomere shortening, and increased oxidative stress (67), which subsequently could lead to cognitive dysfunction. Other proposed mechanisms include cardio-toxicity, which may affect

cognitive functioning through effects on cerebrovascular integrity. Because central nervous system regeneration is limited (68, 69), it is possible that chemotherapy-induced cognitive dysfunction is persistent rather than transient.

In the general population cognitive dysfunction has been associated with brain structural changes such as global and focal gray matter volume (70), white matter lesions (71), and reduced microstructural integrity of the normal appearing white matter (72-74). The relationship between chemotherapy, cognitive functioning and brain structural alterations is yet largely unknown but not unlikely considering that under certain conditions some cytotoxic agents can penetrate the blood brain barrier. Besides our own study, twelve imaging studies, of which two had a prospective design and ten had cross-sectional designs, have been conducted in chemotherapy-exposed patients. These studies showed that shortly up till approximately three years post-treatment, chemotherapy is associated with brain structural changes, mainly detectable as white matter hyperintensities (75-77), lower quality of white matter (32, 51), and gray matter volume reductions (78). Even though chemotherapy-induced brain alterations seem to be at least partially transient (31, 76, 78), the only study that examined the association between chemotherapy and brain structure in patients almost ten years post-treatment showed focal effects of chemotherapy on white matter quality and gray matter (79). The idea that chemotherapy is associated with long-term brain structural alterations is strengthened by animal studies in which delayed myelin destruction of the central nervous system and long-lasting suppression of hippocampal cell proliferation were observed in rodents that had received clinically relevant doses of cyclophosphamide and methotrexate (59, 65).

Scope of the thesis

With the steeply increasing number of long-term breast cancer survivors who are aware of the potential cognitive side effects of cytotoxic systemic therapy, there is need for clarification on the potential long-term cognitive effects of chemotherapy that can adversely affect quality of life (80). Results from the few small studies on the late effects of chemotherapy on either brain function or structure suggest that these effects may not be transient. These outcomes warrant the investigation of the late effects of chemotherapy through large-scale studies that combine imaging, neuropsychological testing, and questionnaires on subjective cognitive complaints. In this thesis I will try to answer the question if standard-dose adjuvant chemotherapy for breast cancer is associated with long-term cognitive functioning and brain structure.

Breast cancer survivors and reference subjects from the general population

The studies described in this thesis are all based on data from almost 200 chemotherapy-exposed breast cancer survivors who have been treated with CMF (Cyclophosphamide, Methotrexate, 5-Fluorouracil) chemotherapy at the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital or the Daniel den Hoed Cancer Clinic, on average more than 20 years

ago.

A reference group of non-exposed control subjects who had never been diagnosed with cancer was sampled from the Rotterdam Study (RS). The Rotterdam Study is a population based cohort study that is ongoing since 1990 in the Ommoord district in Rotterdam, the Netherlands. Ever since its start the study has included almost 15,000 subjects who are invited for follow-up examinations every three to four years. Amongst many others, examinations include questionnaires on medical history, mood, and subjective cognitive functioning, neuropsychological testing, MRI examination and carotid artery ultrasound.

Breast cancer survivors were assessed at the research center of the Rotterdam Study in exactly the same way as the reference subjects following the protocol of the Rotterdam Study.

By sampling a population-based reference group from an existing study we were able to put all our effort and resources into including a large number of chemotherapy-exposed breast cancer survivors. The large number of subjects and the extensive set of examinations enabled us to adjust for several confounders and detect even small effects. **Figure 3** shows how the current study relates to other cross-sectional studies that have been published regarding sample size and mean time since treatment.

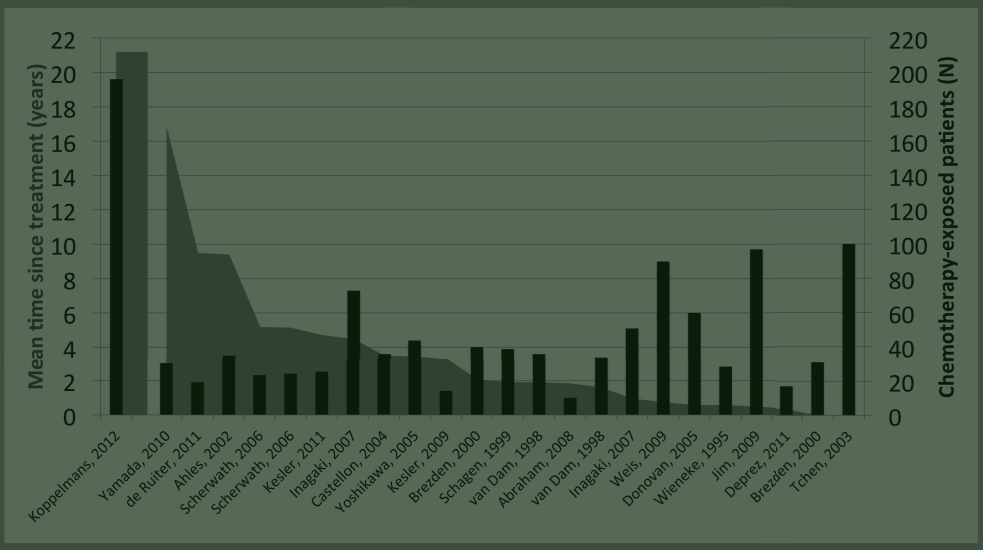


Figure 3. Positioning of the current study among published cross-sectional studies on the late effects of chemotherapy on cognitive functioning

Thesis outline

In **Chapter 2** I give an overview of the few studies that have investigated the effects of chemotherapy in subjects who completed treatment for breast cancer five or more years before and studies that have looked at the risk of dementia after cytotoxic treatment.

Chapter 3 reports on the outcome of our study on the effects of chemotherapy on cognition

in long-term breast cancer survivors. To investigate this effect we compare neuropsychological test outcomes of breast cancer survivors who have been treated with standard-dose CMF chemotherapy to neuropsychological test outcomes of a population-based sample of women from the general population who were never diagnosed with cancer.

In **Chapter 4** the association between CMF chemotherapy and long-term brain structure is discussed. **Chapter 4.1** focuses on the relation between cytotoxic treatment and brain volume, gray matter volume, white matter volume and hippocampal volume. Subsequently the effect of chemotherapy on local gray matter density is explored using Voxel Based Morphometry (VBM). In **Chapter 4.2** I relate chemotherapy and time since treatment to global and local white matter quality using Diffusion Tensor Imaging (DTI) and Tract Based Spatial Statistics (TBSS). **Chapter 4.3** reports on the association between chemotherapy, carotid artery integrity and cerebral blood flow and brain perfusion. In **Chapter 5** I describe the relative risks of incidental findings on brain MRI for breast cancer survivors, who completed chemotherapy on average more than 20 years ago, in relation to the risk of incidental findings in the general population. Finally, in **Chapter 6** I summarize the late-effects of CMF chemotherapy for breast cancer on cognition and brain structure, and I discuss implications and suggestions for future research.

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Chapter 2

A review of the late effects of adjuvant chemotherapy for adult onset non-central nervous system cancer

cognitive impairment, brain structure and risk of dementia

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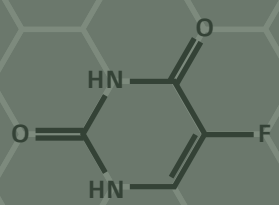

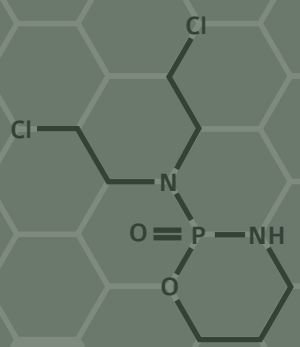

Abstract

Few studies have investigated the effects of chemotherapy on the brain more than five years after cessation of treatment. Here we discuss the studies that have investigated the late (i.e. at least 5 years post-treatment) effects of adjuvant chemotherapy on a) cognitive function (n=6); b) incidence of dementia (n=4); and c) brain structure and function (n=2). Although several methodological issues limit the validity and interpretation of some of the results, taken together these studies suggest that chemotherapy is associated with subtle, yet long-lasting cognitive deficits that might be related to brain structural and functional differences, but not with an increased risk of dementia.

Index of Chapter 2

1 Background

Adjuvant chemotherapy applied in the treatment of adult onset non-central nervous system (CNS) cancer has been associated with cognitive problems up to several years after cessation of treatment (1-3). The majority of studies have examined the cognitive effects of adjuvant chemotherapy given to breast cancer patients and have investigated these effects during treatment or up to two years after completion of treatment (1-3). Several cross-sectional and prospective studies have shown a relation between certain cytotoxic regimens and cognitive problems in subgroups of patients, which could not be explained by anxiety, depression, or fatigue (2, 4). Throughout studies, the most frequently observed cognitive problems are within the domains of memory, processing speed and executive functioning (4).

The mechanisms of chemotherapy-induced cognitive decline are still largely unknown, but several mechanisms have been suggested, including a) direct neurotoxic effects on CNS cells caused by crossing of the blood-brain barrier of chemotherapeutic agents, resulting in enhanced cell death and decreased cell division of in particular neural progenitor cells and oligodendrocytes (5); b) effect on the CNS vessels including reduced blood vessel density in the hippocampus (6); and c) indirect effects resulting from increased levels of cytokines or reduced levels of estrogen and progesterone after chemotherapy-induced menopause or decreased synthesis of neurotransmitter agents or DNA damage as a result of oxidative stress or accelerated telomere shortening. Patient-related factors such as genetic susceptibility, age, vascular risk factors, and baseline cognitive functioning may further influence the risk of cognitive impairment (7, 8).

Imaging studies in patients who had been treated with adjuvant chemotherapy showed structural brain differences such as white matter hyperintensities (9-12), microstructural damage of white matter tracts (13), and local grey matter volume reductions (14, 15). The majority of neuro-imaging studies have been conducted at less than two years post-treatment (9-15). A few studies have showed that chemotherapy associated structural and functional brain changes brain changes are related to worse cognitive performance (8, 16). These observations in humans have been supported by animal studies. Studies in rodents found evidence for chemotherapy induced memory retention and recall problems, and learning disabilities, which subsequently have been linked to long-lasting brain structural alterations, such as white matter damage and decreased hippocampal cell proliferation (17, 18).

The postulated mechanisms and observations of structural brain changes after chemotherapy suggest that chemotherapy-induced cognitive changes can be long lasting or even persistent, especially through chemotherapy accelerated ageing as a result of accumulation of DNA damage and telomere shortening (19). As longitudinal studies in healthy individuals and individuals at risk for Alzheimers disease have shown that cognitive deficits precede dementia (20, 21), adjuvant chemotherapy may also be associated with an increased risk of dementia.

From a patient perspective there is need for clarity on the long-term cognitive sequelae of adjuvant cytotoxic treatment. Large numbers of breast cancer patients receive chemotherapy

as part of primary treatment and the number of long-term elderly survivors of breast cancer is increasing due to increased incidence of breast cancer, (22, 23) and improved survival (24).

2 Objectives

The purpose of this systematic review is to investigate the late effects of adjuvant chemotherapy given for adult onset non-CNS cancer on a) cognitive functioning as assessed with neuropsychological tests more than five years after completion of chemotherapy, b) the risk of dementia, and c) brain structure.

3 Methods of the review

3.1 Search methods

We searched MEDLINE (January 1966 to April 2011) and reference lists of articles in English language. Search terms were:

1. cancer AND ((long AND term) OR long-term) AND (cogn* OR neuropsychological) AND (function* OR impairment OR deficit*)
2. cancer AND (chemotherapy OR (adjuvant AND therapy) OR cytotoxic) AND dementia
3. cancer AND (chemotherapy OR (adjuvant AND therapy) OR cytotoxic) AND brain AND (imaging OR MRI) AND (structur* OR function*) AND ((white AND matter) OR (gray AND matter) OR (fMRI))

3.2 Selection of studies

Studies were selected that investigated:

a. cognitive functioning of cancer patients who had completed cytotoxic treatment for adult-onset non-CNS cancer at least five years earlier, who were cancer-free and who were assessed with a neuropsychological test-battery
b. brain structure and function in cancer patients who had completed cytotoxic treatment for adult-onset non-CNS cancer at least five years earlier, who were cancer-free
c. risk of dementia in subjects who had been treated with chemotherapy for adult-onset non-CNS cancer.

3.3 Cognitive domains

For the purpose of this review, we categorized the neuropsychological tests used in the different studies into the following cognitive domains, according to the classifications made by the researchers or the original test manual: language, attention, memory, visuo-spatial functioning, executive functioning and motor function (see **Supplementary Material**).

4 Studies on long-term cognitive functioning after chemotherapy

4.1 Study designs

Seven studies matched our criteria of investigating cognitive functioning in cancer patients who had been diagnosed more than five years before (range throughout studies: on average 5.1 to 21.2 years) (16, 25-30). **Table 1** summarizes patient and tumour characteristics, group sizes, time since treatment, and type of chemotherapy per study.

Six of these seven studies explicitly investigated the effects of adjuvant chemotherapy on cognitive functioning. The remaining study, which compared twin pairs discordant for cancer, reported on cognitive functioning regardless of treatment history (27). Five studies examined solely breast cancer patients, one study included breast and lymphoma patients (25), and a sixth study assessed cognitive functioning in long-term survivors of ovarian cancer (26). The twin study investigated a heterogeneous group of cancer patients (27) and did not report on the kind of cytotoxic regimen applied. In the remaining studies, Anthracycline based regimens and Cyclophosphamide-Methotrexate-Fluorouracil were the most commonly applied regimens.

All studies assessed cognitive functioning via comprehensive neuropsychological assessment, except for the twin study, which investigated cognitive functioning with a short telephonic cognitive screening instrument.

4.2 Findings

Performance on neuropsychological tests

Table 2 provides an overview of the number and percentage test outcomes on which chemotherapy-exposed subjects performed worse than either study specific controls or published reference data. Results are presented per study and divided in cognitive domains. Five out of seven studies (16, 25-27, 29) reported on the risk or prevalence of cognitive dysfunction; all studies used a different definition for impaired performance (see **Table 3**).

Ahles et al. reported that lymphoma and breast cancer survivors exposed to chemotherapy five years before scored significantly lower on neuropsychological tests in the domains of verbal memory and psychomotor functioning than participants who received local therapy only. Of the chemotherapy-exposed survivors 39% scored within that range compared to 14% of patients who had received local therapy only ($p<.01$).

Scherwath et al. reported that both high-dose and low-dose chemotherapy-exposed breast cancer survivors did not differ significantly from an early-stage breast cancer control group on neuropsychological test outcomes, on average five years after completion of chemotherapy. Compared to normative data, both the chemotherapy-exposed and non-exposed patients performed significantly worse on tests in the domains of attention and executive functioning. No significant differences regarding neuropsychological performance were observed between patients who received either high-dose or low-dose chemotherapy. No differences were observed in the distributions of subjects with impaired test scores across the high-dose,

Table 1. Overview of studies of late effects of cancer and cancer treatment on cognitive functioning

Auteur	Patients	N	Stage	Type Cx	Hx	Age (sd)	TST (sd)	minTST	Controls	N	Stage	Treatment	TST (sd)	Age (sd)
Ahles 2002	BC patients	35	0-IV	Standard dose Cx various regimens	+	59.1 (10.7)	9.4 (4.5)	≥ 5	BC -Cx	35	0-III	Sx or Rx	9.9 (5.8)	60.6 (10.5)
	Lymphoma patients	36	I-IV			55.9 (12.1)	9.5 (4.8)		Lymphoma -Cx	22	I-IV		14.4 (6.3)	48.7 (11.7)
Heflin 2005	Mixed cancer types	405 [a]	n/a	?	?	74.9 (6.3)	14.1 (?)[b]	≥ 5[b]	Cancer free twins	702	n/a	n/a	n/a	74.9 (6.3)
Scherwath 2006	High-risk BC	24 HD	?	EC + CMF	+	51.8 (8.6)	5.2 (1.9)	n/a	Early stage BC -Cx + normative data	29	I-II	Sx & Rx	5.3 (1.2)	54.6 (8.0)
		23 SD	?	EC + CTM	+	53.3 (7.1)	5.1 (1.8)	n/a						
Correa 2010	Ovarian cancer survivors	22	?	?	+	61.8 (9.2)	7.7 (?)	5 to 10	Age and education adjusted Normative data	-	-	-	-	-
de Ruiter 2010	High-risk BC, at least 4 tumor positive lymph nodes, no metastases	19	II-III	4 cycles FEC + 1 cycle CTC	+	56.3 (5.5)	9.5 (0.8)	n/a	Early stage BC -Cx	15	I	Sx & Rx	9.2 (0.5)	58.2 (5.8)
Yamada 2010	Early stage malignant BC, no evidence of metastasis	30	I-IIIa	CMF or doxorubicin	?	72.8 (5.1)	16.8 (2.8)[b]	≥ 10[b]	Matched to a non-cancer comparison participant	30	n/a	n/a	n/a	72.6 (5.5)
Koppelmans 2011	Unilateral BC patients	196	I-IIIa	CMF	-	64.1 (6.4)	21.2 (4.4)	13.7 [b]	Non-cancer subjects from a population based study	1509	n/a	n/a	n/a	57.9 (5.4)

Cx= Chemotherapy; Hx=Hormonal therapy; TST= Mean time since treatment in years; minTST; Minimal time since treatment in years; BC= Breast Cancer; Sx= Surgery; Rx= Radiotherapy; HD= High Dose; SD= Standard Dose; EC= Epirubicin + Cyclophosphamide; CMF= Cyclophosphamide, Metotrexate + 5-Fluorouracil; CTM= Cyclophosphamide, Thiotepa + Mitoxantrone; FEC= 5-Fluorouracil, Epirubicin + Cyclophosphamide; CTC= Cyclophosphamide, Thiotepa + Carboplatin; [a]= number of long-term survivors; [b]= time since diagnosis

Table 2. Outcomes of neuropsychological studies on the late effects of chemotherapy

	Language	Attention	Memory	Visuospatial Functioning	Executive Functioning	Motor Function
	NoT %	NoT %	NoT %	NoT %	NoT %	NoT %
Ahles, 2002	0/3	1/4	1/4	0/1	0/1	0/2
Scherwath, 2006[a]	- -	0/5	0/7	- -	0/4	- -
Scherwath, 2006[b]	- -	2/5	0/7	- -	3/4	- -
Scherwath, 2006[c]	- -	2/5	0/7	- -	1/2	- -
Correa, 2010	- -	1/2	0/4	- -	2/3	0/2
Yamada, 2010	0/2	2/4	0/3	0/2	3/3	- -
de Ruiter, 2011	- -	0/4	0/7	- -	2/6	0/2
Koppelmans, 2011	- -	1/3	2/3	0/1	1/2	1/3

NoT= Number of Tests in which chemotherapy-exposed subjects performed significantly worse; a=standard and high-dose chemotherapy-exposed patients in comparison with a reference group; b=standard-dose chemotherapy in comparison with normative data c=high-dose chemotherapy in comparison with normative data

standard-dose, and the non-chemotherapy-exposed group.

Yamada et al. reported that chemotherapy-exposed survivors performed worse than healthy controls on cognitive tests in the domains of attention, working memory, psychomotor speed and executive functioning, on average 16.8 years after diagnosis.

De Ruiter et al. observed that high-dose chemotherapy-exposed patients performed worse than early-stage breast cancer survivors not treated with chemotherapy on tests of planning and verbal fluency, on average 9.5 years post-chemotherapy. Within the chemotherapy-exposed patients the number of test scores in the impaired range was somewhat, but not significantly higher than in the control group.

The study by *Correa et al.* revealed that long-term recurrence free survivors of ovarian cancer performed significantly worse on tests of attention and executive functioning compared to normative data on average 7.7 years after chemotherapy. Moreover, they reported that of the survivors, 24% were classified as cognitively impaired, which did not significantly differ from the 15% reported in healthy populations using test batteries of similar length.

Koppelmans et al. reported that chemotherapy-exposed survivors, on average 21 years post-diagnosis, performed worse than a population-based reference group of women without a history of cancer on cognitive tests measuring delayed verbal memory, processing speed, executive functioning and psycho-motor speed (28). On an overall measure of cognitive functioning the chemotherapy-exposed survivors scored on average 0.21 standard deviations (p=.007) below the reference subjects, indicating overall slightly worse cognitive performance.

The study by *Heflin et al.* showed that long-term cancer survivors on average 14.1 years after diagnosis were more likely to have cognitive problems than their co-twins (OR=2.71; 95% CI 1.47-5.01).

Cognitive domains that were most frequently impaired were attention and executive

Table 3. Definitions and outcomes of cognitive impairment

Author	Definition of cognitive impairment	Outcome
Ahles 2002	The lower quartile for all study participants of each domain was defined as low performance. Three cut-points were used to distinguish between subjects with and without cognitive impairment: a) three or more; b) four or more and c) five or more domain scores within the lower quartile.	a) 50% of the CEBCS 23% of the CG scored within the low performance range; b) 39% of CEBCS versus 14% of the CG within the low performance range; c) 24% of the survivors who received CT versus 5% of the survivors who received local therapy scored within the low performance range. Logistic regression including treatment, diagnosis, age and education in the model showed a significant difference for all three cut-points (for all analyses p<.01, no odds ratios were reported).
Heflin 2005	Cognitive screening scores and informant reports were used to assign cognitive functioning scores: 0 cognitively intact; 1 minor errors; 2 poor performance; 3 cognitive dysfunction sufficient to interfere with managing everyday life demands.	Long-term cancer survivors had an almost three times higher risk than their co-twins to have a cognitive function score indicating cognitive dysfunction (OR = 2.71, 95% ci = 1.47-5.01, p<.001).
Scherwath 2006	Test scores were normalised on the basis of published test norms. Definition: Z-scores ≤ -1.4 SD bellow the mean of zero. a) The number of subjects with impaired test-scores per cognitive function; b) and the proportion of subjects with ≥ 4 impaired test scores were compared between groups.	a) No significant differences were observed between groups; b) 8% of the high-dose CEBCP had global neuropsychological impairment compared to 13% of the standard-dose CEBCS and 3% of the early-stage breast cancer group that did not undergo chemotherapy. This difference however was not significant (Fisher's exact test, P=0.43).
Correa 2010	Two or more test z-scores at or below -1.5 standard deviations from the normative mean.	No significant differences were observed between the proportion of patients with cognitive dysfunction (24%) and normative data (15%).
de Ruiter 2010	A cognitive test index was considered impaired when a subject scored two standard deviations below the mean of the healthy control group on that index	The CEBCS were impaired on 1.37 ± 1.86 of 16 test indices (8.6%) while the CG was impaired on 0.80 ± 1.15 test indices (5.0%). This difference however was not significant (F(1, 30)= 3.27, P=0.081).
Koppelmans 2011	A summary measure: "Mahalanobis distance" was calculated. MD can be interpreted as the distance to the mean of the multidimensional distribution of cognitive test scores of the reference subjects.	MD was significantly larger for the CEBCS (mean=2.8; sd=2.6) than for the population-based reference group (mean=2.2; sd=2.8) indicating that the former had worse overall performance ((F 1,1648)=7.3; p=.007).

CEBCS= chemotherapy-exposed breast cancer survivors; CG= control group; sd= standard deviation; MD= Mahalanobis Distance

functioning: for both domains five studies reported worse scores in chemotherapy-exposed cancer survivors than reference subjects or normative data (16, 25, 26, 28-30) (see **table 2**). *Ahles et al.* and *Koppelmans et al.* observed worse memory performance in long-term chemotherapy-exposed cancer survivors than in reference subjects (25, 28). None of the studies observed lower than expected performance on tests measuring the domains of language or visuo-spatial functioning.

4.3 Methodological issues

Design

Because of the cross-sectional designs of the studies cognitive differences that might have been present before administration of chemotherapy could not be taken into account. Several prospective studies have reported poorer pre-treatment cognitive functioning of breast cancer patients compared to healthy control subjects (31, 32). In addition, in cross-sectional studies subjects with above average cognitive functions before treatment who experience chemotherapy-associated cognitive decline may still score within the normal range after treatment, and vice versa. For these reasons, the results of cross-sectional studies should be interpreted with caution.

Sample size

The number of chemotherapy-exposed patients per subgroup in the different studies ranged from 19 to 36 but for the study by *Koppelmans et al.* that included 196 subjects. Because of the resulting limited power small effects of chemotherapy on cognition may not have been detected. This is particular relevant as a meta-analysis of a series of cross-sectional studies on cognitive function 6 months up to 9.4 years after cytotoxic treatment suggested that overall effects sizes were only small to moderate (Cohen's *d* -0.07 to -0.50) (33).

Reference group

In cross-sectional studies that compared cognitive function between chemotherapy-exposed cancer patients and control subjects without a history of cancer, it is not possible to distinguish the effect of chemotherapy from the potential effect of cancer itself. However, a longitudinal study on the effects of chemotherapy with baseline measurements, showed that chemotherapy is associated with cognitive functioning, independent of the potential effect of cancer (34). This suggests that the long-term cognitive effects of cytotoxic treatment in the reviewed studies may at least partially be due to chemotherapy.

Cognitive assessment and test selection

Comparison of study outcomes is hindered by the use of different neuropsychological test batteries (**Table 2**). All six studies that used neuropsychological tests implemented tests of

attention, memory and executive functioning. Four studies addressed motor function, three addressed visuo-spatial functioning and two addressed language. Moreover, different tests have been used to measure the same construct. Studies that were conducted up to two years after cessation of treatment and that investigated cognitive effects of chemotherapy, showed that mainly memory, executive functioning and processing speed were affected (4). This might explain why most cognitive tests in the late-effects studies focused on these domains. Because few studies assessed the domains of language and visuo-spatial functioning, it is hard to draw any firm conclusions regarding potential impairments in these domains.

Criteria for cognitive dysfunction

Results from several studies show that chemotherapy induced cognitive decline does not affect every patient that receives chemotherapy. Five out of seven studies that reported on the prevalence of cognitive dysfunction, observed a higher prevalence in chemotherapy-exposed survivors (see **Table 3**), although only in two studies these differences were significant. However, because different definitions of cognitive impairment were used: *Ahles et al.* defined subjects as cognitively impaired if they had four or more test-scores within the lower quartile of test scores of all study participants (25); *Heflin et al.* used an algorithm based on screening scores and informant reports to identify cognitively impaired subjects (27); *Scherwath et al.* defined cognitive dysfunction as a standardized test-score of 1.4 or more standard deviations below zero (29); *de Ruiter et al.* defined a test score of two standard deviations below the mean of the reference group as an impaired score (16); *Correa et al.* defined cognitive dysfunction as two or more test scores 1.5 or more standard deviations below the normative mean (26); and *Koppelmans et al.* calculated a summary measure for all cognitive tests based on the Mahalanobis distance (28). Because of the use of these different measures, the extent of the problem remains unclear.

Confounding factors and statistical procedures

The selective estrogen receptor modulator tamoxifen has been the most widely prescribed antineoplastic agent for the adjuvant treatment of breast cancer for more than two decades (35). It has been associated with (small) cognitive problems in several cognitive domains (36). The study by *Koppelmans et al.*, excluded subjects who used tamoxifen, and *Ahles et al.* and *Scherwath et al.* stratified on the use of tamoxifen. Both latter studies reported no effect of tamoxifen on cognitive functioning.

Scherwath et al. only observed poor cognitive function in chemotherapy-exposed breast cancer survivors when performance was compared to normative data, but not when it was compared to that of a matched early-stage cancer control group. An explanation for this discrepancy might be that the authors applied Bonferroni correction to adjust for multiple testing. Since neuropsychological measures are at least partially correlated, Bonferroni

correction may be too conservative (37). Alternatively, the normative data that *Scherwath et al.* used as reference data might not have been appropriate to adequately adjust for all confounding factors. Therefore, the reported cognitive effects of chemotherapy may actually reflect residual confounding. Because of the contradictory results of this study, it is difficult to draw firm conclusions from it.

5 Studies on long-term brain structure and function after chemotherapy

5.1 Study designs

We identified three imaging studies that had investigated the late effects of chemotherapy on brain structure or function (see **Table 4**). The patient and tumour characteristics, group sizes, time since treatment, and type of chemotherapy for these studies have already been discussed in section 4.1 (see **Table 1**) as all of these studies also evaluated cognitive performance. In the first study, conducted by *de Ruiter et al.*, functional magnetic resonance imaging (fMRI)

Table 4. Overview of neuro-imaging studies on the late effects of chemotherapy

Study	**de Ruiter, 2011a** (16)	**de Ruiter, 2011b** (38)	**Koppelmans** (39)
CT+	19	17	184
CT-		15	-
Ref	-	-	368
years (sd)	9.5 (0.8)	9.5 (0.8)	21.1 (4.4)
Measures	Functional Magnetic Resonance Imaging; BOLD activation measured during an executive functioning task (EFT) and episodic memory task (EMT)	DTI; White matter lesions (visually checked); 1H-MRS in left centrum semiovale; VBM	Total brain/gray matter/white matter volume; Hippocampal volume; VBM
Outcome	Hyporesponsiveness in CT+ compared to CT- of 1) the dorsolateral prefrontal cortex during EFT; and 2) parahippocampal gyrus during EMT. During both EFT and EMT hyporesponsiveness was observed in CT+ compared to CT- in bilateral posterior parietal cortex.	Whole brain MD and AD were higher in CT+. In several regions FA was lower and MD and RD were higher in CT+. No differences were observed in visually checked WMH. NAA/Cr was lower in CT+ indicating axonal injury. VBM showed gray matter reductions in the posterior parts of the brain in CT+.	CT+ had less brain volume and gray matter volume (3 ml) than reference subjects. This difference was comparable to the effect of approximately four years of age on gray matter reduction. No differences were observed between groups in regional gray matter volume.

CT^+=Breast Cancer patients treated with Chemotherapy; CT^-= Breast Cancer patients NOT treated with Chemotherapy; Ref=population-based reference group; BOLD= Blood Oxygen Level Dependence; WMH= White Matter Hyperintensities; VBM= Voxel Based Morphometry; DTI= Diffusion Tensor Imaging; VBA= Voxel Based Analysis; WM= White Matter; FA= fractional anisotropy; MD= Mean Diffusivity; RD= Radial Diffusivity; AD= Axial Diffusivity; 1H-MRS= Single voxel MR spectroscopy

was used to compare brain functioning of 19 chemotherapy-exposed breast cancer patients treated with high-dose adjuvant chemotherapy 9.5 years before, to 15 breast cancer survivors that only received local therapy (16). In a subsequent study in a subset of these subjects, (two chemotherapy-exposed patients were excluded on the basis of scan artifacts and claustrophobia), the effects of high-dose adjuvant chemotherapy on white matter lesions, focal gray matter volume, and white matter integrity were investigated (38). The third study by *Koppelmans et al.* compared total brain volume, total gray and white matter volume, hippocampal volume and regional brain volume of a set of 184 CMF chemotherapy-exposed breast cancer patients (complete MRI scans were obtained from 184 of the 196 subjects that completed cognitive testing) to that of an age-matched population based control group (n=368) (39).

5.2 Findings

Table 4 presents the outcomes of the neuro-imaging studies on the late effects of chemotherapy. The two studies by *de Ruiter et al.* showed that almost ten years post-treatment, chemotherapy was associated with less gray matter volume in posterior parts of the brain. Within these areas fMRI revealed hypo-activation during neuropsychological task performance. Chemotherapy was furthermore associated with global and focal lower white matter integrity. *Koppelmans et al.* found lower total brain volume and gray matter volume in chemotherapy-exposed patients than the population-based reference group. The lower gray matter volume observed in the breast cancer survivors was comparable to the effect of approximately four years of age on gray matter reduction. No differences were observed in regional gray matter volume or global white matter volume.

6 Studies on the risk of dementia after chemotherapy

6.1 Study designs

Four studies examined the risk of dementia in breast cancer survivors who completed cytotoxic treatment up till 16 years before (see **Table 5**) (40-43). All studies were done retrospectively and using data from the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database (44). This database combines data from population based cancer registries that cover 14% of the United States population from 1992 to 1999 and 25% of the population from 2000 to the present, and the claims database from the Medicare Program. The latter includes financial reimbursement claims from hospital, physician and other medical services for more than 97% of the U.S. population aged 65 years or older (44). In all studies, chemotherapy use was determined on the basis of Medicare claims. Three studies (40-42) investigated the effect of chemotherapy on incidence of dementia by comparing breast cancer patients treated with versus without chemotherapy. In the fourth study, the relative incidence of dementia following different cytotoxic regimens was investigated by comparing dementia incidence in patients who received a specific cytotoxic agent to the incidence of dementia in the patients that had not

Table 5. Overview of studies on the risk of dementia after chemotherapy treatment

Study	N total	N chemo	N control	Max. years follow-up	Age at inclusion	Diagnosed between	BC Stage	Controls	Study outcome
Heck, 2008	18360	6289	12071	10.6	≥65	01-01-1992 - 31-12-1999	II-IV	BC -Cx	Chemotherapy-exposed survivors have a higher incidence of any form of dementia than non-exposed control subjects.
Raji, 2008	6932	6932	-	11	≥68	1994-2002	I-III	none	No difference in the incidence of dementia after different regimens of cytotoxic treatment.
Baxter, 2009	21362	2913	18449	6	66-80	01-01-1992 - 31-12-1999	non-metastatic invasive BC	BC -Cx	No overall difference in incidence of dementia after chemotherapy. In a stratified analysis chemotherapy-exposed survivors aged 76-80 were less likely to develop dementia than non-exposed subjects of the same age.
Du, 2010a	62565	14057	48508	16	65-89	1991-2002	I-IV	BC -Cx	No difference was observed between groups in the incidence of drug-induced dementia. A lower incidence of Alzheimer's disease, vascular dementia and other dementias was seen in chemotherapy-exposed survivors than controls.
Du, 2010b	19504	9752	9752	16	65-89	1991-2002	I-IV	BC -Cx	Results for this analysis in which cases and controls were matched on the probability to receive chemotherapy were not significantly different.

BC= breast cancer patients; -Cx= no chemotherapy; [a]= total sample; [b]= sample matched on the propensity of receiving chemotherapy

received that particular agent (43).

All studies had large sample sizes; the number of chemotherapy-exposed survivors ranged from 2913 to 14057 and the number of controls ranged from 9752 to 48508. Throughout studies, breast cancer patients were diagnosed from 65 (41, 42), 66 (40) and 68 (43) years of age onwards and the maximum follow-up time of the studies ranged from 6 to 16 years. Dementia cases were identified through Medicare claims and based on ICD-9-CM codes (see **Table 6**).

6.2 Findings

Of the three studies (40-42) that investigated the incidence of dementia in chemotherapy-exposed survivors in comparison with non-exposed survivors, only one revealed a significant positive association between cytotoxic treatment and dementia (42). In this study by *Heck et al.*, Cox proportional hazards modeling showed that a diagnosis of any form of dementia was significantly more common in chemotherapy-exposed breast cancer survivors than in survivors who had not received cytotoxic treatment (hazard ratio (HR): 1.20; 95% ci 1.08-1.33).

Baxter et al. found no overall association between chemotherapy and incidence of dementia (40). An age stratified analysis showed that women aged 76-80 years who had

Table 6. ICD-9-CM codes used to identify dementia

ICD-9-CM	Disorder	2008 Heck	2008 Raji	2009 Baxter	2010 Du
290.0x	Senile dementia, uncomplicated	x	x	x	x
290.1x	Presenile dementia	x	x	x	x
290.2x	Senile dementia with delusional or depressive features	x	x	x	x
290.3x	Senile dementia with delirium	x	x	x	x
290.4x	Vascular dementia	x	x		x
290.8	Other specified senile psychotic conditions				x
290.9	Unspecified senile psychotic condition			x	x
292.82	Drug-induced persisting dementia			x	x
292.x	Drug-induced mental disorders				x
294.0	Amnestic disorder in conditions classified elsewhere	x	x		x
294.1	Dementia in conditions classified elsewhere	x	x		
294.8	Other persistent disorders due to condition classified elsewhere	x	x		x
294.9	Unspecified persistent mental disorder due to conditions classified elsewhere				x
331.0	Alzheimer's Disease	x	x	x	x
331.1	Frontotemporal dementia	x	x	x	
331.2	Senile degeneration of the brain	x	x	x	
331.7	Cerebral degeneration in diseases classified elsewhere	x	x		
331.89	Other cerebral degeneration			x	
331.9	Cerebral degeneration, unspecified			x	
349.82 ¶	Toxic encephalopathy (Primarily systemic agents)			x	
797	Senility without mention of psychosis	x	x	x	x

¶= subcode E933

received chemotherapy were less likely to develop dementia than women who had not received chemotherapy (HR: 0.49; 95% ci 0.28-0.88), but no associations between chemotherapy and dementia risk were observed in other age strata (women aged 66-70: HR: 0.83; 95% ci 0.48-1.45; women aged 71-75: HR: 0.74; 95% ci 0.46-1.18).

Du et al. reported that chemotherapy-exposed survivors were 8% more likely to develop drug-induced dementia than survivors who were not treated with adjuvant cytotoxic treatment, but this result disappeared after adjustment for patient and tumor characteristics (including age, ethnicity, tumor stage, and grade). In this study, the incidence of Alzheimer's disease (HR: 0.76; 95% ci 0.67-0.85) and vascular dementia (HR: 0.72; 95% ci 0.65-0.80) were significantly lower in

chemotherapy-exposed breast cancer survivors than in unexposed survivors (41).

A fourth study on the relationship between chemotherapy and dementia that compared patients who completed different cytotoxic regimens mutually found no differences in dementia incidence between women who received either CMF (cyclophosphamide, methotrexate, 5-fluorouracil) chemotherapy (HR: 1.02; 95% ci 0.78-1.33), anthracycline-based regimens (HR: 0.99; 95% ci 0.77-1.30) or taxane-based regimens (HR: 0.99; 95% ci 0.79-1.25), or a combined group of various other regimens (HR: 1.10; 95% ci 0.88-1.39) (43).

6.3 Methodological issues

Design

The studies used subsets from the SEER-Medicare database from largely overlapping time periods, therefore their results cannot be interpreted as independent outcomes.

Dementia was the primary outcome measure for all studies, but different combinations of ICD-9-CM codes were used for case ascertainment (see **Table 6**). *Baxter et al.* classified subjects as demented when they had two or more dementia related claims that were more than 14 days but not more than 6 months apart, whereas *Du et al.* rated women as demented when they had two claims that were more than 30 days apart. The other two studies did not specify a number of claims necessary for a dementia diagnosis. In addition, *Raji et al.* excluded women who were diagnosed with dementia in the three years preceding breast cancer diagnosis. In the studies by *Baxter et al.* and *Du et al.* women who had ICD codes for cognitive impairment at any time before the breast cancer diagnosis were subsequently excluded (40, 41). *Baxter et al.* also excluded women with vascular dementia, even though adjuvant systemic therapy may be a risk factor for dementia with a vascular etiology (40). This may have lead to an underestimation of the effect of chemotherapy on the development of dementia in general.

Because identification of chemotherapy use by means of Medicare claims is misclassified in 12% of the cases (45), the association between cytotoxic treatment and dementia may have been underestimated.

Another factor that makes it difficult to interpret the results of the studies on the risk of dementia following chemotherapy is the variation in maximum follow-up time between the studies (range: 6-16 years, see **Table 5**). Only *Baxter et al* reported the medium follow-up time, which was 4.9 years. No other studies reported their mean or median follow-up time. Dementia is a slowly developing disease with a long pre-clinical phase. Therefore, these studies may have observed the acceleration of dementia development as a result of chemotherapy rather than chemotherapy-induced dementia, which may take longer follow-up time to develop.

Confounding factors, bias and statistical procedures

Because use of tamoxifen has been associated with cognitive decline (36) and cognitive dysfunction is a risk factor for dementia, (46) it is possible that tamoxifen may have confounded

the association between chemotherapy and dementia. Indications for tamoxifen have changed (47) from postmenopausal node-positive breast cancer in the eighties and early nineties (48) to node-negative, estrogen receptor-positive breast cancer in the mid nineties (49). The cancer patients from the four SEER-Medicare studies were diagnosed between 1992 and 2002 (see **Table 5**) (40-43). Because mainly node-positive breast cancer patients received chemotherapy at that time, it is possible that in the early nineties the patients who received chemotherapy also more often received tamoxifen. Since the SEER-Medicare database does not hold information on tamoxifen use, none of the reviewed studies was able to adjust for this potential confounder.

One should consider that confounding by indication may have influenced the results of these studies, as fit elderly breast cancer patients without cognitive problems are more prone to be offered chemotherapy than their less healthy counterparts (50). This might explain why *Baxter et al.* found that chemotherapy-exposed survivors who had been diagnosed at age 76 to 80 had lower rates of dementia than their non-exposed counterparts (40).

Because *Du et al.* excluded women who had cognitive impairments before the cancer was diagnosed, it is difficult to compare their study results with the other studies in which patients with such problems were not excluded. By excluding cognitive impaired subjects *Du et al.* may have underestimated the effect of chemotherapy on the risk of dementia. Some neuropsychological studies suggest that pre-treatment cognitive reserve may be related to cognitive functioning post-therapy (4). Therefore, the risk of dementia after cytotoxic treatment might also differ in persons who already have cognitive problems from those who have not.

Heck et al. were the only investigators that reported a significant positive association between chemotherapy and dementia. However, because the hazard curves crossed the assumption of proportionality is violated (51) and therefore the reported hazard ratio should not be interpreted (42).

Generalizability

Because solely persons 65 years of age and up are eligible for Medicare, only breast cancer patients who were diagnosed at age 65 or older could be followed for subsequent dementia. Therefore these studies may not be generalizable to younger breast cancer patients who were diagnosed with breast cancer before the age of 65.

7 Conclusion

Five out of six neuropsychological studies addressing long-term cognitive functioning of cancer patients showed that adjuvant chemotherapy is associated with subtle worse cognitive functioning in the domains of attention and executive functioning. Further support for this contention comes from a study that did not investigate the effects of cytotoxic treatment but rather compared twins discordant for cancer, and that showed that long-term survivors of cancer were at higher risk of cognitive dysfunction than their non-cancer co-twins.

Because of methodological limitations and differences between studies, we could not perform a formal meta-analysis. Nevertheless, the consistency of the findings across studies is striking, with all studies reporting on average worse cognitive performance in the chemotherapy treated group.

The imaging studies that investigated the effects of chemotherapy on brain structure and function where in line with these observations and indicated that cytotoxic treatment is associated with long-term gray matter reductions, global and focal lower white matter integrity and hypo-activation of brain areas during cognitive tasks.

Studies on the association between chemotherapy and dementia that used data from the SEER-Medicare database did not show clear evidence for such a relationship. The large samples of chemotherapy-exposed breast cancer patients and non-exposed survivors ensured high power to detect small effects of cytotoxic treatment on the incidence of dementia. However, all studies used data from the same database and therefore their outcomes are not independent. In addition, potential misclassification of exposure and misclassification of the outcome measure, the exclusion of subjects at risk to develop dementia in one particular study (41) and the relatively short follow-up time in another study (40) may have diminished the potential to detect an association between chemotherapy treatment and incidence of dementia.

7.1 Clinical implications

Studies on late-cognitive functioning after cytotoxic treatment suggest that chemotherapy may be associated with subtle yet long lasting cognitive deficits, but no increased incidence of dementia. Together with human imaging studies and experimental animal studies that both indicate short and long-term structural brain changes (16, 38, 39, 52, 53) associated with common chemotherapeutic agents, this finding is of clinical relevance. The mechanisms underlying late cognitive dysfunction are still largely unknown (7). However, cytotoxic treatment may have a direct effect on central nervous system cells, as studies have showed that chemotherapy can penetrate the blood brain barrier (8). Also other proposed mechanisms for chemotherapy associated cognitive decline during and shortly after treatment, such as deficient neuronal repair mechanisms and genetic susceptibility, are stable characteristics, and thus may be responsible for cognitive comprise in the long run (7, 8). Currently, no therapy or preventive strategies are available for chemotherapy-induced cognitive changes and actual treatment mainly focuses on coping with symptoms.

Women with a history of breast cancer constitute a large group in the cancer survivor community. Whereas these side effects may not offset the beneficial effects of chemotherapy, they emphasize the need to adequately address the potential cognitive side-effects of cytotoxic treatment in counseling and aftercare.

7.2 Future perspectives

Large prospective longitudinal trials with long follow-up would be optimal to address the relation between chemotherapy and cognitive functioning and the risk of dementia. Unfortunately the feasibility of such studies is limited. Population-based cohort studies with long follow-up that include adequate neuropsychological testing and/or complete dementia work-up may give insight in these long-term adverse effects. Furthermore, incidence of dementia after chemotherapy should also be investigated in subjects that received chemotherapy before the age of 65 and in pre-menopausal women.

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Supplementary material to Chapter 2:

A review of the late effects of adjuvant chemotherapy for adult onset non-central nervous system cancer

Ahles et al., 2002 (1)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
Vocabulary (WAIS III)		VERBAL ABILITY	*Language*
Reading subtest (WRAT-3)		VERBAL ABILITY	*Premorbid intelligence level*
Boston Naming Test		VERBAL ABILITY	*Language*
Controlled Oral Word Association Test (COWAT)		VERBAL ABILITY	*Language*
Block design (WAIS III)		SPATIAL ABILITY	*Visuospatial functioning*
California verbal learning test		VERBAL LEARNING	*Memory*
Logical memory I (WMS-R)	Stories A&B	VERBAL MEMORY	*Memory*
Logical memory multiple choice (WMS-R)	Story B (30'delay)	VERBAL MEMORY	*Memory*
Visual reporduction I + II (30min delay) (WMS-R)		VISUAL MEMORY	*Memory*
Digit symbol (WAIS-III)		PSYCHOMOTOR FUNCTION	*Attention*
Trail making test	A	PSYCHOMOTOR FUNCTION	*Attention*
	B	PSYCHOMOTOR FUNCTION	*Executive functioning*
Finger tapping		MOTOR FUNCTION	*Motor function*
Thumb-finge sequencing		MOTOR FUNCTION	*Motor function*
Vigilance and distractability	Targets	ATTENTION	*Attention*
	Reaction time	ATTENTION REACTION TIME	*Attention*

* Text in printed in SMALL CAPITALS indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper; WAIS III= Wechsler Adult Intelligence Scale-III; WRAT-3= Wide Range Achievement Test 3; WMS-R= Wechsler Memory Scale Revised

Scherwath et al., 2006 (2)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
Trail making test	A	SPEED OF INFORMATION PROCESSING	ATTENTION
	B	DIVIDED ATTENTION/COGNITIVE FLEXIBILITY	ATTENTION
Test Battery for Attentional Performance	Alertness	SIMPLE REACTION TIME AND PHASIC ALERTNESS	ATTENTION
	Go/No-go	SELECTIVE ATTENTION	ATTENTION
Test d2 - Cancellation Test		SELECTIVE ATTENTION	ATTENTION
WMS-R ¶	Digit span	VERBAL WORKING MEMORY	MEMORY
	Visual span	VISUO-SPATIAL WORKING MEMORY	MEMORY
Auditory Verbal Learning Test (Form A) ¶		VERBAL LEARNING	MEMORY
		VERBAL RETROACTIVE INTERFERENCE	MEMORY
		VERBAL DELATED RECALL	MEMORY
		VERBAL RECOGNITION	MEMORY
Rey-Osterrieth Complex Figure Test		VISUAL DELAYED RECALL	MEMORY
Regensburg Word Fluency Test	Letter P	LETTER FLUENCY	EXECUTIVE FUNCTIONING
	Foodstuffs	CATEGORY FLUENCY	EXECUTIVE FUNCTIONING
Achievement Measure System	3	REASONING	EXECUTIVE FUNCTIONING
	4	REASONING	EXECUTIVE FUNCTIONING
Wechsler Adult Intelligence Scale-Revised ¶	General knowledge	GENERAL KNOWLEDGE	PREMORBID INTELLIGENCE LEVEL

* Text in printed in SMALL CAPITALS indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper; ¶ German version/adaptation; WMS-R= Wechsler Memory Scale Revised

Correa et al., 2010 (3)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
Digit span (WAIS)	Forward		Attention
	Backward		Attention
Brief test of attention			Executive functioning
Trail making test	A		Executive functioning
	B		Executive functioning
Hopkins Verbal Learning Test	Delay		Memory
	% Retention		Memory
	Discrimination		Memory
	Total		Memory

* Text in printed in SMALL CAPITALS indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper; WAIS= Wechsler Adult Intelligence Scale

Yamada et al., 2010 (4)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
Abbreviated Scale of Intelligence (WAIS)		Intelligence	IQ
MMSE		General cognitive functioning	Dementia screener
Digit span (WAIS)		Simple/divided attention/working memory	Attention
Letter number sequencing (WAIS)		Simple/divided attention/working memory	Attention
Arithmetic (WAIS)		Simple/divided attention/working memory	Attention
Trail making test	A	Attention/psychomotor	Attention
Controlled Oral Word Association Test (COWAT)		Verbal fluency	Language
Boston Naming Test			Language
Rey Ostreich Complex Figure test	Copy condition		Visuospatial functioning
Facial Recognition test			Visuospatial functioning
Rey auditory verbal learning test		Verbal learning and memory	Memory
Rey Ostreich Complex Figure test	Delay condition	Visual memory	Memory
Benton visual retention test		Immediate memory	Memory
Intradimensional/Extradimensional Shift task			Executive Functioning
Trail making test	B		Executive Functioning
Wisconsin Card Sorting Task			Executive Functioning

* Text in printed in small capitals indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper; WAIS III= Wechsler Adult Intelligence Scale-III

de Ruiter et al., 2011 (5)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
Tower of London		PLANNING ABILITIES	*Executive functioning*
Paired associates task		EPISODIC MEMORY	*Memory*
Trail making test	A	FOCUSSED & SUSTAINED ATTENTION	*Attention*
	B	MENTAL FLEXIBILITY	*Executive functioning*
Digit symbol coding test (WAIS-III)	Correct answers	FOCUSSED & SUSTAINED ATTENTION	*Attention*
Stroop color-word test	Card 1 & 2	FOCUSSED & SUSTAINED ATTENTION	*Attention*
	Card 3 & 4	MENTAL FLEXIBILITY	*Executive functioning*
California Verbal Learning test	Immediate recall	VERBAL MEMORY	*Memory*
	Delayed recall	VERBAL MEMORY	*Memory*
	Recognition	VERBAL MEMORY	*Memory*
Visual reproduction test (WMS-R)	Immediate recall	MEMORY	*Memory*
	Delayed recall	MEMORY	*Memory*
	Recognition	MEMORY	*Memory*
Word fluency test	Animals	VERBAL FUNCTION	*Executive functioning*
	Professions	VERBAL FUNCTION	*Executive functioning*
Fepsy finger tapping test	Dominant hand	MOTOR SPEED	*Motor function*
	Non-dominat hand	MOTOR SPEED	*Motor function*
Dutch Adult Reading Test	General knowledge	GENERAL KNOWLEDGE	*Premorbid intelligence level*

* Text in printed in SMALL CAPITALS indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper; WAIS III= Wechsler Adult Intelligence Scale-III; WMS-R= Wechsler Memory Scale Revised

Koppelmans et al., 2011 (6)

Tests	Subtests	Specific cognitive function *	Cognitive domain *
MMSE		DEMENTIA SCREENER	DEMENTIA SCREENER
15-Word learning test	Immediate recall	LEARNING AND MEMORY	*Memory*
	Delayed recall	LEARNING AND MEMORY	*Memory*
	Recognition	LEARNING AND MEMORY	*Memory*
Letter digit substitution test		PROCESSING SPEED	*Attention*
Stroop test	Card 1	PROCESSING SPEED	*Attention*
	Card 2	PROCESSING SPEED	*Attention*
	Card 3	PROCESSING SPEED & INHIBITION	EXECUTIVE FUNCTIONING
Word fluency test	Animals	VERBAL FLUENCY	EXECUTIVE FUNCTIONING
Design Organization test		VISUO-SPATIAL ABILITY	*Visuo-spatial functioning*
Purdue pegboard test	Both hands	MOTOR SPEED & DEXTERITY	*Motor function*
	Left hand	MOTOR SPEED & DEXTERITY	*Motor function*
	Right hand	MOTOR SPEED & DEXTERITY	*Motor function*

* Text in printed in SMALL CAPITALS indicates terminology of cognitive functions and/or domains that were copied from the original paper. Text in *italics* indicates terminology deduced from cognitive functions or domains used in the original paper because cognitive functions or domains were not mentioned in the original paper

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Chapter 3

Neuropsychological performance in breast cancer survivors more than 20 years after adjuvant chemotherapy

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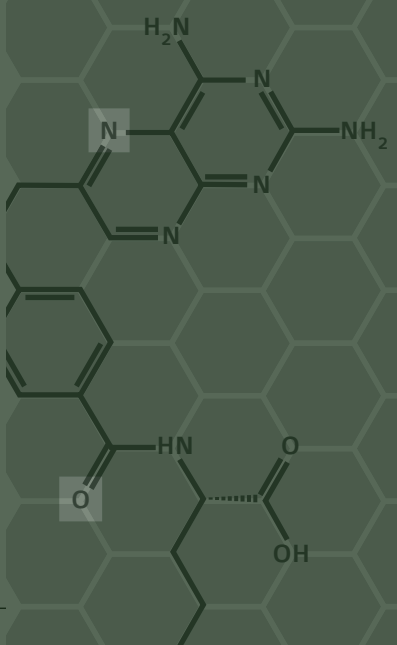


Abstract

Purpose: Adjuvant chemotherapy for breast cancer can have adverse effects on cognition shortly after administration. Whether chemotherapy has any long-term effects on cognition is largely unknown, yet becomes increasingly relevant because of the widespread use of chemotherapy for early breast cancer and the improved survival. We investigated whether CMF chemotherapy for breast cancer is associated with worse cognitive performance more than 20 years after treatment.

Methods: Case-cohort study comparing the cognitive performance of breast cancer patients with a history of adjuvant CMF chemotherapy treatment (six cycles; average time since treatment 21 years) (n=196) to that of a population-based sample of women never diagnosed with cancer (n=1509). Participants were between 50 and 80 years of age. Exclusion criteria were: ever use of adjuvant endocrine therapy, secondary malignancy, recurrence and/or metastasis.

Results: The chemotherapy-exposed women performed significantly worse than the reference group on cognitive tests of immediate (p=.015) and delayed verbal memory (p=.002), processing speed (<.001), executive functioning (p=.013) and psycho-motor speed (p=.001). They experienced less symptoms of depression (p<.001) yet had significantly more memory complaints on two out of three measures that could not be explained by cognitive test performance.

Conclusion: Breast cancer survivors treated with adjuvant CMF chemotherapy more than 20 years ago, perform on average worse on neuropsychological tests than random population controls. The pattern of cognitive problems is largely similar to that observed in patients shortly after cessation of chemotherapy. This study suggests that cognitive deficits following breast cancer diagnosis and subsequent CMF chemotherapy can be long-lasting.

Introduction

Chemotherapy has well-recognized acute side-effects, including nausea and hair-loss. Cognitive impairment is a potential short-term side effect that has gained more attention only in the last decade (1-20). A number of studies have shown that chemotherapy can induce cognitive changes up to five years after treatment (2, 5, 14 ,20). Differences are mainly observed in the domains of memory, processing speed and executive function and are generally not explained by socio-demographic and clinical variables (21). Nevertheless, cognitive dysfunction has also been observed in the domains of visuo-spatial functioning (22) and psycho-motor speed (15). Potential predictors for cognitive problems in chemotherapy-exposed breast cancer patients, such as cognitive reserve and genetic susceptibility, are topics of ongoing research (23). Besides differences in cognitive performance, structural brain differences have been observed in patients who underwent chemotherapy compared to control subjects, including more white-matter hyperintensities and microstructural damage to white-matter tracts, and gray matter alterations (1, 7, 24-30), whereas functional magnetic resonance imaging (fMRI) studies revealed measurable differences in task-specific responsiveness between chemotherapy-exposed patients and control subjects (5, 26, 31). The observational studies in humans are strongly supported by animal studies (32).

Whether chemotherapy has long-term effects on brain function is still largely unknown.

However, this question is becoming increasingly relevant as the number of long-term survivors is rapidly increasing.

We aimed to investigate the late effects of chemotherapy on cognitive functioning by comparing the neuropsychological test performance of women who received adjuvant CMF chemotherapy for breast cancer patients on average more than 20 years before with that of a population sample of women who had never been diagnosed with cancer.

Materials & methods

Participants

Our case group consisted of breast cancer survivors who had undergone adjuvant chemotherapy in either of two specialised cancer clinics in the Netherlands. The reference group was selected from an ongoing population study in the Netherlands. The review boards of the participating institutes (the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Erasmus University Medical Center) approved this study.

Chemotherapy-exposed subjects

From the registries of the Antoni van Leeuwenhoek Hospital/Netherlands Cancer Institute and the Erasmus University Medical Center-Daniel den Hoed Cancer Center we identified consecutive female breast cancer patients who, as part of their primary treatment had received six cycles of adjuvant CMF chemotherapy (Cyclophosphamide 100 mg/m2, taken orally, on days

1-14, Methotrexate 40 mg/m2, given intravenously, on days 1 and 8, 5-Fluorouracil 600 mg/m2, given intravenously, on days 1 and 8) between 1976 and 1995. Eligibility criteria included age between 50 and 80 years at recruitment time in 2008, and sufficient command of the Dutch language. Only those women who never had a relapse, secondary primary tumour or distant metastasis were selected. Exclusion criteria were ever use of adjuvant endocrine therapy and contra-indications for MRI.

Potential participants (n=359) were sent an invitation letter and information on the study. Twenty patients (5.6%) could not be reached either because their current address was unavailable, or they did not react to the invitation or subsequent reminders. Fifteen subjects (4.2%) had a health related contra-indication for MRI, 30 (8.4%) were ineligible for MRI-assessment because of claustrophobia and two (0.6%) patients had insufficient command of the Dutch language. The final number of eligible patients was 292 of whom 196 (67.1%) eventually agreed to participate and provided written informed consent. Examinations were performed between October 2008 and October 2009.

Main reasons for decline were: not wanting to be reminded of the cancer episode (21.9%) and unwillingness to undergo MRI-assessment (26.0%). Decliners were older than participants ($F_{1,290}$)=12.24; p=.001.

To assess possible selection bias, eligible women who declined participation and women for whom claustrophobia was the only contra-indication were invited to complete the interview and the neuropsychological assessments at home. Test results of these 'initial decliners' were compared with the results of those who participated in the current study. Of the 126 invited initial decliners (96 decliners + 30 claustrophobic women) 48 (38.1%) agreed to participate. They were assessed between November 2009 and June 2010.

Reference group

A reference group was selected from the Rotterdam Study, a population-based prospective cohort study ongoing since 1990 in the city of Rotterdam, the Netherlands (33). By the end of 2008, 14,926 subjects had been included in three separate subcohorts. Rotterdam Study-III is the most recent subcohort, which comprises 3932 persons who have been assessed only once, namely between February 2006 and December 2008. To date it is the only cohort that is assessed with an extensive set of neuropsychological tests and therefore the most appropriate reference subcohort.

From Rotterdam Study-III we selected all women without a history of cancer, based on self-reports and linkage with data from the general physician, who were between 50 and 80 years of age at the time of neuropsychological assessment. In total 1509 subjects met these criteria.

Methods

Examination of the participants took place at the Rotterdam Study research center (34).

Participants underwent neuropsychological examinations and an interview identical to those used in the Rotterdam Study. Subsequently blood was drawn, height and weight were measured, and participants underwent MRI of the brain, carotid ultrasound imaging and an electro-cardiogram. Results from the latter measures will be described separately.

Neuropsychological examination

Seven neuropsychological tests were administrated and scored by experienced test-assistants from the Rotterdam Study. These tests yielded 17 outcomes in the following cognitive domains: processing speed, verbal learning, memory, inhibition and word fluency as elements of executive functioning, visuo-spatial ability and psycho-motor speed. In addition, the MMSE was included as a dementia screener. For an overview of the tests and domains see **Table 1** (35-42).

Interview

Participants completed an interview on clinical and socio-demographic factors, which included questions regarding medical history of neurological, psychiatric and cardiovascular diseases. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression scale (CES-D) (43) which was converted to a sum-score according to the standard scoring rules (44). Subjective memory complaints were measured with three 'yes/no' questions: 1) Do you have more problems remembering things than before?; 2) Has there been an increase in the times that you forgot what you were up to?; 3) Do you have more word finding problems than before? Subsequently, participants were asked whether these problems had an acute onset (yes/no) and if the severity of the problems had changed over time (no change/problems increased/problems decreased).

Statistical analysis

We compared differences in socio-demographic variables between groups by means of binary, ordinal and multinomial logistic regression analysis. Group differences in neuropsychological performance and depressive symptoms were investigated with analysis of covariance (ANCOVA), adjusted for age, and education. Although studies on the cognitive effects of chemotherapy shortly after treatment do not show a strong relationship between depressive symptoms and neuropsychological performance, (45) no information is available on this potential association long after chemotherapy. Therefore we subsequently adjusted our analyses for CES-D sum-score. We used Bonferroni correction to account for multiple testing.

The age distribution of the reference group was more skewed towards younger ages than that of the chemotherapy-exposed cancer survivors. To check whether any residual confounding by age remained after standard adjustment for age, we additionally executed all analyses 1) with propensity scores for age; and 2) using an age-matched reference group randomly

Table 1. Outcome measures

Neuropsychological test	Acronym	Functional Area Assessed	Test element	Outcome Measure	Range
Mini Mental State Examination	MMSE	Dementia screener (34, 36)	-	Total correct answers [a]	0-30
15 Word learning test	15 WLT	Learning and memory (35)	Immediate recall (3 trials)	Number of words remembered immediately after each trial [a]	(3x) 0-15
			Delayed recall	Number of words remembered after 20 minutes [a]	0-15
			Recognition	Number of words recognized [a]	0-30
Letter digit substitution test	LDST	Processing speed (40)	-	Number of correctly substituted letters [a]	0-125
Stroop color-word test		Processing speed and inhibition as an element of executive function (abbreviated version) (37)	Word card	Seconds needed to complete the first 4 lines [b]	≥0
			Color-card	idem	≥0
			Color-word card	idem	≥0
Word fluency test	WFT	Verbal fluency (executive function) (39)	-	Number of animals mentioned within 1 minute [a]	≥0
Design Organization test	DOT	Visuo-spatial ability (38)	-	Number of correctly coded blocks [a]	0-56
Purdue pegboard test	PPB	Motor speed and dexterity (33)	Left hand	Number of pins inserted in the board within 1 minute	0-25
			Right hand	idem	0-25
			Both hands	idem	0-25

[a] higher score indicates better performance; [b] lower score indicates better performance.

drawn from the total reference group. Since these additional analyses yielded similar results as the primary analyses, their results are not separately reported.

Even though the different cognitive tests in our battery intend to measure different domains, an individual's scores on cognitive tests are often related. To account for this interdependency between test scores, we calculated for each individual the Mahalanobis Distance (MD) (46) as a summary measure of overall performance (47). The MD takes into account the correlations between test scores and the different variances of the test scores and can be interpreted as the distance to the mean of the multidimensional distribution of the neuropsychological test scores of the reference subjects.

MD was based on all tests, except for the Design Organization test because few women from the reference group completed this test, and except for the MMSE as it is a dementia screener. We calculated age, education and CES-D score adjusted residuals of

the neuropsychological tests while the computation of the relevant means and (co)variances was based on the residuals of the reference group (47, 48). We assigned a value of zero to all residual scores greater than their respective mean score of the reference group such that a positive test score(s) could not compensate for negative scores (49). We transformed the MD with log base 2 because of skewness of its distribution and subsequently used one-way ANOVA to compare MD between the chemotherapy-exposed subjects and the reference group.

Spearman rank correlation coefficients with 2-sided p-values were calculated to obtain the associations between memory complaints, neuropsychological test outcomes and mood. Alpha levels were set at p=0.05 for all analyses.

Results

Table 2 presents the baseline characteristics of the chemotherapy-exposed breast cancer patients and the reference group. On average, breast cancer survivors were older and had completed a higher level of education. They had been diagnosed on average at age 42.9, and received chemotherapy on average 21.2 years before enrollment in this study. No differences were observed in the prevalence of neurologic, psychiatric or cardiovascular diseases.

Table 2. Socio-demographic and clinical characteristics of former breast cancer patients exposed to chemotherapy and the reference group from the general population

	Chemotherapy-exposed patients (n=196)		Reference group (n=1509)		p-value
Mean age in years (Sd)	64.1	(6.4)	57.9	(5.4)	<.001
Education level %					<.001
primary education	8.7		12.5		
lower vocational education	16.3		21.5		
intermediate general education	20.4		24.2		
intermediate vocational education	16.8		16.3		
higher general education	5.6		5.2		
college education	23.5		16.1		
university education	8.7		4.2		
Mean age at BC diagnosis (Sd)	42.9	(5.3)			
Mean time since BC diagnosis in years (Sd)	21.2	(4.4)			

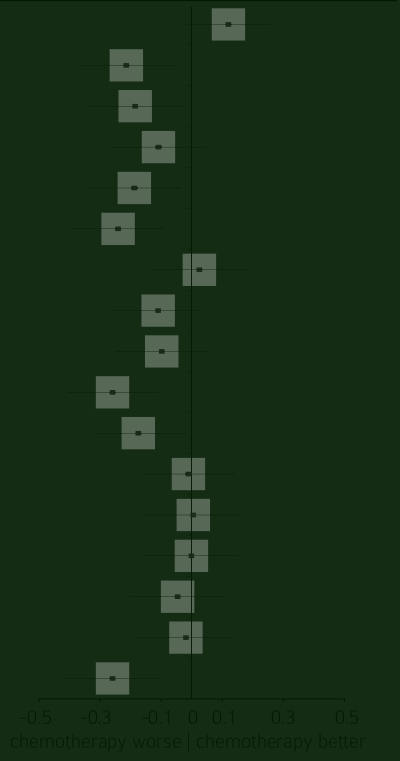
BC= breast cancer; Sd= Standard deviation.

Neuropsychological outcomes

On all neuropsychological tests chemotherapy-exposed breast cancer survivors performed similar or worse than reference subjects. These differences were significant for nearly all trials of immediate and delayed recall of the 15WLT, for the color-card and the color-word card of

Table 3. Neuropsychological test outcomes

	Chemotherapy-exposed breast cancer patients (N=196)			Reference group (N=1518)				
Testoutcome	N	Mean	(Sd)	N	Mean	(Sd)	p-value	difference of the Z-scores (95% c.i.)
MMSE	196	28.4	(2.0)	1507	28.2	(2.2)	.09	
15WLT: trial 1	194	5.5	(2.2)	1397	5.9	(2.4)	.008	
15WLT: trial 2	194	8.6	(2.4)	1397	9.0	(2.7)	.02	
15WLT: trial 3	194	10.3	(2.6)	1397	10.6	(2.9)	.17	
15WLT: total of 3 trials	194	24.3	(6.2)	1397	25.5	(6.9)	.02	
15WLT: delayed recall	194	8.0	(2.9)	1397	8.7	(3.2)	.002	
15WLT: recognition	194	13.8	(1.8)	1397	13.8	(2.0)	.76	
LDST: total correct	195	31.8	(6.7)	1497	32.5	(7.5)	.14	
Stroop: Word card	195	16.8	(3.3)	1404	16.5	(3.7)	.14	
Stroop: Color-card	195	23.3	(4.4)	1404	22.2	(4.9)	.001	
Stroop: Color-word card	195	45.8	(12.6)	1404	43.5	(14.0)	.02	
Word fluency: total	194	24.1	(6.1)	1490	24.2	(6.8)	.89	
Word fluency: after 15 sec.	194	13.8	(4.8)	1490	13.8	(5.4)	.95	
DOT: total correct	195	28.9	(9.2)	511	28.9	(9.7)	.99	
PPB: Both hands	195	11.1	(1.6)	1494	11.2	(1.8)	.56	
PPB: Dominant hand	195	13.8	(1.9)	1490	13.8	(2.1)	.81	
PPB: Non-dominant hand	195	12.9	(1.8)	1490	13.4	(2.0)	.001	



c.i.= confidence interval; MMSE = Mini Mental State Examination; 15WLT = 15 Word Learning Test; LDST = Letter Digit Substitution Test; DOT =Design Organization Test.

the Stroop test, and for non-dominant-hand performance on the Purdue pegboard test (**Table 3**). After Bonferroni corrections, differences on the 15WLT delayed recall, the Stroop color-card and the Purdue pegboard test for the non-dominant-hand condition remained significant. MMSE scores did not differ between groups. Excluding participants with neurologic or psychiatric diseases did not change the outcome of the analyses.

The base-2-log of the Mahalanobis distance was significantly larger for chemotherapy-exposed survivors (mean=2.8; sd=2.6) than for the reference group (mean=2.2; sd=2.8; (($F_{1,1648}$)=7.3; p=.007) indicating that the former had worse overall cognitive performance.

Time since diagnosis was not associated with neuropsychological performance in chemotherapy-exposed survivors.

Table 4. Subjective cognitive complaints in chemotherapy-exposed breast cancer patients and a reference group from the general population

	Chemotherapy-exposed patients	Reference group		95% c.i. for OR *		
Memory complaints	**% ***	**% ***	**OR**	**Lower**	**Upper**	**p**
More problems remembering [a]	52.8	46.1	1.32	.96	1.82	.09
Forgetting (daily) pursuits [b]	42.9	35.2	1.41	1.01	1.96	.042
Word finding problems [c]	38.2	30.2	1.46	1.04	2.05	.030
Rapid onset of problems [d]	10.7	13.4	.76	.36	1.62	.48
Change in problems [e]						
worsened over time [f]	30.5	19.6	1.68	.99	2.82	.05
improved over time [f]	5.8	6.5	.98	.36	2.65	.97

c.i.= confidence interval; OR= Odds Ratio; * depression score (CES-D depression inventory) and age adjusted; [a] Do you have more problems remembering things than before?; [b] Has there been an increase in the times that you forgot what your were up to?; [c] Do you have more word finding problems than before?; [d] Did the problems occur suddenly?; [e] Have the problems changed over time?; [f] Reference category=no change over time.

Depressive symptoms and memory complaints

The reference group reported significantly more depressive symptoms than the chemotherapy-exposed breast cancer survivors (age-adjusted mean sum-score on the CESD=6.7, sd=8.4 versus 4.7, sd=8.0; ($F_{1,1696}$)=9.54; p=.002). There was a low correlation between memory complaints and total score on the CES-D (ρ=.275; p=<.001) in chemotherapy-exposed survivors.

The proportion of subjects who reported problems with remembering did not differ between groups, yet chemotherapy-exposed breast cancer survivors were more likely to report an increase in word finding problems and in the frequency of forgetting pursuits (**Table 4**). These

subjective memory complaints were not related to neuropsychological performance.

Chemotherapy-exposed breast cancer survivors who participated at the research center of the Rotterdam Study did not differ from participants who declined participation at the research center, but agreed to cognitive testing in their own home regarding age, education level, Bonferroni-corrected cognitive scores or mood status. Without correction for multiple testing, home participants performed worse than center participants on one out of the seventeen cognitive measures, namely the word card of the Stroop test (p=.011).

Discussion

To our knowledge, this is the first report on the cognitive effects of adjuvant CMF chemotherapy in breast cancer survivors who completed this treatment on average more than 21 years before. Compared to women from the general population without cancer, chemotherapy-exposed breast cancer survivors performed worse on cognitive tests covering the domains of learning, immediate and delayed verbal memory, information processing speed, inhibition and psychomotor speed. No differences were observed in scores on a dementia screener. The results persisted after controlling for several confounders including age, education-level and depression score. After subsequent correction for multiple comparisons, chemotherapy-exposed survivors still performed worse on tests measuring delayed verbal memory, processing speed and psychomotor speed. Also on a summary measure of the neuropsychological tests that takes correlations between multiple measures into account, chemotherapy-exposed survivors performed significantly worse than women from the general population.

Further, chemotherapy-exposed breast cancer survivors more often reported memory complaints, which were not associated with test performance, but were weakly correlated with mood. Chemotherapy-exposed survivors had less depressive symptoms than the reference group although both groups scored below the cut-off score of 16, indicative for clinical depression (43).

Strengths of our study are the large sample size, the long interval since chemotherapy, the homogeneous study population regarding cytotoxic agents (regimen, cycles), and the large population-based reference group without cancer. Possible selection bias within the chemotherapy-exposed group has been investigated, and was found to be unlikely.

We compared chemotherapy-exposed breast cancer survivors to a population-based sample of healthy control subjects without a history of cancer because we wanted to investigate to which extent chemotherapy-exposed breast cancer survivors deviate from the norm regarding cognitive functioning. Subsequently, as tamoxifen was not part of standard treatment in the Netherlands until the mid nineteen-nineties, it was not possible to include a comparison group of long-term tamoxifen-exposed survivors. Because of our design, we were unable to distinguish the effect of chemotherapy on cognition from the possible effect of breast cancer itself.

It has been suggested that breast cancer patients may already do worse on tests of cognitive

function compared to healthy controls before the start of chemotherapy (8-10, 15, 18, 50, 51). Since we do not have pre-treatment assessments to adjust our results for, our findings might partially reflect group differences already present before chemotherapy. The mechanisms for pre-treatment differences are largely unknown, although the prevalence of cognitive problems at baseline has been associated with breast-cancer stage (51). Suggested explanations for pre-treatment problems include diminished cognitive reserve, stimulation of proinflammatory cytokines (18), and the effect of anesthetic drugs received for breast surgery (52). Because the effect of anesthesia is transient (52) we consider its influence on cognition more than 20 years post treatment unlikely. Moreover, follow-up studies demonstrated a larger prevalence of cognitive decline from baseline in chemotherapy-exposed patients than patients who only underwent loco-regional therapy, indicating that at least a part of the deficits are indeed associated with cytotoxic treatment (3, 15, 53-56).

Although information on hormone replacement therapy was not available, we do not think this can have majorly confounded our findings as the use of HRT in the Netherlands tended to be low in the years under study (<2.5% of women aged 40-74) (57).

An important question is to what extent our observations extent to other chemotherapy regimens. The CMF regimen is no longer the most optimal adjuvant chemotherapy for early breast cancer. However, it has been the standard regimen up to the nineteen-nineties, and it is the only regimen that enables the investigation of the very late effects of chemotherapy in sufficiently large numbers of subjects. In addition, there is still an elaborate group of women that has been treated with CMF in the late nineties of whom some may experience its cognitive side effects in the future. Furthermore, cyclophosphamide and 5-fluorouracil continue to be incorporated in currently used regimens for early breast cancer. Even if the findings of our study would be specific to CMF, they therefore remain relevant.

Several studies have found impairments in cognitive domains in cancer patients shortly after treatment with chemotherapy (4, 58-65). Impairments frequently observed in chemotherapy-exposed breast cancer patients are learning problems and deficits in memory retrieval with more preserved retention, as well as problems with information-processing speed and more complex aspects of attention. Imaging studies showed that some chemotherapy regimens may induce structural brain alterations (1, 7, 24, 25, 27, 28).

The current study resembles this pattern: chemotherapy-exposed breast cancer survivors from our study also had more problems with learning and memory retrieval while retention was intact. The combination of worse processing speed, inhibition problems and problems with fine motor functioning we observed in chemotherapy-exposed survivors adds to this profile. This profile is suggestive for disruption of the frontal-subcortical network and matches the profile observed in other studies (65).

The fact that chemotherapy-exposed breast cancer survivors performed worse on the non-dominant condition of the Purdue pegboard test, but not on the dominant condition, has been

observed before in patients treated with chemotherapy (7) and other patient populations. It has been related to neurological damage (66) and may possibly be related to interhemispheric transfer deficits (67).

Our neuropsychological test-battery was identical to the one used in the Rotterdam Study, but less extensive than some used in previous studies (16, 22, 54, 68, 69). Some domains (e.g. visual memory), which are known to be affected by cytotoxic treatment, were not explicitly examined (15, 22, 70). Even though we found several significant differences in cognitive functioning between chemotherapy-exposed survivors and the reference group, we may have underestimated the effects of CMF chemotherapy on cognitive functioning. The effects of chemotherapy might extend to more cognitive domains than we showed in this study.

When we compare our study outcomes with other studies investigating the cognitive effects of CMF-chemotherapy there are several similarities. One study showed that, patients who underwent CMF at least 10 years ago performed worse on tests measuring executive functioning, psychomotor speed and attention than healthy controls (20). Another study found that a subgroup of patients treated with CMF showed impaired information processing speed five years after completion of treatment (71). Animal studies support our findings and have pointed out that methotrexate, cyclophosphamide and the combination of 5-fluorouracil and methotrexate are associated with impaired learning and memory and structural brain changes (32, 72-76).

In conclusion, the cognitive functioning of breast cancer survivors on average 21 years after adjuvant CMF chemotherapy is worse than that of women from the general population who have never been diagnosed with cancer. These data suggest that cognitive deficits following breast cancer diagnosis and subsequent CMF chemotherapy are at least partially long-lasting. Our results are highly relevant in the field of cancer survivorship as with the current treatment strategies the number of long-term breast cancer survivors is increasing due to improved recognition of early stage breast cancer, ageing of the population and improved survival after breast cancer diagnosis (77, 78). Further studies into the very late effects of adjuvant chemotherapy for cancer are needed in order to corroborate these results and to gain further insight in the mechanisms underlying these observations.

Acknowledgement

In loving memoriam of Chad Michael Gundy

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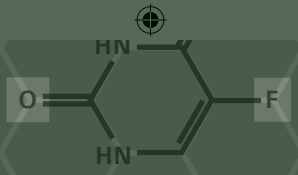
Chapter 4

Late effects of chemotherapy on brain structure



Chapter 4.1

Global and focal brain volume in long-term breast cancer survivors exposed to adjuvant chemotherapy

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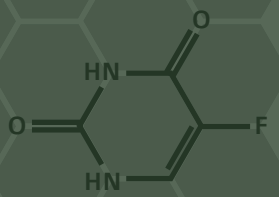

Abstract

Introduction: A limited number of studies have associated adjuvant chemotherapy with structural brain changes. These studies had small sample sizes and were conducted shortly after cessation of chemotherapy. Results of these studies indicate local gray matter volume decrease and an increase in white matter lesions. Up till now, it is unclear if non-CNS chemotherapy is associated with long-term structural brain changes. We compared focal and total brain volume of a large set of non-CNS directed chemotherapy-exposed breast cancer survivors, on average 21 years post-treatment, to that of a population-based sample of women without a history of cancer.

Methods: Structural MRI (1.5T) was performed in 184 chemotherapy-exposed breast cancer patients, mean age 64.0 (sd=6.5) years, who had been diagnosed with cancer on average 21.1 (sd=4.4) years before, and 368 age-matched cancer-free reference subjects from a population-based cohort study. Outcome measures were: total brain volume and total gray and white matter volume, and hippocampal volume. In addition, voxel based morphometry was performed to analyze differences in focal gray matter.

Results: The chemotherapy-exposed breast cancer survivors had significantly smaller total brain volume (-3.5 ml, p=.019) and gray matter volume (-2.9 ml, p=.003) than the reference subjects. No significant differences were observed in white matter volume, hippocampal volume, or local gray matter volume.

Discussion: This study shows that adjuvant chemotherapy for breast cancer is associated with long-term reductions in total brain volume and overall gray matter volume in the absence of focal reductions. The observed smaller gray matter volume in chemotherapy-exposed survivors was comparable to the effect of almost 4 years of age on gray matter volume reduction. These volume differences might be associated with the slightly worse cognitive performance that we observed previously in this group of breast cancer survivors.

Introduction

Treatment of breast cancer with cytotoxic agents has been associated with structural and functional brain changes (**Table 1**) (1). White-matter pathology has been observed within months up to ten years post-treatment. Compared to healthy controls and breast cancer patients who never received cytotoxic treatment, chemotherapy-exposed patients had more white matter hyperintensities (2, 3) and decreased integrity of major white matter tracts in frontal and temporal regions of the brain (4, 5). The integrity of the genu of the corpus callosum also was lower in chemotherapy-exposed patients (6).

Few studies have investigated the association between chemotherapy and gray matter volume. One study reported that patients one year post-treatment had smaller local gray matter volumes than cancer patients who never received chemotherapy. This was not observed in another group of patients who were three years post-treatment (7). A study that strictly examined hippocampal volume did not find differences between cancer patients who received chemotherapy three years before and those who did not (8). We recently showed that breast cancer survivors who completed high-dose chemotherapy almost ten years before had less focal gray matter than survivors who never received chemotherapy (4). A prospective study observed focal gray matter volume decrease one month after cessation of chemotherapy, which recovered in some, but not all regions at one year post-treatment (9).

The four studies described above performed brain volumetrics from one month up to 10 years post-treatment using different imaging protocols and analytic procedures. Their sample sizes were relatively small: the number of chemotherapy-exposed patients ranged from 5-73 (**Table 1**) (2-4, 7-10).

To date, it is unclear if standard-dose chemotherapy is associated with long-term effects on brain structure. This issue becomes increasingly important as the number of long-term, hence elderly cancer survivors is steeply increasing (11) and recent literature shows that chemotherapy is associated with cognitive problems in long-term survivors of breast cancer (12). Because central nervous system (CNS) regeneration is limited (13), it is possible that chemotherapy-induced structural brain changes are persistent rather than transient.

We evaluated whether breast cancer patients who had been exposed to adjuvant chemotherapy on average more than 20 years before, had smaller brain volumes than women from the general population without cancer. Hereto we compared 1) brain tissue volumes; 2) hippocampal volume; and 3) regional gray matter volume of 184 invasive breast cancer survivors who had been exposed to chemotherapy and radiotherapy to those of 368 age-matched healthy control subjects from a population-based study.

Materials & methods

Participants

The current study is embedded in a study investigating the late effects of adjuvant

Table 1. Overview of MRI studies that investigated the association between chemotherapy for breast cancer and brain volume

	Number of subjects			Time since end of CT	White matter		Gray matter		
Study	**CT^+**	**CT^-**	**HC**	**years (sd)**	**Measure**	**ROI**	**Measure**	**ROI**	**Outcome**
Brown 1995 (2)	13	-	13	1.0 (0.5)	WMH (ml)	Whole brain	-	-	High dose CT is associated with WMH
Brown 1998 (10)	8	-	-	1,3,6,9,12 months	WMH (ml)	Whole brain	-	-	White matter changes occur as soon as 2 months after chemo and stabilize after 6 months to 1 year. WMH still present at 1 year seem to be permanent
Choi 2001 (3)	5	1	-	during treatment	Visually checked	Whole brain	Visually checked	Whole brain	All patients leukoencephalopathy, visible as diffuse periventricular WMH
Yoshikawa 2005 (8)	44	31	-	3.5 (1.1)	-	-	Volume (ml)	Hippocampus	No differences in hippocampal volume
Inagaki 2006 (7)	51	54	55	0.3 (0.1)	VBM	Whole brain	VBM	Whole brain	Smaller right prefrontal and parahippocampal gyrus in CT^+ patients compared to CT^- patients
	73	59	37	3.3 (1.0)	VBM	Whole brain	VBM	Whole brain	No volume differences between groups
Abraham 2008 (6)	10	-	9	1.8 (0.8)	DTI	Genu and Spelnium	-	-	Patients had lower FA in the genu but not in the splenium of the corpus callosum
Deprez 2010 (5)	17	10	18	0.4 (0.1)	DTI; VBA	Whole brain	-	-	Compared to HC, CT^+ had lower FA in frontal and temporal WM tracts. In frontal WM patients had increased MD compared to HC. RD values for the above reported regions were higher in C^+ than in HC. Compared to CT^-, CT^+ had lower FA, higher MD, and higher RD in the above reported regions
McDonald 2010 (9)	17	12	18	0,1,12 months	-	-	VBM	Whole brain	Patients had a decline in gray matter from baseline to 1 month compared to HC. At 1 month CT^+ had decreased gray matter in bilateral frontal, temporal and cerebellar regions and in the right thalamus. Recovery was seen at 1 year in some, but not all regions, indicating persistent decrease
de Ruiter 2011 (4)	17	15	-	9.5 (0.8)	DTI; visually checked; 1H-MRS	Whole brain; 1H-MRS in left centrum semiovale	VBM	Whole brain	Whole brain MD and AD were higher in CT^+. In several regions FA was lower and MD and RD were higher in CT^+. No differences were observed in visually checked WMH. NAA/Cr was lower in CT^+ indicating axonal injury. VBM showed gray matter reductions in the posterior parts of the brain in CT^+.

CT^+=Breast Cancer patients treated with Chemotherapy; CT^-= Breast Cancer patients NOT treated with Chemotherapy; HC= Healthy Control Subjects; ROI= Region of Interesert; WMH= White Matter Hyperintensities; VBM= Voxel Based Morphometry; DTI= Diffusion Tensor Imaging; VBA= Voxel Based Analysis; WM= White Matter; FA= fractional anisotropy; MD= Mean Diffusivity; RD= Radial Diffusivity; AD= Axial Diffusivity; 1H-MRS= Single voxel MR spectroscopy

chemotherapy on brain function and structure in elderly breast cancer survivors. It compares chemotherapy-exposed invasive breast cancer survivors with female subjects without a history of cancer from the Rotterdam Study (RS). Written informed consent was obtained from all participants. The institutional review boards of the two participating institutions (the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Erasmus University Medical Center) approved the study.

Chemotherapy-exposed subjects

We selected consecutive female patients with unilateral invasive breast cancer from the registries of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Daniel den Hoed Clinic of the Erasmus Medical Center, who had been treated with 6 cycles of CMF chemotherapy (Cyclophosphamide 100 mg/m^2 on days 1-14; Methotrexate 40 mg/m^2 on days 1 and 8; 5-Fluorouracil 600 mg/m^2 on days 1 and 8) between 1976 and 1995.

We included patients who were between 50 and 80 years of age, in whom invasive breast cancer was their first and only malignancy, who had remained disease-free since treatment for breast cancer, and who had sufficient command of the Dutch language. Exclusion criteria were use of adjuvant endocrine therapy and MRI contra-indications.

A complete overview of the subject inclusion has been described earlier (14). In short, of the 291 patients who were eligible, 195 (67.0%) agreed to participate. Of these 195 women, four aborted the scan because of claustrophobia. Three scans were unusable due to motion artifacts. Another four were excluded on the basis of cortical infarctions, leaving 184 scans to be analyzed.

Decliners were older than subjects who were willing to participate when invitation letters were sent (($F_{1,289}$)=11.13; p<.05).

Healthy reference subjects

Reference subjects were selected from the Rotterdam Study; a population-based prospective cohort study that is ongoing since 1990 (15). Among other diseases in the elderly, the study targets neurological and psychiatric diseases, and includes an extensive MR brain imaging protocol. As of 2008, the study has included 14,926 subjects. To date, 4,898 participants of the Rotterdam Study have been invited for the Rotterdam Scan Study (RSS). Exclusion of individuals with MRI contraindications (n=389) left 4,509 eligible persons, of whom 4,102 (91%) agreed to participate. Due to physical inabilities, imaging could not be completed in 44 individuals.

Each chemotherapy-exposed breast cancer survivor was matched on age to two randomly selected women without a history of cancer of the 4,058 participants of the RSS who completed MRI examination. This resulted in a total of 368 reference subjects.

Methods

MRI Acquisition

Multi-sequence MRI for both cancer survivors and reference subjects was performed on the same 1.5-Tesla MRI scanner (General Electric Healthcare, Milwaukee, Wisconsin). During the study period, no software or hardware upgrades were performed on the system. Our full scan protocol has been described in detail earlier (16).

For this study we used a high-resolution axial MRI sequence, i.e. a T1-weighted 3-dimensional fast radiofrequency spoiled gradient recalled acquisition in steady state with an inversion recovery prepulse sequence (TR=13.8 ms, TE=2.8 ms, inversion time=400 ms, FOV=25×17.5 cm^2, matrix=416×256 [interpolated to 512×512], flip angle=20°, NEX=1, bandwidth [BW]=12.50 kHz, 96 slices with a thickness=1.6mm zero-padded in the frequency domain to 0.8 mm, interpolated voxel size=0.5×0.5×0.8=0.2 mm^3; duration=6 min.).

Remaining scan sequences such as cerebral blood flow and diffusion weighted imaging will be described separately.

Acquisition of medical and demographic data

Demographic information and medical data that are associated with brain structure were collected for all participants. Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. We used the average of these two measurements (17). Data on diabetes, education level, and smoking status were obtained, as they are part of the core interview of the Rotterdam Study (15). Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression scale (CES-D), which was converted to a sum-score according to the standard scoring rules. (18) Education level was subdivided into three levels: 1) lower vocational education or less; 2) lower secondary education/intermediate vocational education/general secondary education; 3) higher vocational education or better. Smoking status was subdivided into three levels: current, ever and never smoker.

Pre-processing and segmentation

Non-uniformity correction and automatic reorientation to the anterior commissure was applied to all scans (17). Reorientation was visually inspected and manually corrected if necessary. Images were segmented into gray matter, white matter and cerebrospinal fluid (CSF) using the 'new segment' module in the Statistical Parametric Mapping software version 8 (SPM8).

Regional gray-matter differences

Regional brain differences were analyzed using voxel-based morphometry (VBM), following diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) under SPM8 implemented in Matlab R2010b (MathWorks, Natick, Massachusetts). DARTEL is a

fully deformable registration method that is effectively unconstrained by number of degrees of freedom. It has proven good segmentation and registration accuracy in comparison with other algorithms (19, 20).

First, an initial template was created by averaging all segmentations per tissue class. Subsequently, a study-specific template was created on the basis of the individual deformation from the initial template of all 184 chemotherapy-exposed survivors and 184 randomly assigned age-matched subjects from the total group of 368 reference subjects. This selection was made to ascertain equal contributions of both groups to the template. Subsequently, the deformation fields of all 552 subjects were warped to the template. Through Jacobian modulation preservation of the initial volumes was achieved. The modulated-warped images were smoothed with an 8-mm full-width at half-maximum Gaussian kernel to increase signal to noise ratio.

Total brain tissue volume

A study-specific brain mask was computed by summing the DARTEL gray matter, white matter, and CSF templates and thresholding this image at a probability of 0.5. Tissue volumes in milliliters were calculated from masked tissue segmentations in DARTEL space by summing all voxels (0.2 mm^3 each) of the corresponding tissue class across the whole brain.

Hippocampal volume

Left and right hippocampal volumes were segmented separately using an automated segmentation method (21). Briefly, this method was developed in-house and uses both a statistical intensity model and a spatial probability map. The intensity model describes the typical intensities of the hippocampus and the background. The spatial probability map contains for every voxel the probability that it is part of the hippocampus. Both the intensity model and spatial probability map were learned from a set of 18 manually labeled hippocampi. Included in the region of interest were the dentate gyrus, CA1-4, and the alveus (21).

Analysis

Intracranial volume (ICV) was defined as the sum of gray matter, white matter, and CSF. Total brain volume (TBV) was defined as the sum of gray matter and white matter. Analysis of (Co) variance (AN(C)OVA) and Chi-square tests were used to compare medical and demographic characteristics between chemotherapy-exposed and reference subjects. We used general linear models to compare groups on gray and white matter volume, CSF, and TBV.

Whole brain voxel-wise comparison in the context of the general linear model with Family Wise Error (FWE) correction was performed in SPM to identify regional gray matter volume differences between groups. All primary analyses were adjusted for ICV, height, age, age-squared, mean systolic and diastolic blood pressure, self-reported prevalence of diabetes, education level, smoking status and symptoms of depression, as these have been associated

Table 2. Population characteristics

	Chemotherapy-exposed breast cancer survivors (n=184)		Reference group (n=368)		
	mean	**sd**	**mean**	**sd**	**p**
Age in years	64.0	(6.5)	64.0	(6.5)	.995
Height in cm	164.9	(6.4)	162.2	(6.0)	<.001
Systolic bloodpressure in mm Hg [a]	140.5	(20.1)	137.7	(20.2)	.13
Diastolic bloodpressure in mm Hg [a]	84.3	(10.5)	80.7	(9.6)	<.001
Depression score (CESD)	4.8	(5.7)	5.9	(7.8)	.11
Age at cancer diagnosis in years	42.9	(5.4)	-	-	-
Time since chemotherapy in years	21.1	(4.4)	-	-	-
	n	**(%)**	**n**	**(%)**	
Diabetes	14	(7.6)	16	(4.3)	.12
Education level					<.001
low [b]	84	(47.5)	242	(65.8)	
intermediate [c]	41	(22.3)	83	(22.6)	
high [d]	59	(32.1)	43	(11.7)	
Smoker status					.28
current	22	(12.0)	67	(18.2)	
ever	97	(52.7)	180	(48.9)	
never	65	(35.3)	121	(32.9)	

sd=standard deviation; CESD=Center for Epidemiologic Studies Depression Scale; [a] =in sitting position; [b] =lower vocational education or less; [c] =lower secondary education, intermediate vocational education and general secondary education; [d] =higher vocational education or better

with brain volume (17, 22, 23). To be able to compare the association between chemotherapy and brain tissue volume with the effect size of age we additionally ran the same general linear model but without including age-square.

Results

Population characteristics are presented in **Table 2**. Chemotherapy-exposed patients had been diagnosed with breast cancer on average 21.1 years before participation in this study at a mean age of 42.9 years. They were taller, better educated and had higher diastolic blood pressure than women from the reference group. No significant differences were observed between the groups regarding age, systolic blood pressure, CES-D score, smoker status, and prevalence of diabetes.

Total tissue volumes are presented in **Table 3**.

Segmentation of the hippocampi failed in seven chemotherapy-exposed patients, leaving 177 subjects to be analyzed. The chemotherapy-exposed survivors had significantly smaller

Table 3. Total brain tissue volumes (in milliliter)

	Chemotherapy-exposed breast cancer survivors (n=184)		Reference group (n=368)				
Tissue / ROI	**Mean**	**sd**	**Mean**	**sd**	**β**	**95% CI for β**	**p**
Intracranial Volume	1318.7	136.5	1315.7	185.3	3.0	-13.9 ; 19.8	.73
Brain volume	1087.0	23.5	1090.7	23.9	-3.5	-6.4 ; -0.6	.019
Gray matter	617.0	15.6	620.0	21.1	-2.9	-4.8 ; -1.0	.003
White matter	470.0	17.4	470.6	23.7	-0.6	-2.8 ; 1.6	.59
Cerebrospinal fluid	235.5	15.5	233.8	21.1	1.6	-0.3 ; 3.5	.10
Left hippocampus [a]	2.9	0.4	2.9	0.6	-0.1	-0.1 ; >0.1	.07
Right hippocampus [a]	2.9	0.4	2.9	0.6	>-0.1	-0.6 ; 0.4	.81

ROI=Region of Interest; sd=standard deviation; CI=Confidence Interval; [a] =n chemotherapy-exposed breast cancer patients=177

total brain volume (-3.5 ml, p=.019) and smaller gray matter volume (-2.9 ml, p=.003) than the reference group. No significant differences were observed in total ICV, white matter volume, cerebrospinal fluid volume, or right or left hippocampal volume between chemotherapy-exposed breast cancer patients and the reference group. In the model without age-square the effect of chemotherapy on total brain volume remained -3.5 ml (p=.018) and on gray matter volume remained -2.9 ml (p=.003), whereas the effect of age on total brain volume was -0.99 ml (p<.001) per year and on gray matter was -0.75 ml (p<.001) per year.

Subsequently, DARTEL revealed no significant regional gray matter volume differences between groups.

Discussion

Here we report the first study on the late effects of standard-dose adjuvant chemotherapy on brain volume in a large sample of breast cancer survivors on average more than two decades after cessation of treatment. Chemotherapy-exposed breast cancer survivors had significantly smaller total brain volume and gray matter volume than reference subjects without a history of cancer. No differences were observed in white matter volume, CSF volume, hippocampal volume or focal gray matter volume.

Strengths of our study are the large sample size, the long time since chemotherapy, the homogeneous study population regarding cytotoxic agents (regimen, number of cycles), and

the large population sample of age-matched women without a history of cancer.

We are aware that our study has some drawbacks that need to be addressed. Because we did not include a non-chemotherapy-exposed breast cancer control group we cannot separate the effect of chemotherapy and cancer itself. However, the only two studies investigating brain structure in breast cancer patients that included both healthy controls and breast cancer patients not exposed to chemotherapy did not report a difference between these two groups, suggesting no effect of cancer itself on brain structure (7, 9).

Another point of discussion is whether the findings of this study could also translate to breast cancer patients treated with contemporary regimens. Since both cyclophosphamide and 5-fluorouracil continue to be implemented in current regimens, and these agents, as well as many other commonly used agents are independently associated with structural brain changes in animals (24, 25), our study results might also apply to contemporary regimens.

Previously two studies applied voxel-based morphometry to investigate the effects of chemotherapy on focal gray matter volume. *Inagaki et al.* reported smaller right prefrontal and parahippocampal gyrus in chemotherapy-exposed patients than non-exposed patients at three months post-treatment. However, no volumetric difference was observed between another sample of chemotherapy-exposed and non-exposed patients who were more than three years post-treatment (7). Likewise, *McDonald et al.* reported that chemotherapy-exposed patients had decreased gray matter in bilateral frontal, temporal and cerebellar regions and in the right thalamus at one month post-treatment, but that recovery was seen at one year in several, although not all regions (9). These studies suggest that chemotherapy may induce transient local gray matter volume reductions that may (partly) recover over time. This is in line with the absence of large differences between chemotherapy-exposed survivors and the general population more than 20 years post-treatment that we observed. The only other study that investigated the association between hippocampal volume and chemotherapy did also not observe a relationship between the two (8).

Of all studies that investigated the effect of chemotherapy on brain structure, none examined total tissue volumes after cytotoxic treatment (2-4, 7-10). We found significant effects of chemotherapy on total brain volume and gray matter volume. In our analysis the lower amount of gray matter in chemotherapy-exposed survivors was comparable to the effect of almost 4 years of age on gray matter volume. The clinical relevance of this volume difference is not straightforward, but considering the effect size, chemotherapy might be associated with cognitive problems that we observed previously in this group of patients (26). Two other recent studies also reported a negative association between chemotherapy and long-term cognitive functioning (12, 27).

Up till now three studies have reported adverse effects of chemotherapy on white matter as measured with diffusion tensor imaging (DTI) (4-6). Therefore it might be that the small effects of chemotherapy on total gray matter volume may be accompanied by microstructural white-

matter changes.

The exact mechanisms for chemotherapy-associated gray matter volume reductions are largely unknown. Postulated explanations are enhanced neural cell death and decreased cell division (28), due to crossing of the blood-brain barrier by certain chemotherapeutic agents and increased levels of oxidative stress (29). Cell death however is less likely to explain the smaller volume, since it is considered irreversible and therefore contradictive to the partial recovery of local gray matter reductions that were reported in a longitudinal study after the effects of chemotherapy. In addition, another study reported smaller gray matter volumes in a group of patients one year post-treatment, but not in a group of patients three years post-treatment (7, 9).

Conclusion

In this study we investigated the very late effects of chemotherapy on the macrostructure of the brain. We observed on average smaller total gray matter volume and total brain volume in chemotherapy-exposed breast cancer survivors than in a population-based reference sample of age-matched women. This volume difference was comparable to the effect of almost 4 years of age on gray matter volume loss. No focal gray matter volume reductions between groups were observed.

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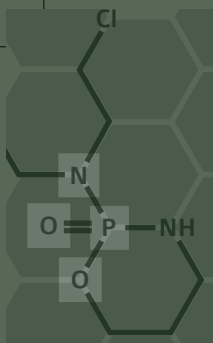
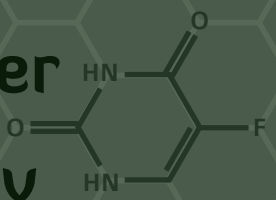


Chapter 4.2

Global and focal white matter integrity in breast cancer survivors 20 years after adjuvant chemotherapy



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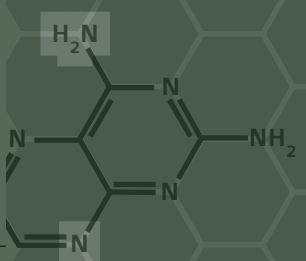

Abstract

Background: To date, only four small studies have investigated the effects of adjuvant chemotherapy for breast cancer on the microstructure of cerebral white matter with magnetic resonance imaging (MRI). These studies that were conducted shortly up to ten years post-treatment, showed that chemotherapy is associated with focal loss of microstructural white matter integrity. We investigated the long-term effect of chemotherapy on white matter microstructural integrity by comparing the brains of chemotherapy-exposed breast cancer survivors to those of a population-based sample of women without a history of cancer.

Methods: Diffusion tensor imaging (DTI) MRI (1.5T) was performed in 187 CMF (cyclophosphamide, methotrexate, 5-flourouracil) chemotherapy-exposed breast cancer survivors, mean age 64.2 (sd=6.5) years, who had been diagnosed with cancer on average 21.2 (sd=4.4) years before, and 374 age-matched cancer-free reference subjects from a population-based cohort study. Outcome measures were: whole-brain microstructural integrity as measured by fractional anisotropy and mean/axial/radial diffusivity; and focal white matter integrity, which was analyzed with tract-based spatial statistics (TBSS). All analyses were adjusted for age, cardiovascular risk factors, education, and symptoms of depression.

Results: No significant group differences were observed in white matter integrity. However, within the breast cancer survivors, time since treatment was inversely associated with lower global and focal white matter integrity.

Conclusions: This cross-sectional study suggests that among chemotherapy-exposed breast cancer survivors white matter microstructural integrity deteriorates with accumulating time since treatment. This warrants further investigation.

Introduction

Over the last decade, several cross-sectional and prospective studies showed that adjuvant chemotherapy for cancers outside the central nervous system (CNS) is associated with reduced performance on neuropsychological tests up to 20 years post-treatment (1-4). Typically, a mild to moderate decline in cognitive functioning is observed across various domains (3). Both direct and indirect neurotoxicity from cytotoxic agents may underlie the cognitive changes, which in general have been associated with white matter pathology (5, 6). Several studies have investigated the effects of chemotherapy on brain structure of breast cancer survivors (7-12). Within months after standard-dose chemotherapy, focal gray matter volume reductions were found that partly recovered with longer follow-up (11, 12). A recent study showed that high-dose chemotherapy is associated with smaller gray matter volume in posterior parts of the brain almost ten years post-treatment (8). We found that standard-dose adjuvant chemotherapy is associated with smaller total gray matter volume in survivors on average more than 20 years post-treatment (13). Conventional MRI also revealed an association of chemotherapy with an increase in white matter lesions up to one year post-treatment (14-16).

However, these macrostructural brain changes may not reflect the more subtle damage to the microstructure of cerebral white matter. Diffusion tensor imaging (DTI) allows the characterization of white matter microstructure in a non-invasive way, (17) providing a potentially more sensitive measure for white matter integrity than conventional MRI. DTI classifies the extent and directionality of the diffusion, i.e. random motion of water molecules in tissue (18-20). In healthy white matter, diffusion is highly anisotropic with the diffusion tensor being larger in directions alongside, than in directions perpendicular to structures. Injury to white matter results in lower anisotropy.

Previous DTI studies have associated conventional-dose chemotherapy with micro-structural damage in the genu of the corpus callosum at 22 months post-treatment (7), and in frontal and temporal white matter tracts at four months after chemotherapy (10). The only prospective DTI study that has been conducted found that at four months post-treatment white matter integrity of chemotherapy-exposed patients had deteriorated in tracts throughout the brain (9). This was not the case in non-chemotherapy-exposed patients or healthy controls. Another study reported that almost ten years after high-dose adjuvant chemotherapy, the treatment was associated with worse white matter integrity (8). Up till now, all studies that have investigated the link between chemotherapy and microstructural white matter damage had small sample sizes with a maximum of 34 chemotherapy-exposed patients.

Whether conventional-dose chemotherapy is associated with long-term adverse effects on white matter structure is unknown, but important to investigate as it may improve our understanding of the cognitive problems that have been observed in these long-term survivors (2). With the steeply increasing number of breast cancer survivors (21, 22) this topic becomes even more important.

This study aimed to investigate the long-term effects of conventional-dose chemotherapy on white matter integrity. DTI was applied to compare global and focal measures of white matter microstrucure between 187 chemotherapy-exposed breast cancer survivors and a population sample of women without a history of cancer.

Materials & methods

Participants

Chemotherapy-exposed subjects

Chemotherapy-exposed breast cancer survivors were derived from the registries of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital and the Erasmus MC-Daniel den Hoed Cancer Center. All women had been treated with 6 cycles of adjuvant CMF chemotherapy (Cyclophosphamide 100 mg/m^2 on days 1-14, Methotrexate 40 mg/m^2 on days 1 and 8, 5-Fluorouracil 600 mg/m^2 on days 1 and 8) between 1976 and 1995. Only those women were screened for eligibility who were known to be alive in 1998 on the basis of a previous study (23).

Inclusion criteria were: age between 50 and 80 years at recruitment; no other malignancy than breast cancer; no recurrence; and sufficient command of the Dutch language. Exclusion criteria were use of adjuvant endocrine therapy for breast cancer, and MRI contra-indications. **Figure 1** gives an overview of the subject inclusion. Women who refused to participate were on average older than participants ($F_{1,289}$=11.13; p<.05).

Reference subjects

Reference subjects were selected from the Rotterdam Study; a population-based prospective cohort study that has been ongoing in the city of Rotterdam since 1990 (24). Among other diseases in the elderly, the study targets neurological and psychiatric diseases. As of 2008, the study has included 14,926 subjects. By the end of the inclusion period of chemotherapy-exposed subjects for this study (October 2009), 4,058 participants had undergone complete brain MRI in the context of the Rotterdam Scan Study (RSS). (25) Each chemotherapy-exposed breast cancer survivor was matched on age to two cancer-free women of the participants of the RSS who completed MRI examination. This resulted in a total of 374 reference subjects.

Informed consent

Written informed consent was obtained from both the chemotherapy-exposed survivors and the reference subjects. The current study was approved by the institutional review boards of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital and the Erasmus MC University Medical Center, and conducted in accordance with the declaration of Helsinki.

Methods

MRI Acquisition

All participants were scanned on the same 1.5-Tesla scanner (General Electric Healthcare, Milwaukee) with a multi-sequence protocol (25). During the study period, no software or hardware upgrades were performed on the system.

For DTI, we performed a single shot, diffusion-weighted spin echo echo-planar imaging sequence (repetition time (TR)=8575 ms, echo time (TE)=82.6 ms, field-of-view (FOV)=21 cm^2, matrix=96×64 (zero-padded in the frequency domain to 256×256), slice thickness=3.5 mm, 35 contiguous slices. Maximum b-value was 1000 s/mm^2 in 25 non-collinear directions (number of excitations (NEX)=1), and three volumes were acquired without diffusion weighting (b-value=0 s/mm^2). Acquisition time was 5:10 min. We further performed three high-resolution axial MRI sequences, i.e. a T1-weighted 3D Fast RF Spoiled Gradient Recalled Acquisition in Steady State with an inversion recovery pre-pulse (FASTSPGR-IR) sequence (TR=13.8 ms, TE=2.8 ms, inversion time (TI)=400 ms, FOV=25 cm^2, matrix=416×256 (interpolated to 512×512), flip angle=20°, NEX=1, bandwidth (BW)= 12.50 kHz, 96 slices with slice thickness 1.6 mm zero-padded in the frequency domain to 0.8 mm), a proton density (PD) weighted sequence (TR=12,300 ms, TE=17.3 ms, FOV=25 cm^2, matrix=416×256, NEX=1, BW=17.86 kHz, 90 slices with slice thickness 1.6 mm), and a fluid attenuated inversion recovery (FLAIR) sequence (TR=8000 ms, TE=120 ms, TI=2000 ms, FOV=25 cm^2, matrix=320×224, NEX=1, BW=31.25 kHz, 64 slices with slice thickness 2.5 mm). All slices were contiguous.

Normal-appearing white matter and white matter lesion segmentation

For the assessment of volumes of normal-appearing white matter (NAWM) and white matter lesions, the structural MRI scans (T1-weighted, PD-weighted, FLAIR) were used. Preprocessing steps and the classification algorithm have been described in detail elsewhere (26). In short, each voxel was automatically segmented as belonging to a brain tissue class, and voxel count was summed and multiplied with voxel size to obtain NAWM volume and white matter lesion volume in mm^3.

DTI metrics

We assessed the following DTI metrics: 1) fractional anisotropy (FA) which represents the orientational dependence of water diffusion, and characterizes the microstructural architecture; 2) axial diffusivity ($\lambda_{\parallel}$) which indicates the extent of diffusion alongside the axis of the main fiber population; 3) radial diffusivity ($\lambda_{\perp}$) which represents the mean diffusivity perpendicular to the main fiber population; and 4) mean diffusivity (MD) which indicates the average diffusion of the three directions ($MD=((\lambda_{\parallel} + 2(\lambda_{\perp}))/3)$).

Pre-processing

Diffusion data were corrected for motion and Eddy currents by affine co-registration of the diffusion weighted volumes to the average b=0 volume. The registrations were performed with Elastix, an open source registration package (27). The rotation component of each transformation was used to realign the gradient vector for each diffusion-weighted volume to compensate for motion during the acquisition (28). Resampling of the transformed diffusion weighted images was done at an isotropic resolution of 1.0 mm. The Brain Extraction Tool (BET) from FSL 4.1 was used to mask away non-brain tissue (29). Tensor fits were performed with a Levenberg-Marquard non-linear least squares optimization algorithm, as available in ExploreDTI (30). Data quality was examined by visual inspection of axial FA slices, every 4 mm, combined with two coronal and two sagittal slices around the center of the brain.

Assessment of voxelwise white matter integrity

Voxelwise analysis of the diffusion data was performed with TBSS (tract-based spatial statistics) (31). TBSS registers all FA images to standard space, and then creates a study specific skeleton of the major white matter tracts (threshold for skeleton FA≥0.2). To overcome residual misalignment following the registration, TBSS projects the maximum FA values in a line perpendicular to the tract onto the skeleton for each individual. The projection results in a series of skeletonized images that can be analyzed voxelwise. Additionally, the projection also maps DTI parameters other than the FA on the skeleton, allowing voxelwise analyses of MD, $\lambda_{\perp}$, and $\lambda_{\parallel}$.

Assessment of global white matter integrity

Global FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ values were computed in two ways: a) We averaged the values of all voxels within the TBSS skeleton. b) We averaged FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ inside the cerebral NAWM for each subject. First, for each subject, the T1 scan was co-registered to the FA, using a 12 degrees-of-freedom affine registration, as implemented by FLIRT (32). Visual inspectation of these registrations revealed no errors. The registration was used to resample the NAWM tissue segmentation in the space of the diffusion metrics. Next, diffusion metrics were averaged inside the NAWM tissue class in those regions where FA≥0.2 (6).

Confounding factors

Demographic information and medical data that were considered potential confounders for the association of chemotherapy and brain structure were collected for all participants. Height and weight were measured and used to calculate body mass index (BMI) (weight/height^2). Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. We used the average of these two measurements (33). Data on diabetes, education level, and smoking status were obtained by interview (24). Maximum attained education level was

subdivided into three levels: 1) lower vocational education or less; 2) lower/general secondary education and intermediate vocational education 3) higher vocational education or better. Smoking status was rated as current, ever or never smoker. Depressive symptoms were assessed with the Center for Epidemiologic Studies-Depression scale (CES-D), which was converted to a sum-score according to the standard scoring rules (34).

Analysis

Analysis of variance (ANOVA) and Chi-square tests were used to compare medical and demographic characteristics between chemotherapy-exposed and reference subjects. We used analysis of covariance (ANCOVA) to investigate the effect of chemotherapy on global FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ values. Within the group of chemotherapy-treated women we investigated the effect of time since treatment on global FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ values using linear regression analysis.

Non-parametric permutation testing (5000 permutations) in the framework of the general linear model (FSL randomise) was used for voxelwise group comparisons and time-since-treatment analysis of skeletonized FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ values (35).

All analyses were adjusted for total white matter volume, white matter lesion load, height, age, age-squared, systolic and diastolic blood pressure, diabetes, education level, smoking status, and CES-D score. All voxelwise analyses were additionally corrected for multiple comparisons. White matter lesion volume was natural log-transformed because of skewness of the untransformed measure. For TBSS we present two models: 1) a model that is adjusted for age; 2) a model that is adjusted for all the above-described covariates. Spatial clustering was performed with threshold-free cluster enhancement (TFCE) with default settings for skeletonized data. (36) The skeletonized results were thickened for better visibility.

Results

Population characteristics are presented in **Table 1**. Chemotherapy-exposed patients had been diagnosed with breast cancer on average 21.1 years before participation (range: 13.7–30.1 years) at a mean age of 42.9 years. They were taller, better educated, had a higher systolic and diastolic blood pressure, had less symptoms of depression, and less often were current smokers than reference subjects. No significant group differences were observed regarding age, BMI, prevalence of diabetes, total white matter volume or white matter lesion volume.

Table 2 presents the average DTI parameters across the entire white matter skeleton for chemotherapy-exposed survivors and reference subjects. No global differences were observed between the two groups regarding FA, MD, $\lambda_{\perp}$ or $\lambda_{\parallel}$, obtained from either the white matter skeleton or the entire NAWM. Within the patient group, time since treatment was significantly associated with a lower FA, and higher MD and $\lambda_{\perp}$ (**Table 3**) within both the white matter skeleton and the entire NAWM. To address whether this latter association was potentially due to residual confounding by age, we performed an additional analysis, where we restricted the

Table 1. Population characteristics

	Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)		
	mean	(sd)	mean	(sd)	p
Age in years	64.2	(6.5)	64.2	(6.5)	.99
Height in cm	164.9	(6.4)	162.8	(6.2)	<.001
Systolic bloodpressure in mm Hg [a]	141.0	(20.2)	136.2	(20.6)	.009
Diastolic bloodpressure in mm Hg [a]	84.3	(10.5)	81.5	(10.4)	.003
Depression score (CESD)	4.8	(5.7)	6.4	(7.4)	.012
Body Mass Index (BMI)	27.0	(4.5)	27.7	(4.5)	.07
Total white matter volume (ml)	388.0	(49.2)	387.3	(50.7)	.88
White matter lesion volume (ml) [b]	1.2	(1.0)	1.1	(0.8)	.36
Age at cancer diagnosis in years	42.9	(5.3)	-	-	-
Time since chemotherapy in years	21.2	(4.4)	-	-	-
	n	**(%)**	**n**	**(%)**	
Diabetes	14	(7.5)	21	(5.6)	.46
Education level:					<.001
low [c]	86	(46.0)	246	(65.8)	
intermediate [d]	42	(22.5)	73	(19.5)	
high [e]	59	(31.6)	55	(14.7)	
Smoker status:					.011
current	22	(11.8)	75	(20.1)	
ever	98	(52.4)	153	(40.9)	
never	67	(35.8)	146	(39.0)	

sd=standard deviation; CESD=Center for Epidemiologic Studies Depression Scale; [a] =in sitting position; [b] =natural log-transformed; [c] =lower vocational education or less; [d] =lower secondary education, intermediate vocational education and general secondary education; [e] =higher vocational education or better

variation in age while maintaining the spread in time since treatment. For this, we selected all cancer survivors around the mean age of 64.2 within a narrow age-range (61.0-67.0) years). Because the distribution of age was normal, selecting around the mean age yielded the largest sample. Within this subset of 73 cancer survivors, the range of time since treatment was 13.8-28.3 years. The observed relation between time since treatment and white matter integrity within this subset was not different from that in the total group.

Table 2. Average DTI parameters across the entire white matter

		Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)				
Measure	**Parameters**	**Mean**	**sd**	**Mean**	**sd**	**β**	**95% CI for β**	**p**
Mean DTI parameters within the TBSS skeleton	Fractional anisotropy (FA)	4.3×10^{-1}	1.8×10^{-2}	4.3×10^{-1}	1.5×10^{-2}	8.0×10^{-4}	[-1.8×10^{-3} 3.4×10^{-3}]	.55
	Mean diffusivity (MD) *	7.2×10^{-4}	2.4×10^{-5}	7.2×10^{-4}	2.1×10^{-5}	-1.3×10^{-6}	[-4.8×10^{-6} 2.2×10^{-6}]	.47
	Radial diffusivity ($\lambda_{\perp}$) *	5.3×10^{-4}	2.6×10^{-5}	5.4×10^{-4}	2.3×10^{-5}	-1.2×10^{-6}	[-5.1×10^{-6} 2.6×10^{-6}]	.54
	Axial diffusivity ($\lambda_{\parallel}$) *	1.1×10^{-3}	2.4×10^{-5}	1.1×10^{-3}	2.1×10^{-5}	-1.4×10^{-6}	[-4.9×10^{-6} 2.1×10^{-6}]	.44
Mean DTI parameters in the normal appearing white matter (NAWM)	Fractional anisotropy (FA)	3.8×10^{-1}	1.2×10^{-2}	3.8×10^{-1}	1.0×10^{-2}	-1.1×10^{-3}	[-2.9×10^{-3} 5.4×10^{-4}]	.18
	Mean diffusivity (MD) *	7.2×10^{-4}	2.1×10^{-5}	7.2×10^{-4}	1.8×10^{-5}	8.3×10^{-7}	[-2.2×10^{-6} 3.9×10^{-6}]	.60
	Radial diffusivity ($\lambda_{\perp}$) *	5.6×10^{-4}	2.1×10^{-5}	5.6×10^{-4}	1.8×10^{-5}	1.4×10^{-6}	[-1.6×10^{-6} 4.4×10^{-6}]	.36
	Axial diffusivity ($\lambda_{\parallel}$) *	1.0×10^{-3}	2.4×10^{-5}	1.0×10^{-3}	2.1×10^{-5}	-2.1×10^{-7}	[-3.8×10^{-6} 3.4×10^{-6}]	.91

DTI= diffusion tensor imaging; TBSS= tract-based spatial statistics; sd=standard deviation; CI=Confidence Interval; β= (beta) values represent the effect of chemotherapy adjusted for age, age squared, height, blood pressure, diabetes status, education level, symptoms of depression, white matter volume and white matter lesion load; *= in millimeter^2/second

Table 3. Effects of time since treatment on average DTI parameters across the entire white matter in 187 chemotherapy-exposed survivors

Measure	Parameters	β	95% CI for β	p
Mean DTI parameters within the TBSS skeleton	Fractional anisotropy (FA)	-7.6×10-4	[-1.4×10-3 -1.6×10-4]	.013
	Mean diffusivity (MD) *	1.1×10-6	[2.1×10-7 1.9×10-6]	.015
	Radial diffusivity ($\lambda_{\perp}$) *	1.2×10-6	[2.9×10-7 2.2×10-6]	.010
	Axial diffusivity ($\lambda_{\parallel}$) *	7.6×10-7	[-6.9×10-8 1.6×10-6]	.072
Mean DTI parameters in the normal appearing white matter (NAWM)	Fractional anisotropy (FA)	-4.6×10-4	[-8.1×10-4 -1.1×10-4]	.011
	Mean diffusivity (MD) *	7.9×10-7	[1.1×10-7 1.5×10-6]	.023
	Radial diffusivity ($\lambda_{\perp}$) *	8.4×10-7	[1.9×10-7 1.5×10-6]	.012
	Axial diffusivity ($\lambda_{\parallel}$) *	6.6×10-7	[-1.4×10-7 1.5×10-6]	.105

CI=Confidence Interval; β= (beta) values represent the effect of time since treatment per year increase, adjusted for age, age squared, height, blood pressure, diabetes status, education level, symptoms of depression, white matter volume and white matter lesion load; *= in millimeter^2/second

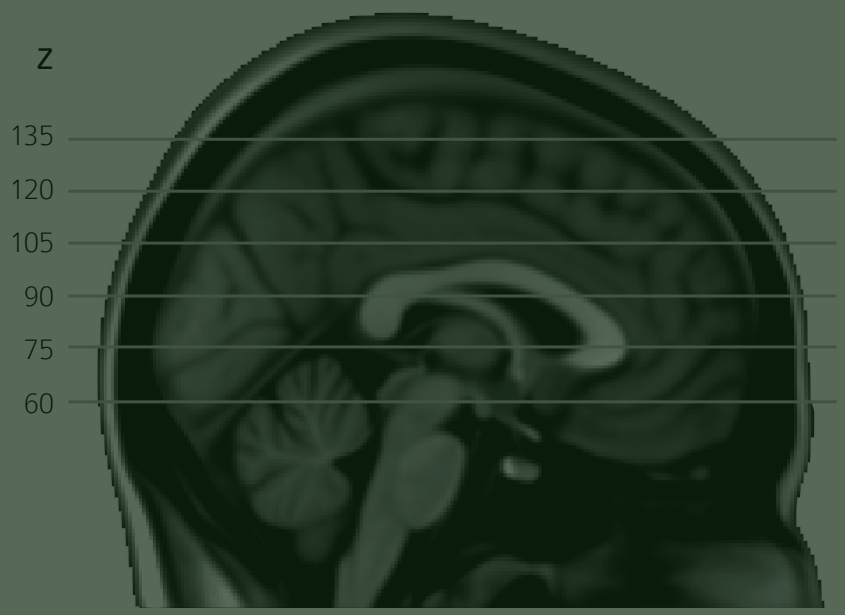

Figure 2. Position of slices in Figures 3 and 4 on the MNI standard brain. Numbers indicate Z-coordinates.

TBSS

No significant differences between chemotherapy-exposed breast cancer patients and reference subjects were observed in either measure of microstructural integrity of white matter (FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$). However, within the group of chemotherapy-exposed survivors time since treatment was significantly associated with a widespread lower FA and a higher MD and $\lambda_{\perp}$, but not $\lambda_{\parallel}$ (**Figure 3**). This association became stronger after additional adjustment for the predefined potential confounders (model 2) (**Figure 4**) and was again similar in the subset of 73 patients with a narrow age range and a large range of time since treatment.

Discussion

Here we report the first study on the very late (>20 years post-treatment) effects of adjuvant CMF chemotherapy for non-CNS cancer on white matter structure in a large sample of breast cancer survivors. We found evidence for an adverse effect of conventional dose cytotoxic treatment on white matter integrity, as time since treatment correlated negatively with global and focal white matter organization within the group of breast cancer survivors.

This association could not be attributed to residual confounding by age, as adjusting for age as well as restriction of the age range while maintaining a large range of time since treatment yielded identical results.

We found no group differences in white matter microstructural integrity between chemotherapy-exposed breast cancer survivors and reference subjects. This may be due to the fact that our study is a cross-sectional study and we were unable to look at change over time. It is conceivable that apart from the cancer diagnosis, our group of chemotherapy-exposed survivors was actually healthier at time of chemotherapy than our reference group; indeed, the current study shows that our breast cancer patients were taller, smoked less, had less symptoms of depression, and were better educated than reference subjects. If baseline white matter integrity was better in the chemotherapy-exposed survivors than in the reference subjects several decades before, this could explain why we found an effect of time since treatment, but no group differences.

Up till now four small studies have reported effects of chemotherapy on white matter structure (7-10). One study examined the FA of white matter in the genu and the splenium of the corpus callosum in ten breast cancer survivors almost two years after treatment with doxorubicin-cyclophosphamide with/without taxane. They found lower FA in the genu, but not in the splenium (7), compared with healthy controls. Another study used a voxel-based analysis to compare 17 chemotherapy-exposed breast cancer patients (fluorouracil, epirubicin, cyclophosphamide (FEC), with/without taxol) three months post-chemotherapy, 10 non-chemotherapy-exposed patients and 18 healthy controls. Lower FA and higher MD and $\lambda_{\perp}$ in frontal and temporal white matter tracts was observed in chemotherapy-exposed patients than in non-exposed patients or healthy controls. Regardless of chemotherapy, breast cancer

patients had higher MD in frontal white matter than healthy controls (10). A recent longitudinal study by the same group compared 34 chemotherapy-exposed patients (FEC with/without taxol) to 16 non-chemotherapy-exposed breast cancer patients, and 19 age-matched healthy controls. FA values in frontal, parietal and occipital white matter tracts decreased from pre-chemotherapy to approximately four months post-chemotherapy. In contrast, FA did not change in white matter tracts of non-exposed patients or healthy controls within the same time-intervals (9). The only other study that applied TBSS was a study by members of our group in 17 breast cancer patients without disease recurrence, ten years after adjuvant high-dose chemotherapy (4 cycles standard-dose FEC + 1 cycle high-dose cyclophosphamide, thiotepa, carboplatin (CTC)) (8). In this study, both global and focal effects of chemotherapy on FA, MD and $\lambda_{\perp}$ in widespread anterior and posterior parts of the brain were found.

It is difficult to directly compare the findings of these studies with our current results as the mean time since treatment in these studies differed substantially from that in our study, as well as the regimens under study and the MR scanner that was used (3-Tesla compared to our 1.5-Tesla) (37).

Strengths of our study are the large sample size of chemotherapy-exposed survivors, the long time since cytotoxic treatment, the homogeneous study population regarding cytotoxic agents (regimen, number of cycles), and the large population-based reference sample of age-matched women without a history of cancer.

Our study is limited by the fact that we did not include a reference group of breast cancer survivors who only underwent local therapy. Therefore we cannot distinguish the effect of chemotherapy and that of cancer itself on white matter structure. In addition, cytotoxic regimens have changed over the last decade. To what extent the results of our study can be generalized to the effects of contemporary cytotoxic regimens on white matter integrity is therefore a matter of debate. We could not invest the effect of chemotherapy on white matter over time because of lack of pre-chemotherapy measurements.

Conclusion

In the absence of group differences in white matter microstructural integrity between chemotherapy-exposed breast cancer survivors and a population sample, we found evidence for adverse effects of adjuvant CMF chemotherapy given for breast cancer on microstructural white matter integrity more than 20 years post-treatment. The observed inverse relation between time since treatment and white matter integrity within the breast cancer group suggests that adjuvant CMF chemotherapy does affect white matter integrity and warrants further investigation.

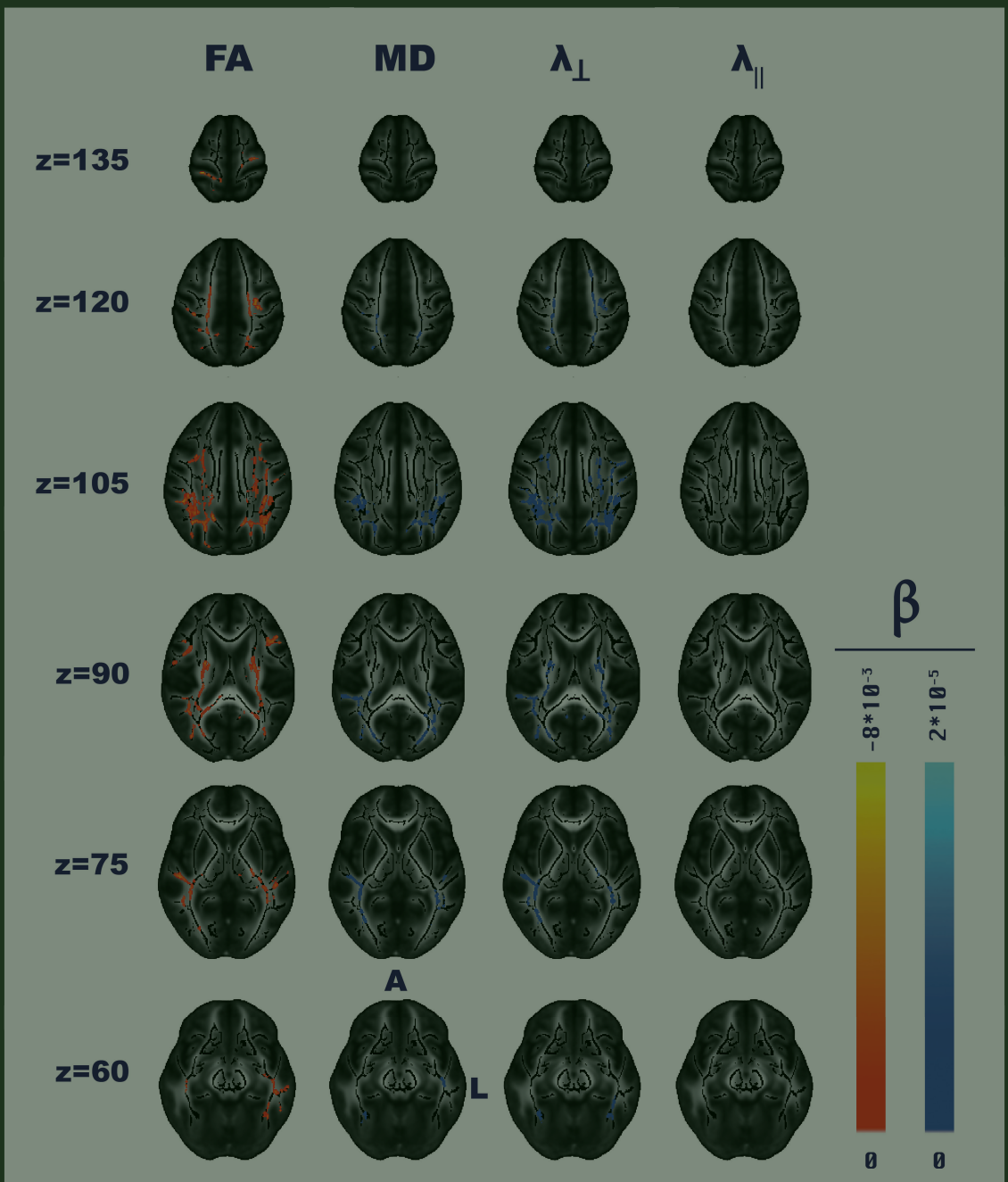

Figure 3. Association between time-since-treatment and fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity ($\lambda_{\perp}$), and axial diffusivity ($\lambda_{\parallel}$) values of white matter in 187 chemotherapy-exposed patients. Analyses are adjusted for age. Images are shown in the Montreal Neurological Institute (MNI) stereotactic space, with MNI coordinates for axial levels (z) depicted for each row. The white matter skeleton (black) is projected onto the axial MR images. The position of slices on the MNI brain is depicted in **Figure 2**. Yellow-to-red colors represent beta values (β) of the effect of time since treatment in regions with FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ levels that significantly reduce with longer time-since-treatment. Blue-to-light-blue colors represent beta values (β) of the effect of time since treatment in regions with FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ levels that significantly increase with longer time-since-treatment.

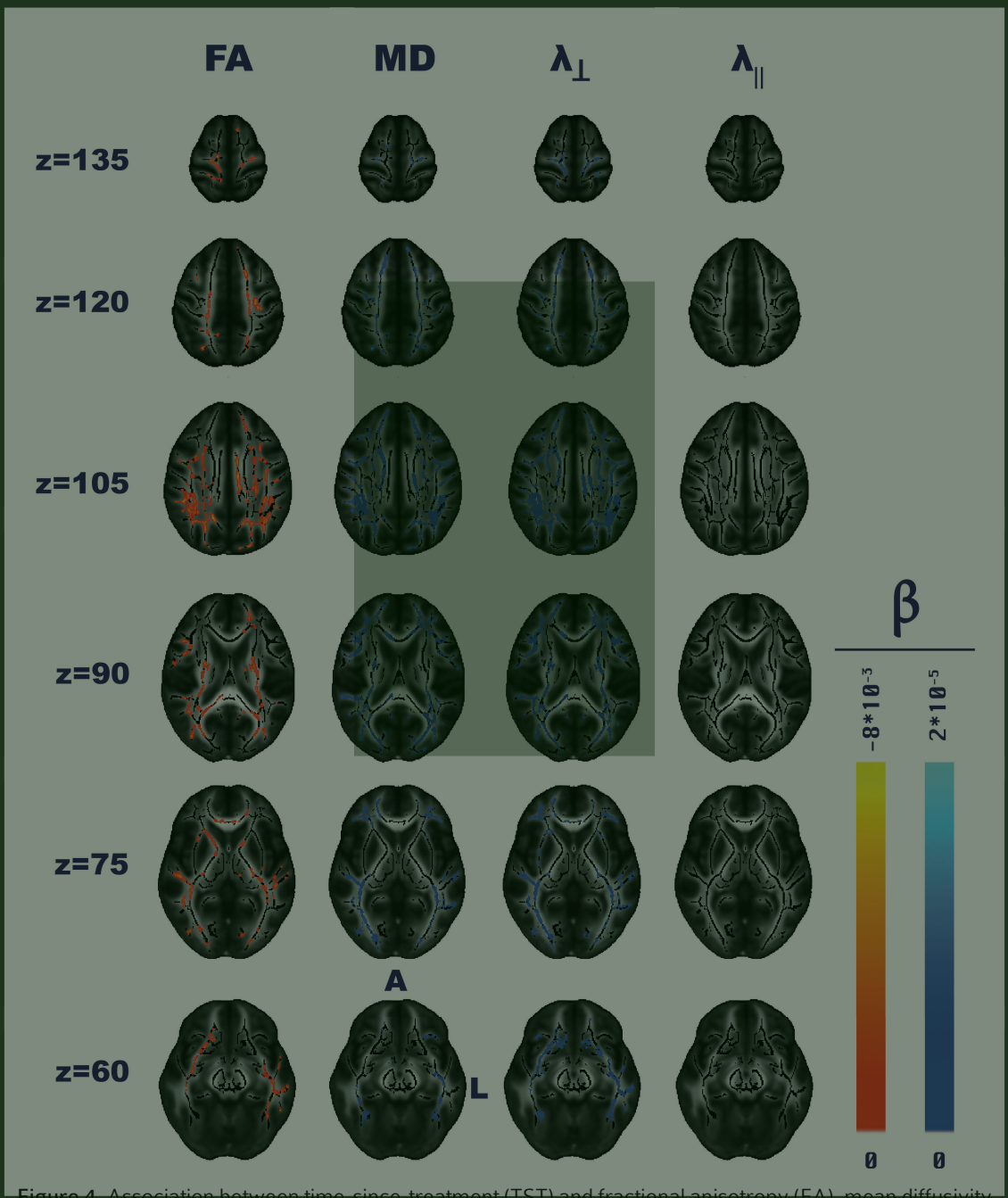

Figure 4. Association between time-since-treatment (TST) and fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity ($\lambda_{\perp}$), and axial diffusivity ($\lambda_{\parallel}$) values of white matter in 187 chemotherapy-exposed patients. Analyses are adjusted for age, age^2, height, blood pressure, diabetes status, education level, symptoms of depression, white matter- volume and lesion load. Images are shown in the Montreal Neurological Institute (MNI) stereotactic space, with MNI coordinates for axial levels (z) depicted for each row (see **Figure 2**). The white matter skeleton (black) is projected onto the axial MR images. Yellow-to-red colors represent beta values (β) of the effect of TST in regions with FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ levels that significantly reduce with longer TST. Blue-to-light-blue colors represent beta values (β) of the effect of TST in regions with FA/MD/$\lambda_{\perp}$/$\lambda_{\parallel}$ levels that significantly increase with longer TST.

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Chapter 4.3

Long-term effects of adjuvant chemotherapy for breast cancer on carotid artery plaques, cerebral blood flow and cerebral perfusion

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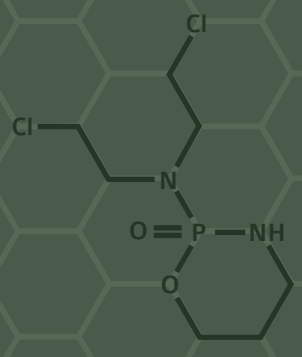

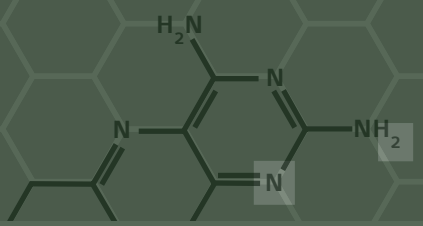

Abstract

Objective: Chemotherapy has been associated vascular damage to the carotid artery. Degree of carotid stenosis and size of carotid artery plaques have been related to cerebral blood flow in patients with stenosis. It is however unclear if adjuvant chemotherapy is associated with carotid artery plaques and with cerebral perfusion and blood flow. Therefore, we compared the prevalence of carotid plaques, cerebral blood flow, and cerebral perfusion in long-term breast cancer survivors treated with chemotherapy to those in a population-based sample of women without a history of cancer.

Methods: The study comprised 187 CMF (cyclophosphamide-methotrexate-fluorouracil) chemotherapy-exposed breast cancer survivors, mean age 64.1 (±6.5) years, who had been diagnosed with cancer on average 21.2 (±4.4) years before, and 374 age-matched cancer-free reference subjects from the prospective population-based Rotterdam study. Outcome measures were plaque score (range 0-12), total cerebral blood flow (tCBF), and brain perfusion (tCBF/brain volume*100). Presence of plaque was examined at twelve sites using ultrasonography of both carotid arteries: the near/far walls of the left/right a) internal carotid artery, b) carotid bifurcation, and c) common carotid artery. For tCBF measurement, 2D phase-contrast MRI (1.5T) was performed.

Results: No differences were observed between chemotherapy-exposed breast cancer survivors and reference subjects regarding carotid artery plaque scores, total cerebral blood flow or brain perfusion. Adjustment for vascular risk factors did not change the results.

Conclusion: Adjuvant CMF chemotherapy for breast cancer is not associated with carotid artery plaques, cerebral perfusion or cerebral blood flow, on average 21 years post-treatment.

Introduction

Chemotherapy has been related to vascular damage. Vascular damage to the common carotid artery (CCA) has been observed in breast cancer survivors after adjuvant anthracycline-based chemotherapy (1). Another study reported 5-fluorouracil (5-FU) induced global but reversible endothelial injury in patients who were treated for colorectal cancer (2). Experimental studies have revealed that several common chemotherapeutic agents such as cyclophosphamide and methotrexate may induce endothelial damage and cell death of blood vessels shortly after administration (3, 4). Whether chemotherapy may also affect vessel integrity of intracranial blood vessels is currently not known, although this is not unlikely as some cytotoxic agents such as 5-FU and methotrexate (MTX) can cross the blood brain barrier in conventional dosages (5-FU) or in higher dosages (MTX) (5-7). In addition, animal research has shown that MTX may reduce hippocampal blood vessel density (8).

Vessel integrity is associated with brain perfusion (9). The carotid and the vertebral arteries are the main arteries that supply blood to the brain. In a study by Douglas et al. it was observed that in patients with carotid artery stenosis, the size of carotid artery plaques and the percentage of carotid stenosis were predictors for blood flow in the internal carotid artery (ICA) (9).

In the general population vascular risk factors such as hypertension (10), arteriosclerosis of the carotid arteries (11), and low total brain perfusion (12) have been linked to pathology of the white matter of the brain. Adjuvant chemotherapy for breast cancer has also been associated with white matter lesion volume (13-15) and reduced white matter integrity (16-18). Knowledge of the underlying mechanisms of chemotherapy-induced structural brain changes is still limited (5). To date, no study has investigated the relationship between adjuvant chemotherapy applied in the treatment of breast cancer, and carotid vascular damage and brain perfusion.

In the current study we therefore compared breast cancer survivors treated with adjuvant CMF (Cyclophosphamide-Methotrexate-Fluorouracil) chemotherapy on average more than 20 years ago to a population based reference group with regard on plaque scores in the carotid arteries, total cerebral blood flow and brain perfusion.

Materials & methods

Participants

Chemotherapy-exposed subjects

We selected consecutive female patients with unilateral invasive breast cancer from the registries of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Daniel den Hoed Clinic of the Erasmus Medical Center, who had been treated with 6 cycles of CMF chemotherapy (Cyclophosphamide 100 mg/m^2 on days 1-14; Methotrexate 40 mg/m^2 on days 1 and 8; 5-Fluorouracil 600 mg/m^2 on days 1 and 8) between 1976 and 1995.

We included patients who were between 50 and 80 years of age, in whom invasive breast cancer was their first and only malignancy, who had remained cancer-free since treatment for

breast cancer, and who had sufficient command of the Dutch language. Exclusion criteria were use of adjuvant endocrine therapy and MRI contra-indications.

A detailed overview of the subject inclusion has been described previously (19). In short, of the 291 eligible patients 195 (67.0%) agreed to participate. Of these 195 women, four aborted the MRI scanner because of claustrophobia. In another four subjects carotid artery ultrasound images in one or two vessel beds were unusable and therefore total plaque score could not be calculated for these subjects. Therefore, 187 subjects were included in the analyses. Women who declined to participate were on average older than subjects who participated ($F_{1,289}$=11.13, p<.05).

Population-based reference subjects

Reference subjects were selected from the Rotterdam Study (RS); a prospective population-based cohort study that has been ongoing in the city of Rotterdam since 1990 (20). Among other diseases in the elderly, the study targets neurological and psychiatric diseases. As of 2008, the study has included 14,926 subjects. By the end of the inclusion period of chemotherapy-exposed subjects for this study (October 2009), 4,058 participants (participation rate 91%) had undergone complete brain MRI in the context of the Rotterdam Scan Study (RSS). Each chemotherapy-exposed breast cancer survivor was matched on age to two randomly selected women without a history of cancer of the 4,058 participants of the RSS who completed both MRI examination and carotid artery ultrasound. This resulted in a total of 374 reference subjects.

Informed consent

Written informed consent was obtained from both the chemotherapy-exposed breast cancer survivors and the reference subjects. The current study was approved by the institutional review boards of the two participating institutions (the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital and the Erasmus MC University Medical Center) and conducted in accordance with the declaration of Helsinki.

Methods

All examinations for both the chemotherapy-exposed breast cancer survivors and the reference subjects took place at the research center of the Rotterdam Study and were conducted by the same technicians.

Carotid Artery Ultrasound

Assessment of carotid plaques has been described previously (21). In short, ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer and a duplex scanner (Acuson 128; Siemens and Esaote, Pie Medical Imaging, Maastricht, The Netherlands). Presence of plaques, defined as focal widenings relative to adjacent segments with the protrusion

into the lumen, was examined at twelve sites: the anterior (near) and posterior (far) walls of the left and right a) internal carotid artery, b) carotid bifurcation, and c) common carotid artery. For the six vessel beds plaque scores were scored as present (in both or either the anterior and posterior wall) or absent (absent in both the anterior and posterior wall). Plaque scores were added for all sites, hence total plaque score ranged from zero to six.

MRI Acquisition

MRI was performed on a 1.5-Tesla MRI scanner (General Electric Healthcare, Milwaukee, Wisconsin). During the study period, no software or hardware upgrades were performed.

Our full scan protocol has been described in detail previously (20). In short, for cerebral blood flow measurement, 2D phase-contrast imaging was performed. First, a sagittal 2D phase-contrast MRI angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular both to the precavernous portion of the internal carotid arteries and to the middle part of the basilar artery was chosen (repetition time=20ms, echo time=4ms, field of view=19cm^2, matrix=256×160, flip angle=8°, number of excitations=8, bandwidth=22.73kHz, velocity encoding=120cm/sec, slice thickness=5mm).

Subsequently three high-resolution axial MRI sequences were acquired: a T1-weighted three-dimensional fast radio frequency spoiled gradient recalled acquisition in steady state with an inversion recovery prepulse (FASTSPGR-IR); a proton density-weighted sequence; and a fluid attenuated inversion recovery sequence (FLAIR). All slices were contiguous.

Measurement of cerebral blood flow

As previously described, we calculated flow from the phase-contrast images using interactive data language-based custom software (Cinetool version 4, General Electric Healthcare, Milwaukee, WI, USA). (20) Regions of interest (ROIs), encompassing the entire lumen of the vessel, were drawn manually around both carotids and the basilar artery on the phase-contrast images. The mean signal intensity in each ROI reflects the flow velocity in the vessel (cm/sec). Flow (in ml/sec) was calculated by multiplying the average velocity with the cross-sectional area of the vessel. To calculate total CBF (tCBF) (in ml/min), flow rates for the carotid arteries and the basilar artery were summed and multiplied by 60 secs/min. Two independent experienced technicians performed all manual ROI drawing and flow measurements. Total CBF strongly depends on the amount of brain tissue (12). To account for this, we calculated brain perfusion (in ml/min per 100 ml) by dividing tCBF (ml/min) by brain volume (ml) and multiplying the obtained result by 100.

Measurement of total brain volume

For the assessment of total brain volume (TBV), defined as the sum of gray matter and white matter, the structural MRI scans (T1-weighted, PD-weighted, FLAIR) were used. Preprocessing

steps and the classification algorithm have been described in detail elsewhere (22). In short, each voxel was automatically segmented as belonging to a brain tissue class, and voxel count was summed and multiplied with voxel size to obtain white matter volume, gray matter volume and cerebro-spinal fluid volume in mm^3.

Cardiovascular determinants

Information on potential cardiovascular confounders was collected for all participants. Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. We used the average of these two measurements (12). Height and weight were assessed. Subsequently, body mass index (BMI) was calculated by dividing weight by height-squared. Self reported data on diabetes, and smoking status were obtained. Maximum attained education level was obtained by interview and subdivided into seven levels: 1) primary education; 2) lower vocational education 3) lower secondary education; 4) intermediate vocational education; 5) general secondary education; 6) higher vocational education; 7) university. Smoking status was subdivided into three levels: current, ever and never smoker. In addition, information on use of lipid-lowering medication and anti-hypertensive medication was collected.

Analyses

Analysis of variance (ANOVA) and $\chi 2$ tests were used to compare chemotherapy-exposed breast cancer patients and reference subjects on age, height, blood pressure, BMI, TBV, prevalence of diabetes, smoker status and highest attained education level (**Table 1**).

We used negative binomial regression to compare the distribution of plaque scores between groups, and general linear models to compare groups on tCBF and brain perfusion. All analyses were adjusted for age, age2, BMI, and height. Subsequently we adjusted for the potential confounding effects of diastolic blood pressure, smoker status, prevalence of diabetes and age at menopause. We adjusted for age at menopause because CMF chemotherapy can induce early menopause (23), which is associated with presence of carotid plaques (24) and cerebral blood flow (25). Additionally, we adjusted for education level because of the different distribution of education level between groups in the current study and its association with cardiovascular diseases in general (26).

Results

Population characteristics are presented in **Table 1**. Chemotherapy-exposed patients had been diagnosed with breast cancer on average 21.2 years before participation in this study, at a mean age of 42.9 years. They were taller, better educated, had higher diastolic blood pressure, were older at the time of carotid artery ultrasound, and transitioned into menopause at younger age than women from the reference group. No significant differences were observed between the groups regarding age at MRI assessment, systolic blood pressure, BMI, total brain volume,

Table 1. Population characteristics

	Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)		
	mean	(sd)	mean	(sd)	p
Age in years	64.1	(6.5)	64.1	(6.4)	.98
Height in cm	165.1	(6.2)	162.8	(6.0)	<.001
Systolic blood pressure in mm Hg [a]	140.1	(19.3)	137.5	(18.9)	.14
Diastolic blood pressure in mm Hg [a]	84.0	(10.2)	80.9	(9.8)	.001
Body mass index (BMI)	26.8	(4.5)	27.5	(4.2)	.09
Total brain volume (in milliliter)	901.0	(75.5)	888.1	(81.6)	.069
Age at menopause in years	43.9	(5.2)	49.3	(5.9)	<.001
Age at cancer diagnosis in years	42.9	(5.3)	-	-	-
Time since chemotherapy in years	21.2	(4.4)	-	-	-
	n	**(%)**	**n**	**(%)**	
Diabetes	13	(7.0)	14	(3.7)	.10
Anti-hypertensive medication	66	(35.3)	117	(31.3)	.20
Lipid lowering medication	34	(18.2)	89	(23.8)	.16
Education level:					<.001
less than lower vocational education	17	(9.1)	67	(17.9)	
lower vocational education	31	(16.6)	91	(24.3)	
lower secondary education	40	(21.4)	98	(26.2)	
intermediate vocational education	30	(16.0)	61	(16.3)	
general secondary education	10	(5.3)	12	(3.2)	
higher vocational education	43	(23.0)	36	(9.6)	
university	16	(8.6)	9	(2.4)	
Smoker status:					.028
never	67	(35.8)	154	(41.2)	
ever	98	(52.4)	154	(41.2)	
current	22	(11.8)	66	(17.6)	

sd=standard deviation; OR=odds ratio; ref=reference category; [a] =in sitting position

smoker status, prevalence of diabetes, and use of anti-hypertensive medication or lipid lowering medication.

No association was observed between chemotherapy and carotid artery plaque score (**Table 2**). This finding did not change after adjustment for blood pressure, smoker status, prevalence of diabetes, use of anti-hypertensive medication, lipid-lowering medication, age at menopause, and education level. Total cerebral blood flow and brain perfusion were also not associated with chemotherapy (**Table 3** and **Table 4**). This relationship did not change significantly after

Table 2. Effects of chemotherapy on plaque scores

	Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)		95% CI for OR			
	Mean	Sd	Mean	Sd	OR	Lower	Upper	P
Model 1	1.8	(1.7)	1.7	(1.5)	1.1	0.9	1.3	.39
Model 2	2.3	(2.9)	2.2	(3.4)	1.1	0.9	1.3	.47
Model 3	2.4	(2.9)	2.2	(3.8)	1.1	0.9	1.2	.58

CI=confidence interval; OR=odds ratio; Sd=standard deviation; Model 1=analysis adjusted for age, age^2, body mass index (BMI), and height; Model 2=as Model 1, plus: diastolic blood pressure; systolic blood pressure; smoking status; prevalence of diabetes; use of anti-hypertensive medication and lipid-lowering medication; Model 3= as Model 2, plus: level of highest completed education

additional adjustment for cardiovascular risk factors, and education level.

Discussion

To our knowledge, this is the first report on the association between adjuvant CMF chemotherapy, carotid artery plaque scores and brain perfusion in breast cancer survivors who completed systemic treatment on average more than 21 years previously. Chemotherapy-exposed breast cancer survivors did not significantly differ from population-based reference subjects who were never diagnosed with cancer regarding the number of carotid artery plaques, total cerebral blood flow or brain perfusion. These results did not change after controlling for

Table 3. Effects of chemotherapy on total cerebral blood flow (tCBF)

	Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)		95% CI for β			
	Mean	Sd	Mean	Sd	β	Lower	Upper	P
Model 1	515.5	(91.2)	518.1	(90.7)	-2.6	-18.7	13.6	.75
Model 2	503.4	(140.8)	502.7	(183.6)	0.8	-15.7	17.3	.92
Model 3	505.7	(145.5)	507.2	(199.1)	-1.5	-18.4	15.5	.86

CI=confidence interval; β=(beta) represents the difference in total cerebral blood flow between the chemotherapy-exposed survivors and the reference group; Sd=standard deviation; Model 1=analysis adjusted for age, age^2, body mass index (BMI), and height; Model 2=as Model 1, plus: diastolic blood pressure; systolic blood pressure; smoker status; prevalence of diabetes; use of anti-hypertensive medication and lipid-lowering medication; Model 3= as Model 2, plus: level of highest completed education

Table 4. Effects of chemotherapy on brain perfusion (ml blood per min per 100 ml brain tissue)

	Chemotherapy-exposed breast cancer survivors (n=187)		Reference group (n=374)		95% CI for β			
	Mean	Sd	Mean	Sd	β	Lower	Upper	P
Model 1	57.8	(9.4)	58.2	(9.3)	-0.5	-2.1	1.2	.58
Model 2	57.6	(14.4)	57.8	(19.0)	-0.2	-1.9	1.5	.86
Model 3	57.7	(15.0)	58.0	(20.6)	-0.2	-2.0	1.5	.79

CI=confidence interval; β=(beta) represents the difference in brain perfusion (ml blood per min per 100 ml brain) between the chemotherapy-exposed survivors and the reference group; Sd=standard deviation; Model 1=analysis adjusted for age, age^2, body mass index (BMI), and height; Model 2=as Model 1, plus: diastolic blood pressure; systolic blood pressure; smoker status; prevalence of diabetes; use of anti-hypertensive medication and lipid-lowering medication; Model 3= as Model 2, plus: level of highest completed education

confounders including age, height, BMI, blood pressure, smoker status, prevalence of diabetes, use of anti-hypertensive medication and lipid-lowering medication, age at menopause, and education level.

Strengths of our study are the large sample of breast cancer survivors with a long interval since chemotherapy, the homogeneous study population with regard to the cytotoxic agents received (regimen, cycles), and the large population-based reference subjects without a history of cancer.

A limitation of our study is that we did not have information on the use of hormonal replacement therapy (HRT). HRT has been associated with decreased occurrence of carotid atherosclerotic plaques (27, 28) and with improvements in cerebral blood flow (29-31) in post-menopause women. However, considering the low prescription of HRT in the Netherlands, which was approximately 13% at the time the patients in this study were diagnosed (32) and further decreased over time, and the clear absence of an association in the current study, we believe that adjusting for HRT use in the analyses presented here would not have changed the outcomes significantly.

Up till now, no other study investigated the acute or long-term effect of non-CNS directed adjuvant chemotherapy on the amount of carotid artery plaques, cerebral blood flow or cerebral perfusion. Although size of carotid plaques and degree of stenosis have been associated with cerebral blood flow in patients with stenosis (9), in our study the number of carotid artery plaques was not related to tCBF or brain perfusion. It is possible that presence of carotid artery plaques only relates to cerebral perfusion in patients who already have stenosis of the carotid arteries. Because of the relatively low incidence of carotid occlusion and the relatively small

number of cases in the present study, we were not able to study the effect of chemotherapy on carotid occlusion and subsequent brain perfusion or cerebral blood flow.

Harila-Saari et al. investigated the effects of intrathecal and intravenous methotrexate on brain perfusion in 25 children with acute lymphoblastic leukemia (33). In contrast to the present study, they did observe an association between chemotherapy and cerebral perfusion; manual inspection of SPECT scans revealed perfusion deficits in 44% of these chemotherapy-exposed patients. Many differences between the present study and the study by Harila-Saari et al. ,i.e. time since treatment, measurement of cerebral blood flow, administration route and dose of chemotherapy, age of the patients, type of cancer, might explain the different outcomes.

We recently reported that CMF chemotherapy-exposed breast cancer survivors performed worse on neuropsychological tests (34) and had less gray matter volume (35) than cancer-free reference subjects from the Rotterdam Study. The breast cancer survivors in these studies and the current study are the same individuals. The results of the current study suggest that the effect of chemotherapy on brain perfusion is not likely to be responsible for the previously observed cognitive dysfunction and brain structural alterations in chemotherapy-exposed breast cancer survivors.

Clinical relevance and future perspectives

An important question is to what extent our observations apply to other cytotoxic regimens. The CMF regimen is no longer the most optimal adjuvant chemotherapy for breast cancer, but it has been the standard regimen world wide up to the nineteen-nineties (36). Therefore it is currently the only regimen that enables the investigation of the very late effects of chemotherapy in sufficiently large numbers of subjects. Current regimens often include anthracyclines, which have also been associated with endothelial damage, but through a different mechanism than cyclophosphamide, methotrexate and fluorouracil (37). Therefore it is possible that the results of this study will not be similar to those when anthracycline-based regimens are the topic of investigation.

Conclusion

Adjuvant CMF chemotherapy is not associated with the presence of carotid artery plaques, total cerebral blood flow or brain perfusion in breast survivors on average 21 years post-treatment.

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Chapter 5

Incidental findings on brain MRI in long-term survivors of breast cancer treated with adjuvant chemotherapy

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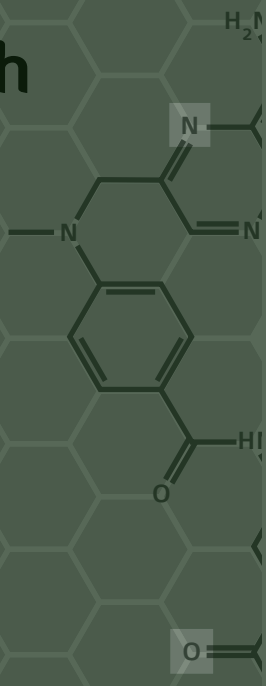

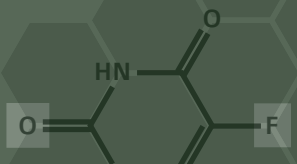

Abstract

Purpose Incidental brain findings defined as previously undetected abnormalities of potential clinical relevance that are unexpectedly discovered at brain imaging and are unrelated to the purpose of the examination are common in the general population. Because it is unclear whether the prevalence of incidental findings in breast cancer patients treated with chemotherapy is different to that in the general population, we compared the prevalence in breast cancer survivors treated with chemotherapy to that in a population-based sample of women without a history of any cancer.

Patients and methods Structural brain MRI (1.5T) was performed in 191 female CMF (Cyclophosphamide, Methotrexate, 5-Fluorouracil) chemotherapy-exposed breast cancer survivors. A reference group of 1590 women without a history of cancer was sampled from a population-based cohort study. All participants were aged 50 to 80 years. Five trained reviewers recorded the brain abnormalities. Two experienced neuro-radiologists reviewed the incidental findings.

Results The cancer survivors had completed chemotherapy on average 21 years before. Of the 191 subjects, 2.6% had an aneurysm and 3.7% had a meningioma. The prevalence of meningiomas and aneurysms was not different between the groups. The prevalence of pituitary macro adenomas in the breast cancer survivors (1.6%) was higher than that in the reference group (0.1%) (OR=23.7; 95% CI 2.3–245.8).

Conclusion Contrary to commonly held opinions, we did not observe an increased prevalence of meningiomas in cancer survivors. Breast cancer survivors previously treated with chemotherapy are more likely to develop pituitary adenomas than persons without a history of cancer and chemotherapy treatment.

Introduction

Over the past decade there has been an increase in the number of Magnetic Resonance Imaging (MRI) studies investigating chemotherapy associated structural and functional brain changes in cancer patients without central nervous system disease (1-9). The focus of these studies has been mainly on brain volumes, white matter lesions and integrity of normal appearing white matter. An implication of the use of brain imaging is the chance of discovering incidental findings, defined as previously undetected abnormalities of potential clinical relevance that are unexpectedly discovered and are unrelated to the purpose of the specific outcome measures under study (10).

The majority of these incidental findings are asymptomatic and little is known about their clinical relevance or prognosis (11). Frequently detected incidental findings in the general population are benign primary tumors and aneurysms (11). Whether the prevalence of such abnormalities in cancer patients is similar to that in the general population is unclear. None of the studies that examined structural or functional brain changes associated with chemotherapeutic treatment (1-9) reported on the occurrence of incidental findings.

We evaluated whether breast cancer patients who have been exposed to chemotherapy have an increased prevalence of incidental intracranial findings. We investigated this by comparing the prevalence of incidental findings in a large sample of chemotherapy-exposed breast cancer survivors with that in a large sample of women who had never been diagnosed with cancer from the general population.

Materials & methods

Participants

We used data from a study after the late effects of CMF chemotherapy on brain function and structure in older breast cancer survivors. This study compares chemotherapy-exposed breast cancer survivors with a population-based sample of women without a history of cancer, on several outcome measures and implements including neuropsychological tests and MRI of the brain. We selected a reference group from an ongoing population-based cohort study. Examination of the breast cancer survivors took place in the research center of this cohort study with the same protocol and by the same technicians.

From the registries of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Erasmus University Medical Center-Daniel den Hoed Cancer Center we selected consecutive female patients who had been treated between 1976 and 1995 for unilateral invasive breast cancer with 6 cycles of CMF (Cyclophosphamide 100 mg/m^2 on days 1-14, Methotrexate 40 mg/m^2 on days 1 and 8, 5-Fluorouracil 600 mg/m^2 on days 1 and 8) chemotherapy (12). All women underwent local radiotherapy. We included women who were between 50 and 80 years of age at time of study enrollment, who had sufficient command of the Dutch language, who had had invasive breast cancer as their first and only neoplasm, and who were disease-free since

primary cancer treatment. Exclusion criteria encompassed use of adjuvant endocrine therapy for breast cancer or MRI contra-indications.

On the basis of information from patient files, 359 women were eligible for participation and were hence sent an invitation letter signed by their treating physician. From the 359 patients, 20 (5.6%) could not be reached, 16 subjects (4.5%) had a health related contra-indication for MR imaging, 30 (8.4%) persons were ineligible for MRI assessment due to claustrophobia, and 2 women (0.6%) had insufficient command of the Dutch language. This left us with 291 eligible patients of whom 195 (67.0%) agreed to participate. Four of the 195 participating women aborted the scan session because of claustrophobic complaints. The final number of patients who completed MRI examination was 191. Written informed consent was obtained from all participants. The institutional review boards of the two participating institutions approved the study.

A reference group was selected from the Rotterdam Study; a population-based prospective cohort study ongoing since 1990 in the Ommoord district, Rotterdam, The Netherlands. (13) Of the 4,058 participants of the Rotterdam Study who completed an MRI examination until September 2009, we selected all women (n=2206; 54.4%) who were between 50 and 80 years of age (n=1881; 85.3%). Next, we excluded all participants with a cancer history based on self-report and record linkage with general practitioners (n=291; 15.5%), leaving a total reference group of 1590 women.

Methods

Brain MRI acquisition

All scans were obtained at the Rotterdam Study research center in Rotterdam, the Netherlands, using a 1.5-T scanner with an eight-channel head coil (GE Healthcare). Two trained technicians performed all examinations in a standardized way. The MRI protocol was identical for all participants has been described previously by *Vernooij et al.* (11).

Assessment of incidental findings

All scans were read for incidental findings of potential clinical relevance by one of five trained reviewers. Examples include brain tumors, aneurysms, subdural fluid collections, and arachnoid cysts. Reviewers were blinded for information on the subjects. Brain findings that were not considered clinically relevant and were not recorded as incidental findings included simple sinus disease and variations from the norm, such as pineal cysts, ventricular asymmetry, and enlarged Virchow–Robin spaces (11). Diagnoses were not confirmed by histologic studies but were made on the basis of MRI findings characteristic of each lesion. Case definitions for each incidental MRI finding have been described previously by *Vernooij et al.* (11). Two experienced neuro-radiologists reviewed and reached a consensus on all initially reported abnormalities (11). The management of incidental findings followed the protocol of the Rotterdam Study and

was defined before the start of the study. Depending on the detected abnormality and after consultation with involved clinicians, persons with incidental findings requiring additional clinical workup or medical treatment were informed and referred to a relevant medical specialist.

Statistical analysis

Prevalence of incidental brain finding were compared between the chemotherapy-exposed women and the women from the reference group using age-adjusted binary logistic regression analysis. We subsequently examined the effect of type of menopause and age at menopause on the risk to develop incidental findings. In addition, within the breast cancer survivors we investigated whether radiotherapy field was associated with the development of incidental findings. Alpha levels were set at p=0.05 for all analyses.

Results

Eligible breast cancer patients who declined participation were older than subjects who were willing to participate at the time invitation letters were sent ($F_{1, 289}$=11.13, p<.05).

Table 1 presents the characteristics of the breast cancer survivors and the reference group. Chemotherapy-exposed subjects were older than women from the reference group (F=59.6; p=<.001). The mean age at breast cancer diagnosis was 42.9 years and time since treatment was on average 21.2 years. Of the 191 chemotherapy-exposed participants 161 (85.3%) became menopausal following breast cancer treatment at a mean age of 43.0 years. For the whole sample, breast cancer patients menopause occurred on average at age 43.8 years (sd=6.1), which is significantly earlier than for women from the reference group who reached menopause at a mean age of 48.6 years (sd=6.0) (F=105.0; p=<.001). Of the 191 breast cancer survivors, 85.3% received parasternal radiotherapy, 9.4% received radiotherapy at the breast or chest wall, 3.7% underwent radiotherapy according to the McWhirter protocol and for three patients (1.6%) radiotherapy field was unknown.

Table 2 presents the prevalence of age-adjusted incidental findings. Of the breast cancer survivors 2.6% had an aneurysm, 3.7% had a meningioma and 1.6% had a pituitary macro-adenoma. There were no significant differences in the prevalence of meningiomas and aneurysms between women who underwent chemotherapy and the reference group. However, chemotherapy-exposed patients had a higher prevalence of pituitary macro adenoma than the reference group (OR= 23.7; 95% CI= 2.3-245.8). Besides aneurysms, pituitary adenomas and meningiomas in this sample of merely 200 women we did not find any other findings such as gangliomas, vestibular schwannomas or subdural hematomas. We found no association between age and type of menopause (chemotherapy induced versus natural) with any of the incidental findings. In the chemotherapy-exposed patients, radiotherapy field was not associated with the prevalence of any of the incidental findings.

Table 1. Characteristics of the breast cancer survivors and the reference group

	Breast cancer survivors (N=191)		Reference group (N=1590)		p
Number of participants	191		1590		
Mean age in years (sd)	64.1	(6.4)	60.2	(6.6)	<.001
Mean age at menopause: total (sd)	43.8	(6.1)	48.6	(6.0)	<.001
sponteaneous menopause in years (sd)	47.5	(4.3)	50.2	(4.3)	<.001
induced menopause in years (sd)	43.0	(7.2)	45.7	(7.1)	.002
Mean age of cancer diagnosis in years (sd)	42.9	(5.3)			
Mean time since treatment in years (sd)	21.2	(4.4)			
Radiotherapy field:					
parasternal (%)	163	(85.3)			
breast / chest wall (%)	18	(9.4)			
McWhirter (%)	7	(3.7)			
unknown (%)	3	(1.6)			

sd= standard deviation

Table 2. Prevalence of age-adjusted incidental findings

	Reference group (N=1590)		Breast cancer survivors (N=191)			
	n	**(%)**	**n**	**(%)**	**OR**	**95% CI**
Aneurysm	37	(2.3)	5	(2.6)	1.1	0.42 – 2.91
Meningioma	36	(2.3)	7	(3.7)	1.4	0.62 – 3.33
Pituitary macro adenoma	1	(0.1)	3	(1.6)	23.7	2.28 – 245.76

OR= odds ratio; CI= confidence interval

Discussion

We found no difference in the prevalence of asymptomatic meningiomas and aneurysms identified on MRI scans in 50 to 80-year-old former breast cancer patients who had been treated with chemotherapy, on average 21 years before, and a population-based sample of women of the same age without a history of cancer. However, the former breast cancer patients had a higher prevalence of asymptomatic pituitary macro adenomas than the reference group.

Up till now, a non-significant positive association between pituitary adenomas and benign breast tumors has been reported (14, 15). To our knowledge, no previous data are available

on a relationship between pituitary adenomas and invasive breast cancer. Therefore it remains undermined whether the excess number of pituitary adenomas in the current group of invasive breast cancer survivors could be explained by their first primary neoplasm.

A possible explanation for the elevated prevalence might be the relation of pituitary adenomas and postmenopausal status. In a case-cohort study *Schoemaker et al.* reported a 3-fold risk increase in postmenopausal women, which was even greater for surgically induced menopause compared to natural menopause (OR 6.7), and was greatest in women who entered menopause before the age of 40 years (OR 7.5) (15).

It is known that CMF may induce menopause in a substantial number of patients. A study by *Goodwin et al.* showed that use of CMF increased the risk of onset of menopause within 1 year after breast cancer diagnosis in 40-year-old women from less than 5% to more than 40%. In 50-year-old women, this risk was increased from approximately 20% to close to 100% (16). In our sample of cancer patients 85.3% became menopausal directly after treatment at a mean age of 43.0 years. The relative early and young mean age at menopause may have put these women at a higher risk for pituitary adenomas. However, type of menopause and menopausal age of onset were no predictors for the risk of incidental findings in our models, although this could also be due to the small number of incidental findings.

Several studies have reported an elevated risk of developing a meningioma after breast cancer and vice versa, with standardized incidence rates ranging from 1.57 to 1.90 (17-22). Proposed explanations for the co-occurrence of these tumors include a) the hormonal dependency of both tumors as estrogen and progesterone receptor expression are frequently present in breast carcinomas as well as in meningiomas, and the observation that meningiomas tend to grow rapidly during pregnancy (23-25), b) the fact that both tumors have a higher incidence in females (20), and c) intake of unsaturated fat as a risk factor for both malignancies (20).

In contrast with the literature, we did not find a higher prevalence of meningiomas in former breast cancer patients compared to the general population. Potential explanations for the divergent observations are the different study designs and populations. The incidental findings in our studies concerned asymptomatic meningiomas whereas other studies focused on symptomatic meningiomas. Moreover, previous studies used data from regional (22) and national (17-20) cancer registries that include almost all consecutive breast cancer patients in a particular time frame whereas we selected a more homogeneous group of breast cancer patients who were all treated with adjuvant chemotherapy and who never developed recurrent breast cancer nor a second malignancy.

Some epidemiologic studies showed that increased estrogen levels are associated with a higher risk for breast cancer in pre- and postmenopausal women (26-29). Because estradiol also might stimulate growth of meningiomas (30) one might expect a higher prevalence of these neoplasms in breast cancer patients. However, the fact that our breast cancer survivors went

through menopause much earlier than women from the general population may have decreased the prevalence of meningiomas in our study group as a result of a significant period of lower estrogen levels. Furthermore, *Wigertz et al.* postulated that sex hormones influence tumor growth rather than tumor initiation (31). These arguments may explain why we did not find a difference in the prevalence of asymptomatic meningiomas between breast cancer survivors and the general population and also the discrepancy of the prevalence of menigiomas that we observed in the breast cancer survivors and the prevalences of symptomatic meningiomas in the published studies.

No difference in the prevalence of aneurysms was observed between chemotherapy-exposed breast cancer survivors and the reference group. In-vitro studies have showed that chemotherapy might induce endothelial cell damage (32, 33), which in rats has been related to cerebral aneurysm formation (34). Data in humans hereon are lacking. Radiotherapy to the head, neck and brain has been associated with intracranial aneurysms (35). To our knowledge, no studies have investigated if ionizing radiation scatter from radiotherapy for breast cancer, for example at the supraclavicular field, is also associated with the formation of intracranial aneurysms. Our results however indicate no association between breast cancer and CMF chemotherapy or radiotherapy-field and the development of intracranial aneurysms.

We are aware that our study has some drawbacks. As a result of the inclusion criteria that we applied, our population under study is a selection, since we have only included breast cancer patients who underwent cytotoxic treatment, who did not develop breast cancer recurrence and who were never diagnosed with a second primary cancer. This limits the generalizability of the study results, because breast cancer patients who have developed a second malignancy may be at higher risk to subsequently develop intracranial neoplasms than those who have not (36).

Moreover, we cannot separate the effect of chemotherapy and breast cancer itself on the risk of developing intracranial neoplasms or aneurysms. Finally, although our sample of former breast cancer patients was large enough to investigate the more common incidental findings, the number of subjects was too small to investigate less common incidental e.g. gangliomas.

Another point of discussion is whether the findings of this study apply to breast cancer patients treated with contemporary regimens, since it is unclear whether the current observation on the associations between adjuvant chemotherapy for breast cancer and the development of incidental findings is exclusively linked to the CMF regimen. If cancer rather than its treatment is a risk factor for incidental findings, risk differences between the CMF regimen and contemporary regimens may not exist. If differences in hormone levels are in the causal pathway of incidental findings, the risk may be different for contemporary regimens, as the occurrence of premature treatment- induced menopause varies by regimen. (37) When development of incidental findings is caused by cytotoxic treatment itself, similar risks may be expected for contemporary regimens compared to the CMF regimen; both cyclophosphamide

and 5-fluorouracil are frequently implemented in current regimens and these agents as well as many other commonly applied agents are independently associated with structural brain changes (38, 39) and comprised vessel integrity in animals (40, 41).

A major strength of this study is the large reference group from which we obtained a precise estimate of the prevalence of incidental findings in women from the general non-cancer population. Up till now, no other study has looked at the relation between breast cancer or adjuvant chemotherapeutic treatment and asymptomatic intracranial neoplasms or aneurysms. The long time since treatment enabled us to look at neoplasms and aneurysms that normally take a long time to develop and of which the initiation or progression may have been triggered by the cytotoxic treatment.

Clinical implications

The number of studies implementing MRI in the field of cancer and cognition is rapidly increasing and as a result the number of incidental findings will progress similarly. No strict guidelines on the management of incidental findings are available and investigators vary greatly in the way they handle them (11, 42). For the interpretation of the prevalence of incidental findings data from an appropriate reference population is of crucial importance. Up till now 3 studies have presented date on the prevalence of incidental findings in healthy adults (43-45), and only one study has described prevalences of incidental findings in the general population (11). This is the first study that presents the prevalence of incidental findings in breast cancer survivors who have been treated with chemotherapy. The observation of an increased incidence of pituitary macro adenomas, possibly in relation to an early postmenopausal status needs confirmation as with the current treatment strategies the number of long-term breast cancer survivors is increasing.

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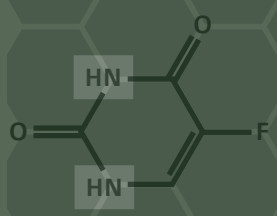
Chapter 6

General discussion



Adjuvant chemotherapy is widely used in the treatment of breast cancer (1). Adverse effects that have been ascribed to the cytotoxic treatment include cognitive dysfunction and brain structural alterations, which have been observed shortly up till several years post treatment (2-7). Not much is known about the long-term consequences of adjuvant cytotoxic treatment on the brain. However, as the number of long-term survivors is increasing (8-11) this topic is gaining more interest (12).

In this thesis I investigated the late effects of adjuvant CMF (Cyclophosphamide, Methotrexate, 5-Fluorouracil) chemotherapy, given for breast cancer, on brain function and structure. In this chapter I will summarize and interpret the main findings of the studies that have been conducted in the framework of this thesis. Subsequently I will get back to the research question that was formulated in Chapter 1: "*Is adjuvant CMF chemotherapy given for early stage breast cancer associated with long-term impaired cognitive functioning and brain structure?*". Furthermore, I will discuss methodological considerations that limit the interpretation of the studies, and consider the clinical implications that result from these studies. Finally, I'll make suggestions for future research following this thesis.

Breast cancer patients that participated in the studies on which this thesis is based had been treated with CMF chemotherapy, on average more than 20 years before, in the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital or the Daniel den Hoed Cancer Center of the Erasmus MC University Medical Center. Reference subjects were sampled from the Rotterdam Study (13); an ongoing population-based cohort study. By sampling reference data from the Rotterdam Study I was able to compare the test results of the chemotherapy-exposed breast cancer survivors to those of the general population. Because of the extensive set of cognitive, psychological, cardiovascular and neuro-imaging measures that are collected in the framework of the Rotterdam Study it was possible to investigate the impact of CMF chemotherapy on these various interdependent outcome measures with subsequent correction for several confounding factors. In addition, it enabled me to focus all our efforts and resources on including as much breast cancer survivors as possible. The eventual inclusion of large numbers of subjects allowed me to adjust for multiple confounders.

Summary and interpretation of main findings

Previous studies

When I started this study, little was known about the potential late effects of adjuvant chemotherapy on cognitive functioning. In Chapter 2 I reviewed the literature on the long-term outcomes of adjuvant chemotherapy given for non-central nervous system cancer, on cognitive functioning, brain structural and functional alterations, and the risk of dementia. Studies were selected if the cancer patients under investigation were at least five years post-treatment. Six studies, including our own, were identified that assessed patients with neuropsychological tests five or more years after cessation of adjuvant cytotoxic treatment. These studies showed that chemotherapy is associated with long-term subtle cognitive dysfunction in some, but not all patients. The effects were mainly observed in the domains of verbal memory and executive functioning. Three neuro-

imaging studies in patients that were five or more years post-treatment were identified. Results from these studies are in line with the previously described late cognitive effects studies and show that cytotoxic treatment is associated with less long-term gray matter volume, worse global and focal white matter integrity, and hypo-activation of brain areas during cognitive tasks. Four studies on the risk of dementia following chemotherapy showed no increased risk for dementia following cytotoxic treatment, yet had severe methodological limitations and cannot be considered as conclusive. The results from this review suggest that five or more years after cessation of treatment, various regimens of adjuvant chemotherapy are associated with impaired cognitive functioning possibly as a result of chemotherapy-induced structural brain changes. The majority of the reviewed neuropsychological studies had small sample sizes and investigated the effects of adjuvant chemotherapy in patients who completed cytotoxic treatment on average less than ten years before. Reliable estimates of the very late effects of adjuvant chemotherapy are therefore largely unknown.

Cognitive functioning

To investigate if conventional-dose adjuvant chemotherapy is associated with long-term cognitive functioning I compared neuropsychological test outcomes of 196 breast cancer patients treated with the adjuvant CMF regimen on average more than 20 years previously (range ~14-30 years) to the neuropsychological test outcomes of more than 1500 women from the general population without a history of cancer. CMF chemotherapy has been the main choice of adjuvant treatment for breast cancer up till the early nineteen nineties. It is therefore currently the only regimen of which the late cognitive adverse effects can be investigated in sufficiently large numbers (14). In Chapter 3 I reported the results of this study, which showed that chemotherapy-exposed breast cancer survivors performed worse on several neuropsychological tests than population based reference subjects without a history of cancer. Significant differences were observed between groups on cognitive tests of immediate and delayed verbal memory, processing speed, executive functioning and psycho-motor speed. Also on a summary measure of the neuropsychological test results that allowed for mutual correlation, the 'Mahalanobis Distance' (15-18), the reference group significantly outperformed the breast cancer survivors. Mean scores on a dementia screener (i.e. the Mini Mental State Examination (MMSE)), did not differ between groups. Besides having worse cognitive functioning, the chemotherapy-exposed survivors also reported more often memory complaints. Similar to studies conducted shortly after cessation of cytotoxic treatment, no significant correlation was found between objective cognitive functioning measured with the neuropsychological tests and self reported memory complaints (19). Memory complaints were associated with symptoms of depression; an association that has been observed previously in chemotherapy-exposed survivors (19), and various other patient populations (20, 21).

The mechanisms by which chemotherapy may induce cognitive dysfunction are currently under intensive investigation. Studies so far have shown that many common chemotherapeutic agents

directly affect central nervous system cells (22). For long, it has been thought that chemotherapy cannot enter the brain, because of the blood-brain barrier that restricts the diffusion of toxins into the brain parenchyma (23). However, under several conditions specific types of drugs are able to cross the blood-brain barrier (24, 25). Both methotrexate and 5-fluorouracil have proven to be able to cross the blood brain barrier in low doses (26, 27). Because neuronal regeneration is limited (28), the effects of chemotherapy on brain structure that have been found shortly after cessation of treatment might be long-lasting (2, 3, 5, 6, 29, 30).

Brain structure

To investigate if adjuvant chemotherapy is associated with long-term brain structure I compared almost two hundred breast cancer survivors who had been treated with CMF chemotherapy, on average more than 20 years before, to an age-matched reference group that I sampled from the Rotterdam Scan Study (31). The two groups were compared on several state of the art neuro-imaging outcome measures, including total brain volume, total gray and white matter volume, focal gray matter volume, and global and focal white matter microstructural integrity. I used two different techniques to assess focal gray matter volume; a widely used hypothesis free technique known as 'Voxel Based Morphometry' (32) and an in-house developed automated region of interest (ROI) analysis in which I specifically looked at hippocampal volume (33). The effect of chemotherapy on Hippocampal volume is of particular interest as animal studies showed a detrimental effect of cytostatic agents on hippocampal structure and learning (34). Moreover, together with the prefrontal cortex, this brain structure is of major importance for consolidation of episodic and semantic memory (35, 36) and several neuropsychological studies, including the one discussed in Chapter 3, showed a negative relationship between cytotoxic treatment and memory function (7, 37). In Chapter 4.1 I discussed the outcomes of my volumetric imaging study. The results of these analyses showed that CMF chemotherapy-exposed breast cancer survivors had less total brain volume and less gray-mater volume than the population based reference subjects. These results did not change after subsequent adjustment for potential confounders such as age, height, cardiovascular risk factors, symptoms of depression, and education level.

Apart from the potential late effects of adjuvant CMF chemotherapy on the macrostructure of the brain I was also interested in the effect of chemotherapy on the integrity of the microstructure of the white matter, as damage to the latter has been associated with cognitive dysfunction (38). Microstructural white matter integrity can be characterized using diffusion tensor imaging (DTI). DTI visualizes the extent and directionality of the diffusion of water molecules within brain tissue at the voxel level. Generally, water diffusion in white matter fiber bundles is anisotropic as it is hindered by tissue architecture (axonal membranes and myelin) and therefore more prominent along the fiber direction. Although the biological background is complex, increased diffusivity is often interpreted as being related to myelin damage, demyelination or axonal injury (39-41). I used tract based spatial statistics (TBSS) (42) to analyze the long-term differences in focal and global white matter integrity

between the previously described sample of adjuvant CMF chemotherapy-exposed breast cancer survivors treated on average more than 20 years before (range ~14-30 years), and an age-matched reference group derived from the Rotterdam Scan Study (31). In Chapter 4.2 the results of this study are discussed. No differences were observed in global or focal microstructural white matter integrity between groups. However, within the group of chemotherapy-exposed breast cancer survivors microstructural white matter integrity was significantly worse with longer times since treatment. A possible explanation for these results is that the group of chemotherapy-exposed cancer survivors was healthier at baseline than the reference group. This idea is supported by the fact that the breast cancer patients were taller, smoked less, had lower BMI, had less symptoms of depression, and were better educated than the reference subjects. This may have resulted partially from the fact that cancer survivors are prone to adopt a healthier lifestyle after the cancer diagnosis (43, 44). Because education level, BMI, and smoking habits all have been associated with white matter integrity (45-47), it is possible that at baseline the chemotherapy-exposed survivors had better white matter integrity than the reference subjects.

As stated, our understanding of the potential mechanisms underlying chemotherapy-induced brain structural changes is still limited. We considered that the difference in total cerebral gray matter volume that I observed between chemotherapy-exposed breast cancer survivors and reference subjects (see Chapter 4.1) might be related to chemotherapy-induced differences in cerebral perfusion. Reductions in total cerebral blood flow (tCBF) have been associated with cortical gray matter atrophy (48). In addition, adjuvant chemotherapy has been linked to endothelial damage and intima-media thickness of the common carotid artery (49, 50). Because degree of carotid stenosis and size of carotid artery plaques have been related to cerebral blood flow (51), one could hypothesize that chemotherapy-exposed patients have reduced tCBF as a result of worse carotid artery integrity. To investigate if adjuvant chemotherapy is associated with carotid artery plaques and with cerebral perfusion and blood flow, I compared the prevalence of plaques scores and tCBF and perfusion between 187 breast cancer survivors treated with adjuvant CMF chemotherapy on average more than 20 years before, and a twice as large age-matched population-based sample of women without a history of any cancer. The presence of carotid artery plaque scores was measured with ultrasound and tCBF was measured with non-invasive 2D phase contrast MRI. Because tCBF is strongly related to brain volume (52), I subsequently calculated cerebral perfusion by dividing tCBF by brain volume. In Chapter 4.3 I presented the outcomes of these analyses. No differences were observed between the groups on any of the outcome measures, indicating that chemotherapy is neither associated with long-term carotid artery integrity, nor with cerebral perfusion or total cerebral blood flow, and as such can not explain the differences in gray matter volume and white matter microstructural integrity between groups that I observed.

Incidental findings

An implication of the use of neuro-imaging is the chance of discovering incidental findings,

defined as previously undetected abnormalities of potential clinical relevance that are unexpectedly discovered and are unrelated to the purpose of the specific outcome measures under study (53). The majority of incidental findings are asymptomatic and little is known about their clinical relevance or prognosis (54). Frequently detected incidental findings in the general population are benign primary tumors and aneurysms (54). Whether the prevalence of such abnormalities in cancer patients is similar to that in the general population is unclear. None of the neuro-imaging studies on the association between chemotherapy and brain structural alterations reported on the occurrence of incidental findings (2, 3, 5, 6, 29, 30, 55-60). To study if the prevalence of intracranial incidental findings in chemotherapy-exposed breast cancer patients is similar to that of the general population I compared the prevalence of incidental findings in 191 CMF chemotherapy-exposed breast cancer survivors, treated on average more than 20 years ago and aged 50 to 80 years, to that of 1590 cancer-free subjects within the same age range sampled from the population-based Rotterdam Scan Study (31). All incidental findings were recorded by experienced medical doctors and reviewed by two neuro-radiologists. In Chapter 5 I present the results of the group comparisons. Within the chemotherapy-exposed breast cancer survivors I observed three different types of intracranial incidental findings; aneurysms, meningiomas, and pituitary macro adenomas. I observed a higher age-adjusted prevalence of pituitary macro adenomas in the breast cancer survivors than in the population-based sample. No significant differences were observed between groups regarding aneurysms, or, contrary to commonly held opinions (61-66), the prevalence of meningiomas. These study results warrants further investigation of the prevalence of incidental findings in chemotherapy-exposed cancer patients and emphasizes the need for strict protocols on the assessment and follow-up of incidental findings.

Answer to my research question and general conclusion

Adjuvant CMF chemotherapy given for breast cancer is associated with long-term worse cognitive functioning, less total gray matter volume, and worse white matter microstructural integrity, but not with total cerebral blood flow, cerebral perfusion, or plaques in the carotid artery. By comparing chemotherapy-exposed breast cancer survivors to a reference population without a history of cancer it is not possible to distinguish the effects of chemotherapy and cancer itself. My findings suggest that chemotherapy-induced gray matter volume reductions and worse white matter microstructural integrity may be part of the mechanisms underlying the observed long-term cognitive dysfunction. This is in line with previous studies in the general population that showed that both worse microstructural white matter integrity (38), and smaller cerebral gray matter volume (67) are associated with cognitive dysfunction. I did not observe differences in cerebral perfusion between the chemotherapy-exposed survivors and the reference group and therefore it is unlikely that chemotherapy-induced altered cerebral perfusion is causing the observed brain structural differences nor is it responsible for the differences in cognitive functioning or brain structure between the groups. Previous studies that simultaneously investigated brain functioning

using neuropsychological tests and structural and (2, 5, 6, 55) functional MRI (56, 58, 68), positron emission tomography (PET) (69), or electro-encephalography (EEG) (70, 71), support the idea that cerebral alterations in chemotherapy-exposed breast cancer patients are associated with cognitive dysfunction.

I observed global but not focal effects of adjuvant CMF chemotherapy on gray matter volume and white matter integrity. Other studies on the effects of cytotoxic treatment on brain structure observed focal effects of chemotherapy on brain volume (6, 55), brain function (56, 58), and white matter integrity (2, 5, 55), but never reported on the presence or absence of global effects of cytotoxic treatment. It may be that the combined focal effects of chemotherapy underlie a global effect in brain structure. The fact that neuro-imaging studies shortly after cessation of treatment found chemotherapy-induced focal differences in brain structure that at least partially recover at longer times since treatment (6, 30, 59), suggests that at very long times after completion of treatment no focal effects of chemotherapy may be observed.

The fact that I did not observe differences in the mean score on a dementia screener between the chemotherapy-exposed breast cancer survivors and the reference subjects suggests that although the breast cancer survivors have more cognitive problems that are associated with chemotherapy and/or the disease, it does not lead to an accelerated incidence or increased risk of dementia, at least not within the age range covered in this study.

Chemotherapy-induced menopause

Early menopause may be an intermediate factor in the effect of adjuvant CMF chemotherapy on brain function (22). Adjuvant CMF is known for its ability to induce menopause. *Goodwin et al.* showed that within 1 year after breast cancer diagnosis, in 40-year-old women who had been treated with CMF, the onset of menopause increased from less than 5% to more than 40%. In 50-year-old women, this risk was increased from approximately 20% to close to 100% (72). In our sample of cancer patients 85.3% became menopausal directly after treatment at a mean age of 43.0 years (see Chapter 5). Some studies have reported effects of surgically and chemically induced menopause on cognitive functioning, in particular on verbal and working memory, although several others did not report such a relationship (73, 74). Nevertheless, within my study population I found no differences in cognitive functioning between women who reached menopause before or after the age of 45, and no correlation between time since menopause and white matter microstructural integrity.

Methodological considerations

Here I will discuss the methodological considerations that generally apply to the studies in this thesis. Methodological considerations that concern individual studies have been discussed in the respective chapters.

Study design

The studies presented in this thesis are the first that assessed the very late effects of conventional-dose adjuvant chemotherapy on brain structure and function in large numbers of patients. The patients under investigation in this study had all been treated more than 13 years up to 30 years prior to participation. A prospective cohort study would be the most optimal design to answer my research question. However, the long follow-up of large numbers of breast cancer patients, taken into account the dropout of subjects, would be a cost-inefficient endeavor, which is why the current cross-sectional design was initially selected. The downside of this design is that I could not investigate any changes in cognitive performance and brain structure that result from cytotoxic treatment. Even though the large samples of breast cancer survivors and reference subjects from the general population enabled precise estimates, I do not know if these women were comparable at baseline (i.e. at the time of the breast cancer diagnosis). My data suggest that the group of chemotherapy-exposed survivors and the reference subjects were not fully comparable at baseline. For example, the chemotherapy-exposed breast cancer patients on average completed higher levels of education than the reference subjects. Education level has been associated with cognitive functioning (75), and brain structure (46, 76) and therefore, at baseline, cognitive function and brain structure may have been better in breast cancer survivors than reference subjects. In addition, breast cancer patients are prone to adopt a healthier lifestyle after the cancer diagnosis (43, 44), which has been associated with better brain function, structure and metabolism (45, 47, 77). Hence, the negative effect of CMF chemotherapy may have been underestimated.

Selection of chemotherapy-exposed breast cancer survivors

To be able to investigate the long-term adverse effects of chemotherapy in sufficiently large numbers, I included women who had been treated with CMF chemotherapy, as this was the regimen of choice from the mid nineteen seventies up until the mid nineteen nineties (14). On average these women had been diagnosed with breast cancer 21 years before entering the study. It is not impossible that selection bias within the group of eligible breast cancer survivors could have led to an underestimation of the effect of chemotherapy. Generally, healthy subjects are more prone to participate in scientific studies than their less healthy counterparts (78). This type of selection bias is often referred to as the healthy worker effect. Of the 292 subjects that were eligible to participate in the studies described in this thesis, 96 declined participation. These women were older than women that participated. To investigate if there was a possible selection bias I invited the 96 women that declined participation and all women who could not complete MRI assessment because of claustrophobia, but who were otherwise eligible for the main study (n=30), for an at-home testing session. Of these 126 women, two had passed away and two could not be contacted. Of the 122 eligible participants 48 women (38%) agreed to participate. They subsequently underwent the same neuropsychological assessment and interview as used in the main study, at their own home. Home-participants had worse processing speed compared to center-participants,

although after adjustment for multiple comparisons there were no differences between groups, suggesting no selection bias regarding cognitive functioning. Furthermore, after adjustment for multiple testing, center participants did not differ from home participants on socio-demographic variables, cardiovascular risk factors or neurologic diseases. Although I cannot completely rule out selection bias, as not all decliners participated in the at-home testing session, and because I did not have information on brain structure of the declining breast cancer survivors, I found no suggestion for selection bias regarding cognitive functioning.

Reference population

To date, the Rotterdam Study has included almost 15,000 subjects from the Ommoord area over three cohorts (13, 31). The first cohort (RS-I) that was recruited from 1990 onwards comprises of 7,893 subjects 55 years of age and older, of whom 203 underwent brain imaging in the framework of the Rotterdam Scan Study (RSS). In 2000 the second cohort (RS-II) was recruited that comprises of 3,011 subjects who had become 55 years of age or came to live into Ommoord. Of RS-II, 895 subjects completed MRI examination. Finally, in 2006, the third cohort (RS-III) of 3,932 subjects aged 45 years to 54 years was recruited. Of these subjects 2,947 completed brain imaging. As the majority (71%) of the scans was obtained from subjects from RS-III who were between 45 and 54 years of age, the distribution of eligible reference subjects for the MRI studies was skewed to the left (see **Figure 1**). Because of the relatively low number of subjects in the higher age categories I was not able to match on education level as adequately as I was able to match on age. As a result, the distributions of highest attained education level between chemotherapy-exposed cancer survivors and reference subjects differed significantly. Although I adjusted for age, I cannot rule out the possible residual effect of education on brain structure (46, 76).

Clinical implications

The goal of my study was to explore the potential long-term effects of standard dose adjuvant CMF chemotherapy given for breast cancer on brain function and structure.

The finding that chemotherapy-exposed breast cancer survivors who were on average more than 20 years post-treatment performed worse than reference subjects on neuropsychological tests measuring verbal memory, information processing speed, executive functioning, and psycho-motor speed has several important implications. First of all, it indicates that chemotherapy for breast cancer is associated with long-term cognitive dysfunction. Whether this effect is due to chemotherapy, breast cancer itself or both is not entirely clear. Nevertheless, since even mild cognitive dysfunction is associated with lower quality of life (79), this outcome is important considering the large and still increasing number of breast cancer survivors (8-11). Although the current findings might not justify informing patients about the potential late effects of chemotherapy at the moment they have to decide whether or not to undergo chemotherapy, they should stimulate clinicians to be perceptive of cognitive problems in patients with a history of adjuvant CMF chemotherapy for breast cancer.

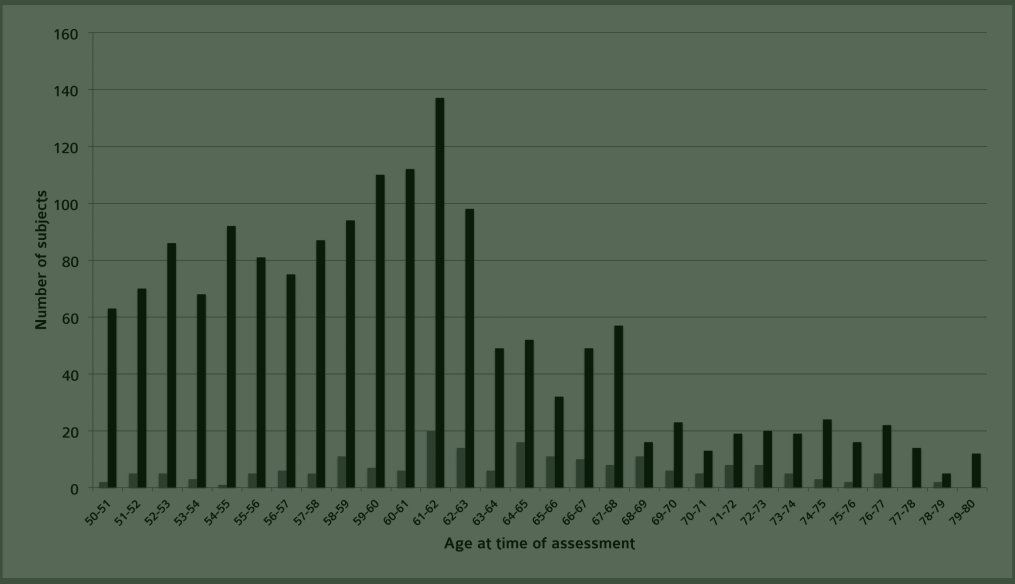


Figure 1. Number of subjects per age class for the chemotherapy-exposed breast cancer survivors and reference subjects. Gray bars indicate the number of chemotherapy-exposed breast cancer survivors; Black bars indicate the number of reference subjects available from the Rotterdam Scan Study

From a patient perspective the results presented in this thesis call for the availability of information for patients on the potential long-term effects of chemotherapy on cognitive functioning. Because this is the first study on the very late effects of chemotherapy on cognitive functioning, replication of our results is necessary before it is justified to inform patients about long-term cognitive consequences of adjuvant cytotoxic treatment. Until then, information on the long-term effects of adjuvant cytotoxic treatment on cognitive functioning may be added to the currently available patient-information leaflet on the adverse-cognitive effects of chemotherapy. Cognitive dysfunction should be considered as a potentially serious adverse effect of chemotherapy and therefore trials investigating new cytotoxic regimens should include standard evaluation of cognitive functioning.

Cytotoxic regimen under investigation

I investigated the long-term adverse effects the CMF regimen. Nowadays, CMF chemotherapy is not the main choice of adjuvant therapy for invasive breast cancer anymore (80). Whether contemporary regimens are also associated with long-term brain structural and functional alterations, and whether these effects are similar the late effects of adjuvant CMF chemotherapy I observed is largely unknown. Nevertheless, as described in Chapter 2, neuropsychological (81-84), and neuro-imaging (6, 55, 58, 60) studies investigating the effects of currently prescribed regimens show that shortly up till several years after cessation of treatment these contemporary regimens

adversely affect brain function and structure. My studies may act as a model to investigate the potential long-term effects of contemporary regimens on cognitive function and brain structure. In addition, the cytotoxic agents cyclophosphamide and 5-fluorouracil that are part of the CMF regimen are still frequently incorporated in conventional contemporary regimens, such as TAC (taxotere, adriamycine, cyclophosphamide) or FEC (cyclophosphamide, epirubicin, fluorouracil) (80). Animal studies, which enabled the investigation of single cytotoxic agents, showed that methotrexate, fluorouracil, and cyclophosphamide may cause brain structural alterations such as reduced hippocampal cell proliferation and delayed myelin destruction (25, 85-88), and may result in learning and memory problems (25, 86, 87, 89, 90). Therefore the late effects of adjuvant CMF chemotherapy reported in this thesis may at least partially apply to contemporary regimens.

In addition, because CMF chemotherapy has been the worldwide adjuvant treatment of choice for invasive breast cancer for almost 20 years up till the late nineteen nineties (91), a large group of breast cancer survivors who have been treated with CMF chemotherapy may experience its cognitive sequelae in the near future. Furthermore, most breast cancer patients are diagnosed after the age of 60 years (80) and a substantial percentage of these patients may already experience some age-related cognitive decline. Any additional adverse cognitive effect induced by chemotherapy, even if these effects are small, may therefore be of clinical relevance. Therefore, the results of the studies in this thesis apply to a large proportion of the breast cancer survivor community for years to come.

Future research

Contemporary regimens

As CMF chemotherapy is no longer the treatment of choice, future studies should focus on the late effects of contemporary regimens, in order to clarify if these regimens are also associated with late cognitive functioning and brain structure. The more recent studies showed that the regimens that are currently prescribed for the adjuvant treatment of breast cancer are also associated with neuro-cognitive dysfunction (55, 92-94). However, because there are several co-existing regimens that are currently applied in the treatment of adjuvant breast cancer (80), it will be difficult to gather a sufficiently large homogenous group of long-term survivors. Furthermore, because since the late nineteen nineties the adjuvant regimen of choice for the treatment of breast cancer has changed several times (80), it will take some time before we will be able to study the effects of contemporary cytotoxic regimens in patients that have been treated more than 20 years ago, simply because these regimens were not available back then. With the addition of neuropsychological assessment to trial protocols for new regimens, the acute and long-term effects of cytotoxic treatment can be monitored directly and enables the investigation of the potential causal relationship between cytotoxic treatment and cognitive dysfunction.

Time since treatment

Even though in the studies in the current thesis there was a large range of time since treatment among subjects, the cross-sectional design of these studies did not allow me to investigate whether the effects of chemotherapy changed with accumulating time since treatment. Follow-up of the population under investigation in the current study and in other studies on the long-term effects of cytotoxic treatment is needed to identify if the late effects of (CMF) chemotherapy change or remain stable at longer times since treatment.

Measurements

For the studies described in this thesis I used reference data that had been collected in the framework of the Rotterdam Study (13, 31). To ensure comparability of outcome measures, the chemotherapy-exposed breast cancer survivors completed the exact same examinations as the participants of the Rotterdam Study, at the same research center and executed by the same technicians. Hence, the examinations were not specifically selected to answer our research question. Although the Rotterdam Study includes a set of neuropsychological examinations and an extensive scan protocol, there are several neuropsychological tests and scan sequences which have proved to be sensitive to pick up the effects of chemotherapy that were not part of the study protocol of the Rotterdam Study. For example, several neuropsychological studies have identified adverse cognitive effects of chemotherapy with tests measuring visual memory, mental flexibility and working memory (82, 95-98). Furthermore, a recent study by our group in which functional MRI was used reported long-term general and cognitive task-specific hypo-activation of brain areas in chemotherapy-exposed breast cancer survivors compared to breast cancer survivors that only received local therapy (56). In addition, the Rotterdam Study does not implement an extensive and validated questionnaire on subjective cognitive functioning. Even though there is no strong correlation between subjective cognitive complaints and objective cognitive functioning (7, 37), self-reported cognitive complaints are important indications of quality of life that add to the information obtained from neuropsychological tests.

By implementing a neuropsychological test battery that is focused on cognitive problems that are frequently observed in chemotherapy-exposed breast cancer survivors, and by adding a sequence for fMRI to the study protocol, future studies on the long-term effects of chemotherapy might pick up larger and/or unidentified late effects of cytotoxic treatment.

Genetic analysis

Several candidate genes have been suggested that may be involved in the effect of cytotoxic treatment on cognitive functioning (22). Currently a number of studies on genetic determinants for chemotherapy-induced cognitive decline are being conducted (99, 100). Preliminary results of these studies show that chemotherapy-exposed patients who are homozygous for the minor allele of the MRE11A gene, or who have at least one Val allele of the Catechol-O-Methyltransferase (COMT)

gene, score lower on neuropsychological tests compared to non-chemotherapy-exposed patients or healthy control subjects. These findings could guide future studies on genetic factors that can increase the risk for chemotherapy-induced cognitive change.

Reference population

For the studies in this thesis I sampled reference subjects from the Rotterdam Study; an ongoing population-based prospective cohort study. By comparing the breast cancer patients to these reference subjects I was able to study the absolute differences in cognitive functioning and brain structure between the general population and long-term chemotherapy-exposed breast cancer survivors. However, by not including a reference group of breast cancer survivors that only received local therapy I was not able to distinguish the effects of chemotherapy and of cancer itself. Although prospective cognitive studies have showed that independently from cancer, chemotherapy is associated with cognitive functioning (94, 101, 102), some authors have suggested a direct link between cancer and neuro-degeneration (103, 104).

Adding an additional reference group of long-term survivors who had only received local therapy (i.e. surgery with or without radiotherapy), may untie the cognitive consequences of a mere history of cancer and the exposure to cancer therapies and may contribute to the understanding of unique or shared mechanisms that cause these cognitive consequences.

Population-based perspectives

Most studies that have been conducted so far had only small sample sizes which limits both the power in the study and the potential to investigate underlying mechanisms in more detail. Investigators should take advantage of the possibilities to participate in currently ongoing large cohort studies to study the chemotherapy-induced brain alterations and cognitive dysfunction and their underlying mechanisms. The Nurses' Health Study (105-107) for example is a prospective population-based cohort study among more than 100,000 women who have been followed over decades. Such a setting offers a huge potential to investigate late effects of cytotoxic treatments in cancer patients. With investigators from the Rotterdam Study (13) we have started to investigate the late effects of chemotherapy on brain structure and function in that cohort. Among participants of the Rotterdam Study, 792 cancers, including 186 breast cancers, had occurred by the end of 2004 (108). Of the majority of these patients prospective neuropsychological data is available, and for several patients there are MRI scans obtained at baseline (i.e. before the cancer diagnosis). Although the cancer survivors within the Rotterdam Study are a heterogeneous group of survivors regarding treatment (e.g. chemotherapy yes/no, regimen, dosimetry, hormonal therapy, radiotherapy) and cancer stage, in contrary to the breast cancer survivors in my studies, the Rotterdam Study offers the opportunity to prospectively investigate the adverse cognitive effects of adjuvant cytotoxic treatment, if necessary stratified by cancer type, and with the possibility to sample various reference groups including patients who only received local therapy and cancer-

free subjects. The current follow-up of MRI examinations in the Rotterdam Scan Study will ensure the longitudinal analysis of the effects of cytotoxic treatment on brain volume, white matter lesion volume, white matter microstructural integrity and cerebral blood flow (31).

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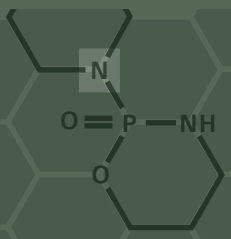


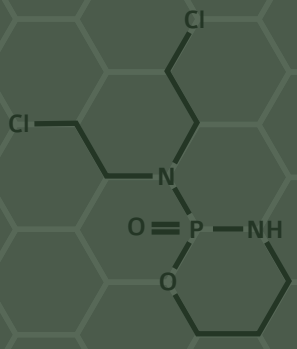
Chapter 7

Summary / Samenvatting

Dankwoord

Publicaties

PhD Portfolio



Summary

Chapter 1 introduces the background and aim of this thesis. Adjuvant chemotherapy is widely used in the treatment of breast cancer. One of its adverse effects that has gained more attention over the last years are cognitive dysfunction and brain structural alterations, which have been observed shortly up till several years after treatment. Not much is known about the long-term cerebral consequences of adjuvant chemotherapy. However, as the number of long-term survivors is increasing this topic is gaining more interest. Therefore, the objective of this study was to investigate the late effects of adjuvant cytotoxic treatment for breast cancer on brain function and structure. In order to do this we compared almost 200 breast cancer patients aged 50 to 80 years who had been treated with CMF (Cyclophosphamide, Methotrexate, 5-Fluorouracil) chemotherapy on average more than 20 years before, to a large set of at least twice as much women without a history of cancer from the Rotterdam Study; an ongoing prospective population-based cohort study. We studied cognitive functioning, brain tissue volume, cerebral white matter integrity, cerebral blood flow, and the prevalence of carotid artery plaques and incidental findings on brain MRI.

Chapter 2 gives an overview of the literature on the long-term effects of adjuvant chemotherapy on cognitive functioning, brain structural and functional alterations, and the risk of dementia. Previous studies showed that chemotherapy is associated with long-term subtle cognitive dysfunction in some, but not all patients. The effects were mainly observed in the domains of verbal memory and executive functioning. The only three neuroimaging studies in patients that were five or more years post-treatment show that cytotoxic treatment is associated with less long-term focal gray matter volume, worse global and focal white matter integrity, and hypo-activation of brain areas during cognitive tasks. The four studies conducted so far on the risk of dementia following chemotherapy showed no increased risk for dementia following cytotoxic treatment, yet had severe methodological limitations and cannot be considered as conclusive. The results from this review suggest that five or more years after cessation of treatment, various regimens of adjuvant chemotherapy are associated with impaired cognitive functioning possibly as a result of chemotherapy-induced structural brain changes.

In **Chapter 3** we compared the neuropsychological tests scores of the chemotherapy-exposed patients and the population-based reference subjects. The patients performed worse than reference subjects on neuropsychological tests of immediate and delayed verbal memory, processing speed, executive functioning and psycho-motor speed. Also on a summary measure of the neuropsychological test results that allowed for mutual correlation, the reference group significantly outperformed the breast cancer survivors. In addition, the chemotherapy-exposed survivors reported memory complaints more often. There were no differences between groups in the mean score on the mini mental state examination: a dementia screenings list. These results show that chemotherapy for breast cancer is associated with cognitive problems and memory complaints, but not with an increased prevalence of dementia.

The mechanisms by which chemotherapy may induce cognitive dysfunction are currently

under intensive investigation. One of the proposed mechanisms is direct neurotoxicity. As both methotrexate and 5-fluorouracil have proven to be able to cross the blood brain barrier in low doses, they may induce long-lasting brain structural alterations.

To investigate if adjuvant chemotherapy is associated with long-term brain structure we compared the group of chemotherapy-exposed survivors to the population-based reference subjects on several neuroimaging outcome measures, including total brain volume, total gray and white matter volume, focal gray matter volume, global and focal white matter microstructural integrity, and cerebral blood flow (**Chapter 4**). In **Chapter 4.1** we reported that CMF chemotherapy-exposed breast cancer survivors had less total brain tissue volume and less gray mater volume than the population based reference subjects. No group differences were observed in focal gray mater volume. The results did not change after correcting for potential confounders such as age, height, cardiovascular risk factors, symptoms of depression, and education level.

In addition to brain atrophy, cytotoxic treatment may also induce cerebral white matter microstructural damage. The latter can be characterized using diffusion tensor imaging (DTI), which visualizes the extent and directionality of the diffusion of water molecules within brain tissue. Generally, water diffusion in healthy white matter fiber bundles is highly anisotropic, as it is hindered by tissue architecture and therefore more prominent along the fiber direction. Increased diffusivity is often interpreted as being related to myelin damage, demyelination or axonal injury. In **Chapter 4.2** we showed that longer time since adjuvant CMF chemotherapy is associated with worse global and focal white matter microstructural integrity. We did not observe group differences in white matter integrity. A possible explanation for these results is that the group of chemotherapy-exposed cancer survivors was healthier and therefore had better white matter integrity at baseline than the reference group. This idea is supported by the fact that the breast cancer patients were taller, and were better educated than the reference subjects.

As stated, our understanding of the potential mechanisms underlying chemotherapy-induced brain structural changes is still limited. Since reductions in total cerebral blood flow have been associated with gray matter atrophy, we considered that the difference in cerebral gray matter volume that we observed between chemotherapy-exposed breast cancer survivors and reference subjects (see **Chapter 4.1**) might be due to chemotherapy-induced differences in cerebral blood flow. Flow deficits could be related to vascular damage, which is why we subsequently looked at the prevalence of carotid artery plaques. As cerebral blood flow is closely related to brain volume, we also looked at brain perfusion, defined as total cerebral blood flow divided by brain volume (× 100). In **Chapter 4.3** we showed that there were no differences between chemotherapy-exposed breast cancer survivors and reference subjects regarding cerebral blood flow, perfusion or prevalence of carotid plaques. This indicates that neither can explain the effects of CMF chemotherapy on gray matter volume that we observed.

An implication of the use of neuroimaging is the chance of discovering incidental findings, defined as previously undetected abnormalities of potential clinical relevance that are unexpectedly

discovered and are unrelated to the purpose of the specific outcome measures under study. Whether the prevalence of such abnormalities in cancer patients is similar to that in the general population is unclear. The brain scans of all participants of our studies were reviewed for incidental findings according to a predefined protocol. In **Chapter 5** we reported that within the chemotherapy-exposed breast cancer survivors three types of intracranial incidental findings were observed; aneurysms, meningiomas, and pituitary macro adenomas. The age-adjusted prevalence of only the pituitary macro adenomas was higher in the breast cancer survivors than in the population-based sample.

In **Chapter 6**, the most important conclusions and implications of the current research is discussed and summarized. Adjuvant CMF chemotherapy given for breast cancer is associated with long-term worse cognitive functioning, less total gray matter volume, and worse white matter microstructural integrity, but not with total cerebral blood flow, cerebral perfusion, or plaques in the carotid artery. By comparing chemotherapy-exposed breast cancer survivors to a reference population without a history of cancer it is not possible to distinguish the effects of chemotherapy and cancer itself. Our findings suggest that chemotherapy-induced gray matter volume reductions and worse white matter microstructural integrity may be part of the mechanisms underlying the observed long-term cognitive dysfunction. We did not observe differences in cerebral perfusion between the chemotherapy-exposed survivors and the reference group and therefore it is unlikely that chemotherapy-induced altered cerebral perfusion is causing the observed brain structural differences nor is it responsible for the differences in cognitive functioning or brain structure between the groups.

The absence of group differences on a dementia screener suggests that adjuvant CMF chemotherapy does not lead to an accelerated incidence or increased risk of dementia, at least not within the age range covered in this study.

Because even mild cognitive dysfunction is associated with lower quality of life, this outcome is important considering the large and still increasing number of breast cancer survivors. The studies in this thesis are the first that investigate the very late effects of chemotherapy on cognitive functioning. These results need replication it is justified to inform patients timely about long-term cognitive consequences of adjuvant cytotoxic treatment. This research therefore should not yet be considered by patients at the moment they have to decide whether or not to undergo chemotherapy. However, it should stimulate clinicians to be perceptive of cognitive problems in patients with a history of adjuvant CMF chemotherapy for breast cancer.

Nowadays, CMF chemotherapy is not the main choice of adjuvant therapy for invasive breast cancer anymore. Whether contemporary regimens are also associated with long-term brain structural and functional alterations, and whether these effects are similar the late effects of adjuvant CMF chemotherapy I observed is largely unknown. However, as animal studies show that cyclophosphamide and 5-fluorouracil, agents that are still widely used in contemporary regimens, are both independently associated with cognitive problems and brain structural alterations, it is

not unlikely that contemporary regimens are also associated with long-term cognitive dysfunction. The cognitive problems and brain structural alterations that have been observed in breast cancer patients treated with contemporary regimens at 10 years post-treatment add to this idea.

Furthermore, CMF chemotherapy has been the worldwide adjuvant treatment of choice for invasive breast cancer for almost 20 years up till the late nineteen nineties, a large group of breast cancer survivors who have been treated with CMF chemotherapy may experience its cognitive sequelae in the near future. In addition, as most breast cancer patients are diagnosed after the age of 60, a substantial percentage of these patients may already experience some age-related cognitive decline. Even small additional adverse cognitive effect induced by chemotherapy may thus be of clinical relevance. Therefore, the results of the studies in this thesis apply to a large proportion of the breast cancer survivor community for years to come.

Samenvatting

Chemotherapie is een veel gebruikte aanvullende behandeling na radiotherapie en chirurgie voor borstkanker. Bijwerkingen van chemotherapie waar de laatste jaren in toenemende mate aandacht aan wordt besteed zijn cognitieve achteruitgang en structurele veranderingen van het brein. Van beiden weten we inmiddels dat zij tot enkele jaren na de behandeling kunnen optreden. Of deze bijwerkingen ook op de lange termijn aanhouden is nog grotendeels onbekend. Doordat het aantal borstkanker patiënten dat lang na de diagnose nog in leven is gestaag toeneemt, is er meer aandacht gekomen voor de kwaliteit van leven –en daarmee het cognitief functioneren– van deze lange overlevers. Het doel van het onderzoek beschreven in dit proefschrift was dan ook inzicht te krijgen in de late effecten van adjuvante chemotherapie voor borstkanker op het functioneren en de structuur van het brein. Wij vergeleken bijna 200 voormalige borstkankerpatiënten tussen de 50 en 80 jaar oud die gemiddeld meer dan 20 jaar eerder werden behandeld met CMF (Cyclofosfamide, Methotrexaat, 5-Flurouracil) chemotherapie, met minimaal twee keer zoveel deelneemsters van ERGO (Erasmus Rotterdam Gezondheids Onderzoek; een prospectieve populatie studie) die nooit kanker hadden gehad. We bestudeerden het effect van chemotherapie op cognitieve functies, brein volume, cerebrale witte stof integriteit, hersendoorbloeding en de prevalentie van plaques in de carotiden. Ook keken wij naar toevalsbevindingen op MRI scans van de hersenen.

In **Hoofdstuk 2** wordt besproken wat er tot nu toe bekend is over de late effecten van chemotherapie wat betreft cognitief functioneren, de structuur van de hersenen en het risico op dementie bij kankerpatiënten behandeld met chemotherapie. Vijf voorgaande studies laten zien dat chemotherapie op de lange termijn is geassocieerd met cognitief disfunctioneren in een subgroep van patiënten. De meeste problemen worden gezien bij het verbaal geheugen en executief functioneren. De enige drie gepubliceerde beeldvormende studies naar de relatie van chemotherapie en de structuur van het brein laten zien dat vijf- of meer jaar na de behandeling, chemotherapie gerelateerd is aan minder focale grijze stof volume, slechtere globale en focale witte stof integriteit, en hypoactivatie van breingebieden tijdens het uitvoeren van cognitieve taken. De tot nu toe vier studies die de relatie onderzochten tussen chemotherapie en dementie vonden geen aanwijzingen voor een verband. Echter, door de methodologische beperkingen van deze studies moeten de resultaten met voorzichtigheid worden geïnterpreteerd.

In **Hoofdstuk 3** vergelijken we de neuropsychologische testscores van de borstkanker overlevers met die van de deelneemsters aan het ERGO onderzoek. De patiëntgroep presteerde significant slechter op tests die een beroep doen op direct en uitgesteld verbaal geheugen, verwerkingssnelheid, executief functioneren en psycho-motore snelheid, als ook op een samenvattende maat voor cognitief functioneren. Tevens rapporteerden de borstkankerpatiënten vaker geheugenproblemen dan de referentiegroep. Gemiddelde scores op een screeningslijst voor dementie verschilden niet tussen de twee groepen. De resultaten van dit onderzoek laten zien dat chemotherapie geassocieerd is met cognitieve problemen en klachten, maar niet met een verhoogd risico op dementie.

De mechanismen waardoor chemotherapie leidt tot cognitief disfunctioneren worden momenteel intensief onderzocht. Eén van de mechanismen is dat chemotherapie een direct neurotoxisch effect kan hebben op de cellen van het centrale zenuwstelsel. Aangezien zowel methotrexaat als 5-fluorouracil de bloed-hersen barrière kunnen passeren, in lage doseringen, is het mogelijk dat zij kunnen leiden tot langdurige structurele veranderingen van het brein.

Om te onderzoeken of adjuvante chemotherapie inderdaad geassocieerd is met de structuur van het brein op de lange termijn vergeleken wij de borstkankerpatiënten behandeld met chemotherapie met de eerder gedefinieerde referentie groep op totaal brein volume totaal witte en grijze stof volume, focaal grijze stof volume, focale en globale microstructurele witte stof integriteit en cerebrale doorbloeding (**Hoofdstuk 4**).

In **Hoofdstuk 4.1** rapporteerden wij dat het brein van borstkankerpatiënten die chemotherapie hadden ondergaan kleiner was dan dat van de referentie groep, en dat dit verschil grotendeels te wijten was aan het kleinere grijze stof volume van de groep overlevers. Focaal vonden wij geen verschillen in het volume van grijze stof. Deze bevindingen veranderden niet nadat we corrigeerden voor factoren die van invloed zouden kunnen zijn op breinvolume, zoals leeftijd, lengte, cardiovasculaire risicofactoren, symptomen van depressie en opleidingsniveau.

Naast volume afname leidt chemotherapie op de lange termijn mogelijk ook tot achteruitgang van de witte stof op microstructureel niveau. Dit laatste kan gemeten worden met behulp van diffusie tensor beeldvorming (DTI), waarmee de mate en richting van diffusie van water moleculen in het brein kan worden geregistreerd. In het algemeen is de diffusie van water in de witte stof anisotroop doordat het gehinderd wordt door de verzelstructuur van de witte stof: de diffusie is sterker in de richting van de vezels dan loodrecht er op. Een toename van diffusie wordt vaak geïnterpreteerd als myeline schade, demyelinisatie of axonale schade. In **Hoofdstuk 4.2** lieten we zien dat tijd sinds behandeling negatief gecorreleerd is met globale en focale witte stof integriteit. Wij vonden geen verschillen tussen de borstkankeroverlevers die chemotherapie hadden ondergaan en de referentie groep wat betreft de microstructuur van de witte stof. Een mogelijke verklaring voor deze bevindingen is dat de overlevers gezonder waren op het moment dat zij werden gediagnostiseerd met borstkanker dan de referentiepopulatie op dat moment, waardoor de integriteit van de witte stof van de overlevers initieel beter was. Dit idee wordt ondersteund door het feit dat de groep overlevers langer waren en hoger waren opgeleid dan de referentiegroep.

Zoals eerder gezegd is ons begrip van de potentiele mechanismen die verantwoordelijk zijn voor de relatie tussen chemotherapie en structurele veranderingen van het brein nog beperkt. Aangezien een afname van cerebrale doorbloeding geassocieerd is met grijze stof atrofie onderzochten wij of adjuvante CMF chemotherapie een effect heeft op de doorbloeding van de hersenen (zie **Hoofdstuk 4.1**). Omdat verminderde doorbloeding samenhangt met vasculaire schade hebben we tevens gekeken naar de prevalentie van plaques in de carotiden. Aangezien totale hersendoorbloeding erg afhankelijk is van het hersenvolume hebben we gekeken naar het effect van chemotherapie op cerebrale perfusie (totale hersendoorbloeding / brein volume × 100).

In **Hoofdstuk 4.3** laten we zien dat er geen verschillen zijn tussen de groep borstkankeroverlevers en de referentiegroep wat betreft totale hersendoorbloeding, cerebrale perfusie en prevalentie van plaques in de carotiden. Dit geeft aan dat geen van deze variabelen een mediërende rol speelt in de relatie tussen chemotherapie en het verminderd grijze stof volume die wij eerder observeerden.

Een implicatie van het gebruik van beeldvormend onderzoek is de kans op het doen van toevalsbevindingen. Deze worden gedefinieerd als eerder niet gedetecteerde abnormaliteiten die potentieel klinisch relevant zijn en onverwacht worden ontdekt bij onderzoek dat niet als doel heeft dergelijke uitkomsten te objectiveren. Of de prevalentie van zulke abnormaliteiten vergelijkbaar is tussen borstkankerpatiënten behandeld met chemotherapie en de normale bevolking, is onduidelijk. De hersenscans van alle borstkankerpatiënten die deel hebben genomen aan onze studies werden daarom volgens een vooraf opgesteld protocol gescreend op toevalsbevindingen. In **Hoofdstuk 5** rapporteren we dat binnen de groep borstkankeroverlevers er drie soorten toevalsbevindingen werden gezien: aneurysmata, meningeomen en macro adenomen van de hypofyse. Alleen de leeftijd-geadjusteerde prevalentie van hypofyse macro adenomen bleek significant hoger in de borstkankeroverlevers dan in de referentie groep afkomstig uit de populatiestudie.

In **Hoofdstuk 6** worden de belangrijkste conclusies en implicaties van het totale onderzoek besproken en samengevat. Adjuvante CMF chemotherapie gegeven voor borstkanker is op de lange termijn geassocieerd met slechtere cognitieve functies, minder grijze stof volume en slechtere integriteit van de witte stof op microstructureel niveau, maar niet met de doorbloeding van de hersenen of de prevalentie van plaques in de carotiden. Doordat wij in dit onderzoek borstkanker patiënten die waren behandeld met chemotherapie vergeleken met een groep vrouwen uit de gewone populatie die nooit kanker hadden gehad was het onmogelijk om onderscheid te maken tussen het effect van chemotherapie en kanker op zich. Onze bevindingen suggereren dat verminderd grijze stof volume als gevolg van chemotherapie en de achteruitgang van de witte stof integriteit mogelijk ten grondslag liggen aan de cognitieve problemen op lange termijn die wij observeerden. Aangezien wij geen verschillen vonden in de doorbloeding van het brein tussen de borstkankeroverlevers en de referentiegroep is het onwaarschijnlijk dat veranderingen in de doorbloeding van de hersenen als gevolg van chemotherapie de oorzaak zijn van de door ons geobserveerde associatie tussen chemotherapie, de structuur van het brein en het cognitief functioneren. De afwezigheid van groepsverschillen wat betreft scores op de mini mental state examination, een screeningslijst voor dementie, wijst er op dat adjuvante CMF chemotherapie niet leidt tot een verhoogd risico op dementie, tenminste niet bij vrouwen in de leeftijd van 50 tot 80 jaar.

Aangezien zelfs subtiele cognitieve problemen geassocieerd zijn met een lagere kwaliteit van leven zijn onze bevindingen belangrijk voor de grote en toenemende groep borstkankeroverlevers. De studies in dit proefschrift zijn de eerste die hebben gekeken naar de zeer late effecten van adjuvante chemotherapie en moeten dan ook worden gerepliceerd alvorens het gerechtvaardigd is om patiënten vroegtijdig voor te lichten. Dit onderzoek zou dan ook niet moeten leiden tot het informeren van patiënten over de mogelijke cognitieve bijwerkingen van chemotherapie op lange

termijn in de beslissingsfase voor chemotherapie. Wel zou het clinici aan moeten zetten oplettend te zijn op cognitieve problemen bij borstkankeroverlevers met een voorgeschiedenis van adjuvante CMF chemotherapie.

Adjuvante CMF chemotherapie is niet meer de standaard behandeling voor borstkanker en het is nog onduidelijk of de hedendaagse cytotoxische therapieën vergelijkbare cognitieve effecten op de lange termijn teweeg brengen. Aangezien dierstudies laten zien dat cyclofosfamide en 5-fluorouracil –componenten die ook in hedendaagse combinatietherapieën zijn geïmplementeerd– beiden afzonderlijk zijn geassocieerd met cognitieve problemen en veranderingen in het brein, is het niet ondenkbaar dat ook de huidige chemotherapie is geassocieerd met cognitieve problemen op de lange termijn. Dat cognitieve problemen en structurele veranderingen in het brein die zijn geobserveerd in borstkankeroverlevers die bijna 10 jaar eerder werden behandeld met hedendaagse chemotherapie sluit hierbij aan.

Adjuvante CMF chemotherapie is tot eind jaren negentig veelvuldig gebruikt. Hierdoor zijn er nog veel vrouwen die mogelijk de late cognitieve effecten van deze behandeling zullen ervaren. De meeste borstkankerpatiënten worden gediagnostiseerd na het zestigste levensjaar, wanneer leeftijds-gerelateerde cognitieve achteruitgang mogelijk al een rol speelt. Elke additioneel nadelig effect van chemotherapie kan daardoor van klinische relevantie zijn. Mede hierdoor zijn de resultaten van dit onderzoek de komende jaren van toepassing op de grote groep borstkankeroverlevers.

Dankwoord

De afgelopen vier jaar heb ik mij intensief bezig gehouden met het onderzoek dat wordt beschreven in dit proefschrift. Al het werk heb ik niet alleen verricht en er zijn velen die ik wil bedanken voor hun begeleiding, bijdragen en ondersteuning.

In de eerste plaats wil ik mijn promotor, Prof.dr. Breteler, en copromotor Dr. Schagen bedanken voor de mogelijkheid die zij mij hebben geboden om met dit onderzoek te starten en voor hun intensieve begeleiding gedurende de afgelopen jaren.

Beste Monique, bedankt voor je stimulerende houding, je kritische blik, maar zeker ook de aanvulling op mijn onderzoek en schrijfsels: ik heb veel bewondering voor de ogenschijnlijke eenvoud waarmee je met een pennenstreek de essentie en relevantie van een stuk weet te benadrukken. Ik hoop ook bij het 'verkopen' van onze laatste papers nog veel van je te leren en wens je het allerbeste in Bonn.

Beste Sanne, bedankt voor je persoonlijke benadering, je waardering, geduld en vertrouwen en de vele uren die je in me hebt geïnvesteerd, ongeacht of dat te maken had met mijn promotieonderzoek, of zaken daarbuiten. Jouw kennis omtrent de literatuur en neuropsychologie lijken haast onuitputtelijk en het was dan ook een plezier om met je over deze zaken te discussiëren.

Dr. de Ruiter, beste Michiel, zonder jou zou ik waarschijnlijk nooit aan dit mooie onderzoek begonnen zijn. Al voordat ik als promovendus met jou samenwerkte begeleide jij mij bij beeldvormend onderzoek en bovenal tipte je mij op het juiste moment over de promotieplek die uiteindelijk uitmondde in dit proefschrift. Over de jaren ben je meer dan alleen een collega gebleken. Dank hiervoor!

Prof.dr. Aad van der Lugt en Prof.dr. Martin van den Bent wil ik graag bedanken voor hun zitting in de leescommissie en de bereidheid te opponeren tijdens de openbare verdediging van mijn proefschrift. I would like to thank Prof.dr. Francine Grodstein for taking part in my thesis-committee and for travelling all the way to the Netherlands for my public defense. Daarnaast wil ik Prof.dr. Frits van Dam, Prof.dr. Roy Kessels en Dr. Caroline Seynaeve bedanken voor het nemen van zitting in mijn promotiecommissie.

De artikelen die ik over de jaren heb geschreven kennen naast mijn promotor en co-promotor veel andere co-auteurs die ik graag zou bedanken voor hun input. Prof.dr. Aad van der Lugt, Dr. Fedde van der Lijn, Prof.dr.ir. Floor van Leeuwen, Dr. Henri Vrooman, Prof.dr. Jacqueline Witteman, Dr. Mariëlle Poels, Maryam Kavousi, Dr. Meike Vernooij en Prof.dr. Wiro Niessen: de oncologische, radiologische, epidemiologische en statistische input die ik van jullie heb gekregen hebben mijn kennis verbreed en hebben mij ongetwijfeld behoed voor lastige vragen van reviewers. Een aantal van mijn co-auteurs zou ik graag in het bijzonder bedanken: Dr. Willem Boogerd, bedankt voor je enthousiasme en interesse in mijn data en papers. De drukte in de kliniek heeft je er nooit van weerhouden mee te denken wanneer dat nodig was. Dr. Caroline Seynaeve, bedankt voor het zo

zorgvuldig doorlezen en becommentariëren van mijn manuscripten en voor de tijd die je nam, ook in vakanties, om jouw bijdragen mondeling toe te lichten. Marius de Groot, bedankt voor de intensieve samenwerking. Ik heb veel van je geleerd wat betreft beeldvormend onderzoek en alles wat daarbij komt kijken. Als entiteit op het gebied van TBSS en met je perfectionistische werkwijze heb je in grote mate bijgedragen aan ons manuscript. Ik heb het enorm leuk gevonden om juist in deze fase samen naar Human Brain Mapping in Canada te kunnen gaan. Chad Gundy, beste Chad, je betrokkenheid en jouw welwillendheid om statistische principes uitgebreid en geanimeerd toe te lichten hebben mij enthousiast gemaakt voor statistiek. Met plezier en interesse heb ik geprobeerd zoveel mogelijk van je op te steken. Wat is het een gemis voor mij, voor onze afdeling en voor het onderzoek in het algemeen dat jij er nu niet meer bent. Ik ben blij dat jouw laatste publicatie met mij in zo'n mooi blad is verschenen!

I am very grateful for the significant amount of time that Marek Molas, Dr. Michael Schaapveld and Dr. Jacobien Kieffer dedicated to my statistical questions and I would like to emphasize how much I enjoyed and learned from our discussions.

Het afdelingshoofd van de afdeling Epidemiologie van het Erasmus MC, Prof.dr. Albert Hofman, en de afdelingshoofden van de afdeling Psychosociaal Onderzoek en Epidemiologie van het Nederlands Kanker Instituut, Prof.dr. Neil Aaronson en Prof.dr.ir. Flora van Leeuwen, wil ik bedanken voor hun toegankelijkheid. Ondanks jullie drukke schema's was er altijd tijd voor vragen, en niet minder belangrijk: antwoorden. Als opvolgend hoofd van de neuro-epidemiologie vakgroep wil ik ook Dr. Arfan Ikram bedanken voor het meedenken over mijn onderzoek: het was fijn dat je deur altijd open stond!

Mijn speciale dank gaat uit naar alle deelnemers van het ERGO onderzoek en in het bijzonder de deelneemsters van mijn eigen onderzoek. Hun belangeloze bijdrage aan mijn onderzoek en de moeite die zij deden om in grote getalen vanuit heel Nederland naar Rotterdam te komen heeft dit onderzoek tot een succes gemaakt.

Al deze deelnemers zijn geïnterviewd, gescand en onderzocht door de medewerkers van het ERGO centrum. In het bijzonder Anneke Korving en Henriette Ensing, maar zeker ook alle andere ERGO medewerkers die hard hebben gewerkt om de inclusie zo soepel en succesvol te laten verlopen, namelijk: Ada Hooghart, Andy van Oosterhout, Bernardina Scheffers, Charlotte Bruidegom, Corina Brussee, Dorien Looren de Jong, Edith Nolet, Hannie van den Boogert, Inge Haumersen, Lydia Buist, Monie Broos, Pauli Eldik, Saskia Hoornweg, Toos Stehman en Trudy Groenewegen, ben ik mijn dank verschuldigd. Als niet ERGO medewerkers wil ik Germaine Verwoert bedanken voor haar bijdrage aan de dataverzameling en Dick Slof voor het vervoeren van een groot aantal deelneemsters vanuit heel Nederland naar Rotterdam en weer terug.

Om het protocol van mijn onderzoek, dat afweek van het ERGO studie protocol, in te passen in de dagelijkse workflow van ERGO heb ik nauw samengewerkt met Dr. Jan Heeringa, Jeanette Vergeer, en de technische mensen van kamer Ee21-91: René Molhoek, Frank van Rooij, Jolande Verkroost en René Vermeeren. Ik wil hen allen hartelijk bedanken voor het plannen van de 'NKI'

dagen in het ERGO rooster, het aanpassen van Pontiac schermen, het beheren van de data en het verzorgen van de stapels testmateriaal.

Naast hen hebben Ellen Crepin, Gabey Ouwens, Jannet Blom, Marianne Kuenen en Nano Suwarno mij belangeloos geholpen met het opstarten van mijn onderzoek. Hiervoor wil ik ze van harte bedanken.

De dames van zowel het Amsterdamse als het Rotterdamse secretariaat, Ellen Keiner, Erica Kroos, Esther Bruining, Hetty Gerritsen, Jacqueline Arnell, Marjon Boltjes en Yvonne Driessen wil ik bedanken voor hun behulpzaamheid.

Als onderzoeker werkzaam op twee afdelingen ben ik veel tijd kwijt geweest met reizen en waren er bijna twee keer zo veel vergaderingen. Daar stond tegenover dat er twee keer zoveel borrels waren, er vaker taart was (mits op de juiste plek op het juiste moment) en dat ik dubbel zo veel collega's had. Met name de vele collega onderzoekers van beide instituten hebben er voor gezorgd dat ik het op beide plekken naar mijn zin heb gehad. Een aantal van hen wil ik in het bijzonder bedanken:

Ten eerste Elisabeth Schrijvers en Renske Wieberdink, mijn kamergenoten van kamer Ee21-79, met wie ik drie en een half jaar het hele promotietraject heb mogen doorlopen. Het was erg prettig om met z'n drieën steeds in min of meer dezelfde fase te zitten. Het voorbereiden van NIHES tentamens en de perikelen van het schrijven van artikelen was allemaal veel aangenamer met jullie.

Elisabeth, als veruit de meest sociale promovendi van de afdeling –wat ook blijkt uit het feit dat jij de afdelingspsycholoog was, en niet ik– was het prettig om bij jou op de kamer te zitten. Ik ben nog steeds onder de indruk van de hoeveelheid werk die jij hebt verzet in zo weinig tijd. Nu je niet meer op onze afdeling bent zie ik je een stuk minder en ik kom je dan ook graag snel een keer bezoeken, op voorwaarde dat je die lekkere risotto maakt!

Renske, met veel plezier denk ik terug aan onze gesprekken over van alles maar zeker het terugkerende onderwerp: hoe schoon en opgeruimd een huis eigenlijk hoort te

Op gelijke hoogte staan Sanne Menning en Myrle (Kempi) Kemperman. Jullie kwamen mijn kamer op het NKI veraangenamen nadat ik lange tijd grotendeels alleen O.3.009 had bewoond. Met zelf gebreide monsters (Gorgé), baksels, haakwerk en foto's overal maakten jullie er een heel gezellige kamer van. Verjaardagen werden nooit vergeten, in tegendeel: jullie maakten mijn eigen super-V snuggie en na congressen namen jullie standaard iets voor me mee. Zo attent als dat jullie zijn kom je niet vaak tegen. Met plezier kijk ik ook terug op de etentjes buiten werktijd bij een van ons thuis.

Sanne, onze trip naar 'the big apple' was één van de hoogtepunten van mijn promotieonderzoek. Ik heb er van genoten om samen met jou heel Manhattan te doorkruisen op zoek naar sneakers en burgers met als kers op de taart 'the Baconator' (foto's op te vragen bij de auteur). Ik hoop dat je dataverzameling snel klaar is en weet zeker dat je hele mooie artikelen gaat schrijven.

Myrle, jij was de perfecte aanvulling

zijn. Bijzonder dankbaar ben ik je voor het eindeloze geduld waarmee je me regelmatig hebt bijgespijkerd in anatomie. Ik ben ervan overtuigd dat je een fantastische neuroloog zult worden als je je net zo bekommert om je patiënten als dat je dat deed om onze plantjes.

Mariëlle Poels, door jouw uitstapjes tijdens je promotieonderzoek liepen we juist in de laatste fase, toen Elisabeth en Renske al van de afdeling waren verdwenen, wat meer synchroon. Ik ben blij dat ik nog zoveel dingen van je heb mogen afkijken in deze periode.

De overige leden van de neuro-groep: Benjamin Verhaaren, Daniel Bos, Dymph Mesker, Elisabeth Devore, Elizabeth Loehrer, Evert van Velsen, Hieab Adams, Jory Hoogendam, Joyce van den Ende, Kèren Zaccai, Meike Vernooij, Mendel Haag, Michiel Bos, Renée de Bruijn, Saloua Akoudad, Sjoerd Euser, Tom den Heijer en Vincent Verlinden, mijn gewaardeerde collega's van de Epidemiologie afdeling, te weten: Abbas Dehghan, Bouwe Krijthe, Daan Loth, Eline Rodenburg, Gabriëlle Buitendijk, Germaine Verwoert, Karin Hek, Lintje Ho, Mariana Selwaness, Mark Eigelsheim, Maryam Kavousi, Milad Solouki, Monica Czudowska, Quirijn van den Bouwhuijsen, Rikje Ruiter, Virginie Verhoeven en Wishal Ramdas, en de fijne mensen van de beeldverwerkers groep: Fedde van der Lijn, Marius de Groot, Renske de Boer, Sandra de Bie en Vera Valkhof wil ik van harte bedanken voor de gezelligheid tijdens lunches, borrels, en promotiefeestjes.

voor onze kamer, niet alleen vanwege je onderzoeksonderwerp, maar juist ook vanwege onze gezamenlijke interesses: als Apple-fan-girl, jaren '80 PC junkie en Hagenees hadden we veel om over te praten en konden we af en toe een trein delen. Ik wens je veel succes met het afronden van je onderzoek!

Vele anderen maakten de PSOE voor mij een gezellige thuisbasis. Naomi Boekel en Sandra van den Broek, jullie begonnen ongeveer rond dezelfde tijd met promoveren en waren altijd in voor epidemiologische vraagstukken of gewoon een praatje. Naomi, bedankt ook voor je immer snelle reacties op al mijn vragen omtrent jouw onderzoekspopulatie, de geschiedenis van de behandeling van het mammacarcinoom, maar ook de trips naar de Albert Heijn. Willem, jij bent de lijm van de afdeling. Met koekjes en praatjes heb je mij en vele anderen op je kamer gelokt voor een goed gesprek in een ontspannen sfeer. Bedankt daarvoor. Ook Anja van Eggermond, Anouk Pijpe, Chantal Lammens, Cherita Sombroek, Christien Schilder, Grace Sigharta, Hanna van Waart, Harmke Groot, Heleen Feenstra, Jacobien Kieffer, Kirsten Douma, Mandy Spaan, Marie-José Blom, Marieke van Leeuwen, Marijke Wevers, Martijn Verheus, Miranda Gerritsma, Rianne van Nimwegen, Riejanne Seigers, Saskia Duijts, Saskia Pelders, Susanne Rebers en Wim Groen wil ik bedanken voor de samenwerking en/of gezelligheid tijdens de vrijdagmiddag borrels, uitjes, kerst lunches, of zomaar tussendoor.

De keuze voor mijn paranimfen Benjamin Verhaaren en Remi Soleman was een eenvoudige. Ik ben blij ben dat jullie naast mij staan op 20 april.

Twee personen die niet mogen ontbreken in dit dankwoord zijn Prof.dr. Robert Schoevers en

Prof.dr. Jack Dekker. Zij introduceerden mij in de wetenschap, betrokken mij bij publicaties en namen me mee naar congressen en symposia. Hiervoor ben ik ze enorm dankbaar.

Familieleden en vrienden die de afgelopen jaren dan wel geen inhoudelijke rol hebben gespeeld, maar die zeker hebben bijgedragen aan het tot stand komen van dit proefschrift en die ik graag bij naam zou noemen zijn: Benjamin Verhaaren, Erik van Dongen, Jeroen Altena, Jordi Simons, Peter Alles, Remi Soleman, Ricky Booms en Roeland Koppelmans. Bedankt voor jullie interesse, de afleidende avonden en weekenden, de goede gesprekken en bovenal de gezelligheid! Voor jullie allemaal geldt dat we elkaar te weinig zie, maar dat wanneer we afspreken het altijd goed voelt. Ik hoop jullie nog velen jaren als vrienden te hebben. Ook de familie en vrienden van Stefanie wil ik bedanken voor hun interesse in mijn onderzoek.

René, Anne, Marijn en Imme bedank ik voor hun interesse en vertrouwen. René, het voelt goed om iemand te hebben die achter je staat. Pa en Ma, bedankt voor jullie vertrouwen en die stimulerende omgeving waarin ik ben opgegroeid. Jullie leerden mij dat met doorzettingsvermogen en ordelijkheid er veel te bereiken is. Dit boekje is een beetje voor jullie.

Lieve Stefanie, jij haalt het beste in me boven en laat me dingen vanuit een ander perspectief zien. Mijn vrije tijd deel ik het liefst met jou, alhoewel dat zeker de laatste tijd misschien anders leek. Jouw reislust brengt me op plekken waar ik anders nooit zou zijn geweest en ik zie er naar uit met jou in een nieuw avontuur te duiken in de VS!

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Koppelmans V, de Groot M, de Ruiter MB, Boogerd W, Seynaeve C, Vernooij MW, Niessen WJ, Schagen SB, Breteler MM. Global and focal white matter integrity in breast cancer survivors 20 years after adjuvant chemotherapy. *Submitted*.

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PhD Portfolio - Summary of PhD training and teaching activities

Name	V. Koppelmans
Erasmus MC Department	Epidemiology
Research School	NIHES
Supervisors	Prof. dr. M.M.B. Breteler, Dr. S.B. Schagen

Research Skills

2008-2010 MSc in Clinical Epidemiology, Netherlands Institute for Health Sciences, Erasmus University Rotterdam, the Netherlands (including courses on methodology, study design, and statistical analysis)

General Academic Skills

2010 Biomedical English Writing and Communication, Erasmus MC, Rotterdam, the Netherlands

In-depth courses

2008 Introduction into clinical and fundamental oncology, Dutch Society for Oncology (NVvO)

National and international conferences

2012 International Cancer and Cognition Task Force, Paris, France -
oral presentation: Effects of adjuvant chemotherapy for breast cancer on brain structure more than 20 years post-treatment

2012 30e Oncologiedagen voor verpleegkundigen, Ede, the Netherlands -
oral presentation: Cognitive problems after chemotherapy

2011 Human Brain Mapping, Quebec, Canada -
poster presentation: Late effects of chemotherapy on brain functioning in the elderly: Incidental findings on brain MRI

2010 Two Faces of Evil: Cancer and Neurodegeneration, Paris, France

2010 International Cancer and Cognition Task Force, New York, US -
poster presentation: Late effects of chemotherapy on brain functioning in the elderly: Incidental findings on brain MRI

2008 International Cancer and Cognition Task Force, Amsterdam, the Netherlands

2008 Research Institute of Diseases in the Elderly symposium, Amsterdam, the Netherlands

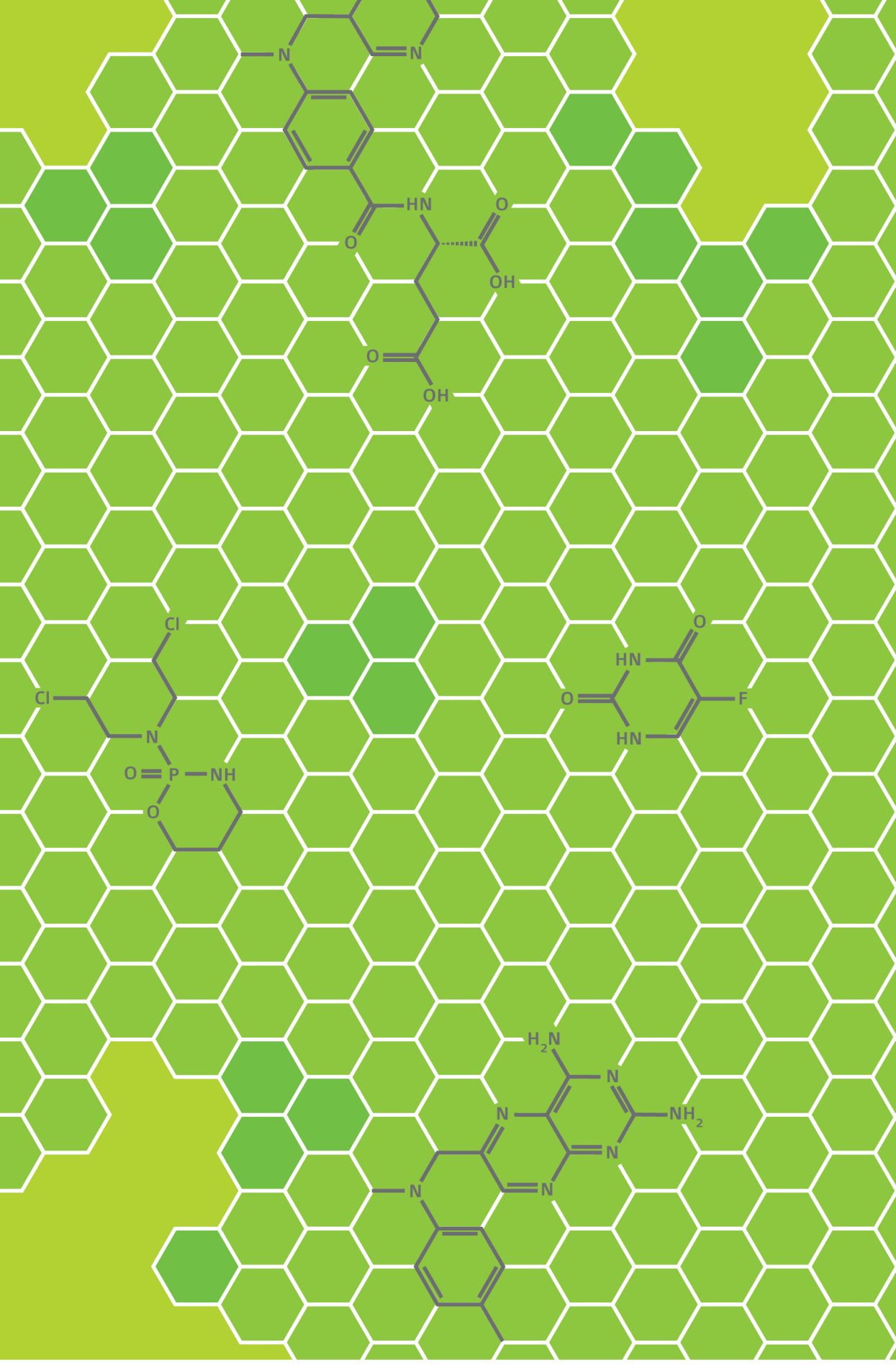
Teaching activities

2011 Supervising practicals in Study Design at NIHES, Erasmus MC, Rotterdam, the Netherlands

2009-2011 Supervising Master students in writing their thesis:
- Anna de Jong - Thesis title: "Long-term effects of CMF chemotherapy on cognitive functioning in breast cancer survivors"
- Kirsten Diek - Thesis title: "Selection bias in a study on long term cognitive effects of chemotherapy in breast cancer survivors"

2008-2010 Teaching practicals in epidemiology to 4th year medical students, Erasmus MC, Rotterdam, the Netherlands

NOTE .....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