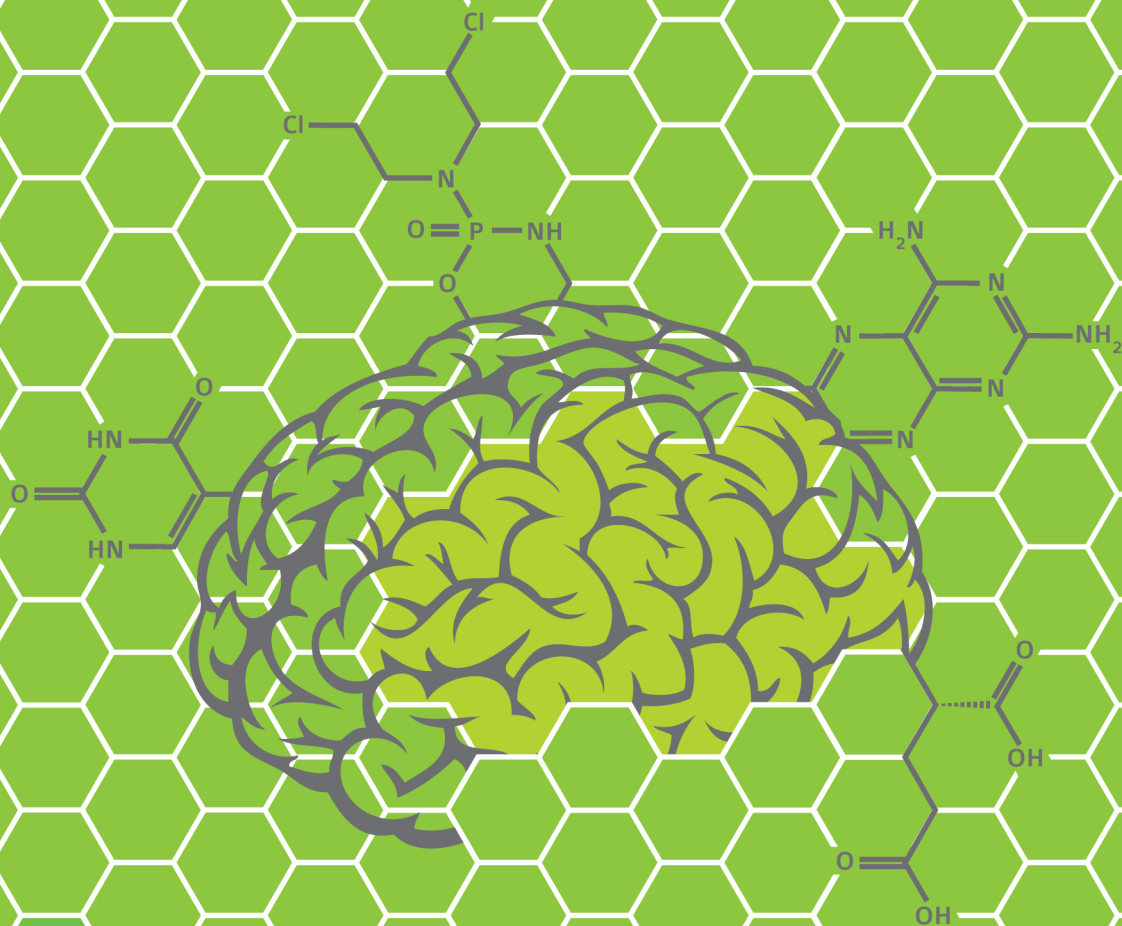# Late Effects of Adjuvant Chemotherapy on Brain Function and Structure

Vincent Koppelmans

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

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie:

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

Overige leden: Prof.dr. A. van der Lugt
                  Prof.dr. M.J. van den Bent
                  Prof.dr. F.D. Grodstein
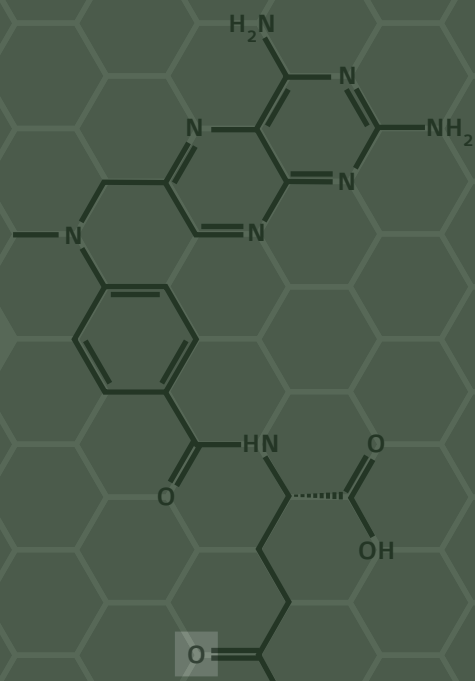
Copromotor: Dr. S.B. Schagen

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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
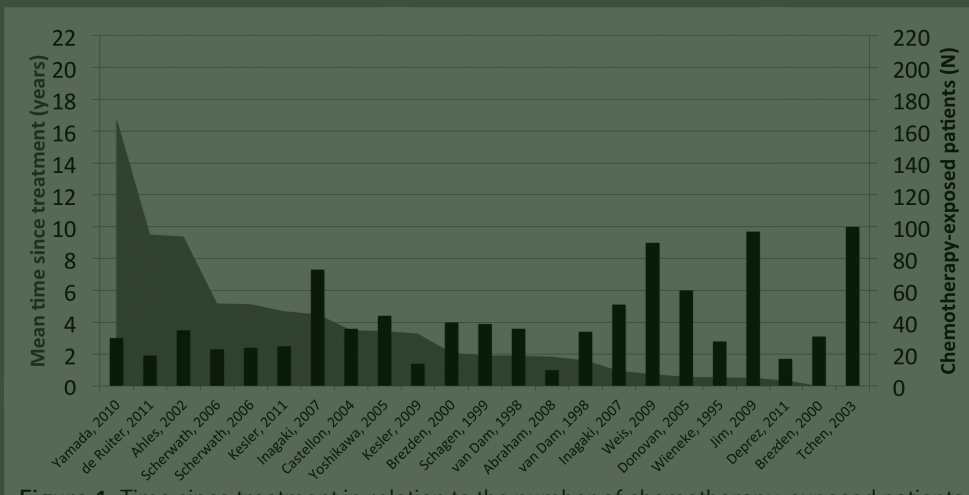

Figure 1. Time since treatment in relation to the number of chemotherapy-exposed patients in cross-sectional studies on the effects of chemotherapy on cognitive functioning.
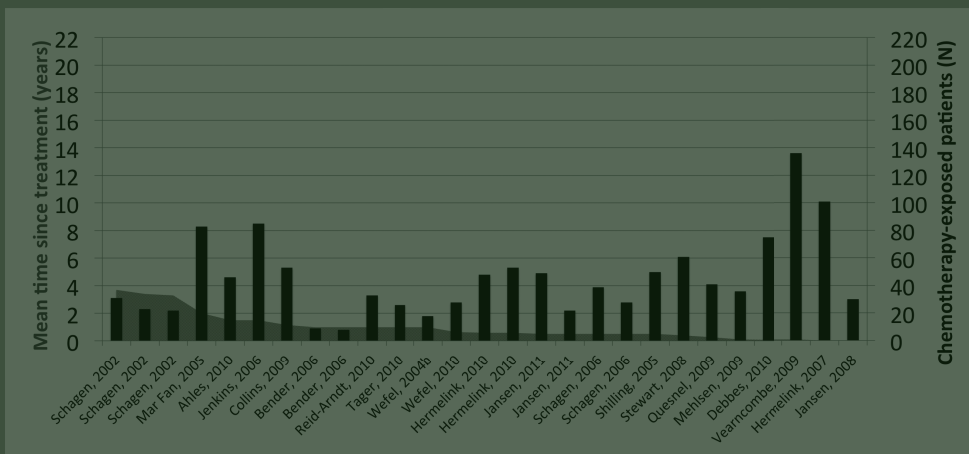

Figure 2. Time since treatment in relation to the number of chemotherapy-exposed patients in prospective studies on the effects of chemotherapy on cognitive functioning.

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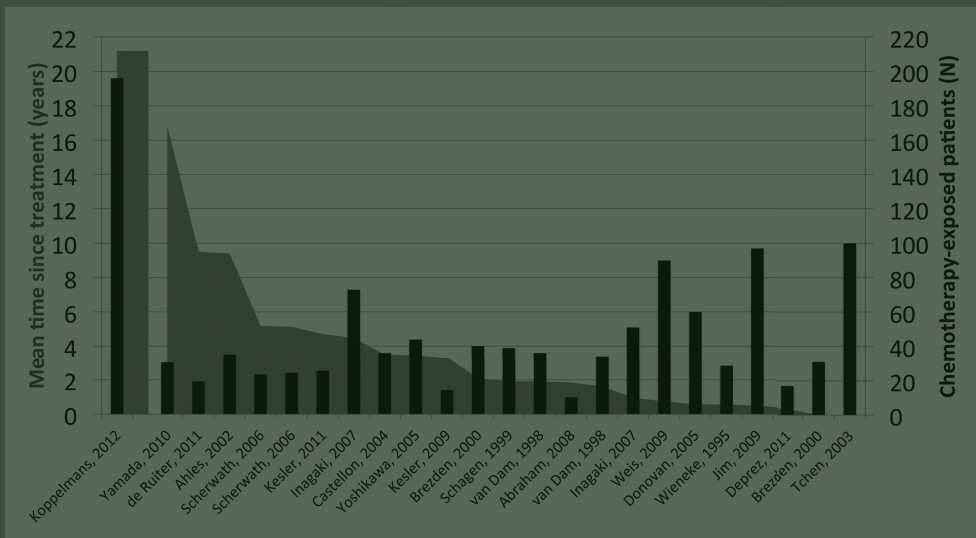
Figure 3. Positioning of the current study among published cross-sectional studies on the late effects of chemotherapy on cognitive functioning

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

Chapter 1

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.

References

1. Comprehensive Cancer Centres. Incidence and mortality of breast cancer in the Netherlands. http://www.cijfersoverkanker.nl. September 7, 2011

2. Cancer Facts and Figures 2011. Atlanta, Georgia: American Cancer Society; 2011.

3. Howel D. Trends in the prevalence of obesity and overweight in English adults by age and birth cohort, 1991-2006. Public Health Nutr 2011;14(1):27-33.

4. Porter PL. Global trends in breast cancer incidence and mortality. Salud Publica Mex 2009;51 Suppl 2:s141-6.

5. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371(9612):569-78.

6. Schokker DF, Visscher TL, Nooyens AC, van Baak MA, Seidell JC. Prevalence of overweight and obesity in the Netherlands. Obes Rev 2007;8(2):101-8.

7. Autier P, Boniol M, Gavin A, Vatten LJ. Breast cancer mortality in neighbouring European countries with different levels of screening but similar access to treatment: trend analysis of WHO mortality database. BMJ 2011;343:d4411.

8. Sant M, Francisci S, Capocaccia R, Verdecchia A, Allemani C, Berrino F. Time trends of breast cancer survival in Europe in relation to incidence and mortality. Int J Cancer 2006;119(10):2417-22.

9. van Schoor G, Moss SM, Otten JD, Donders R, Paap E, den Heeten GJ, Holland R, Broeders MJ, Verbeek AL. Increasingly strong reduction in breast cancer mortality due to screening. Br J Cancer 2011;104(6):910-4.

10. Sjostrom J. Predictive factors for response to chemotherapy in advanced breast cancer. Acta oncologica 2002;41(4):334-45.

11. NABON. Mammacarcinoom - Landelijke richtlijn met regionale toevoegingen - Versie 1.1. In; 2010.

12. Vodermaier A. Breast cancer treatment and cognitive function: the current state of evidence, underlying mechanisms and potential treatments. Women's health 2009;5(5):503-16.

13. Wieneke MH, Dienst ER. Neuropsychological assessment of cognitive functioning following chemotherapy for breast cancer. Psycho-oncology 1995;4:61-66.

14. van Dam FS, Schagen SB, Muller MJ, Boogerd W, vd Wall E, Droogleever Fortuyn ME, Rodenhuis S. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. Journal of the National Cancer Institute 1998;90(3):210-8.

15. Schagen SB, van Dam FS, Muller MJ, Boogerd W, Lindeboom J, Bruning PF. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer 1999;85(3):640-50.

16. Brezden CB, Phillips KA, Abdolell M, Bunston T, Tannock IF. Cognitive function in breast cancer patients receiving adjuvant chemotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2000;18(14):2695-701.

17. Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, Whedon MB, Bivens S, Mitchell T, Greenberg ER, Silberfarb PM. Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2002;20(2):485-93.

18. Schagen SB, Muller MJ, Boogerd W, Rosenbrand RM, van Rhijn D, Rodenhuis S, van Dam FS. Late effects of adjuvant chemotherapy on cognitive function: a follow-up study in breast cancer patients. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2002;13(9):1387-97.

19. Tchen N, Juffs HG, Downie FP, Yi QL, Hu H, Chemerynsky I, Clemons M, Crump M, Goss PE, Warr D, Tweedale ME, Tannock IF. Cognitive function, fatigue, and menopausal symptoms in women receiving adjuvant chemotherapy for breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21(22):4175-83.

20. Castellon SA, Ganz PA, Bower JE, Petersen L, Abraham L, Greendale GA. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. Journal of clinical and experimental neuropsychology 2004;26(7):955-69.

21. Wefel JS, Lenzi R, Theriault RL, Davis RN, Meyers CA. The cognitive sequelae of standard-dose adjuvant chemotherapy in women with breast carcinoma: results of a prospective, randomized, longitudinal trial. Cancer 2004;100(11):2292-9.

22. Donovan KA, Small BJ, Andrykowski MA, Schmitt FA, Munster P, Jacobsen PB. Cognitive functioning after adjuvant chemotherapy and/or radiotherapy for early-stage breast carcinoma. Cancer 2005;104(11):2499-507.

23. Mar Fan HG, Houede-Tchen N, Yi QL, Chemerynsky I, Downie FP, Sabate K, Tannock IF. Fatigue, menopausal symptoms, and cognitive function in women after adjuvant chemotherapy for breast cancer: 1- and 2-year follow-up of a prospective controlled study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23(31):8025-32.

24. Shilling V, Jenkins V, Morris R, Deutsch G, Bloomfield D. The effects of adjuvant chemotherapy on cognition in women with breast cancer-- preliminary results of an observational longitudinal study. Breast 2005;14(2):142-50.

25. Yoshikawa E, Matsuoka Y, Inagaki M, Nakano T, Akechi T, Kobayakawa M, Fujimori M, Nakaya N, Akizuki N, Imoto S, Murakami K, Uchitomi Y. No adverse effects of adjuvant chemotherapy on hippocampal volume in Japanese breast cancer survivors. Breast cancer research and treatment 2005;92(1):81-4.

26. Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, Paraska KK, Ryan CM. Cognitive

impairment associated with adjuvant therapy in breast cancer. Psycho-oncology 2006;15(5):422-30.

27. Jenkins V, Shilling V, Deutsch G, Bloomfield D, Morris R, Allan S, Bishop H, Hodson N, Mitra S, Sadler G, Shah E, Stein R, Whitehead S, Winstanley J. A 3-year prospective study of the effects of adjuvant treatments on cognition in women with early stage breast cancer. British journal of cancer 2006;94(6):828-34.

28. Schagen SB, Muller MJ, Boogerd W, Mellenbergh GJ, van Dam FS. Change in cognitive function after chemotherapy: a prospective longitudinal study in breast cancer patients. Journal of the National Cancer Institute 2006;98(23):1742-5.

29. Scherwath A, Mehnert A, Schleimer B, Schirmer L, Fehlauer F, Kreienberg R, Metzner B, Thiel E, Zander AR, Schulz-Kindermann F, Koch U. Neuropsychological function in high-risk breast cancer survivors after stem-cell supported high-dose therapy versus standard-dose chemotherapy: evaluation of long-term treatment effects. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2006;17(3):415-23.

30. Hermelink K, Untch M, Lux MP, Kreienberg R, Beck T, Bauerfeind I, Munzel K. Cognitive function during neoadjuvant chemotherapy for breast cancer: results of a prospective, multicenter, longitudinal study. Cancer 2007;109(9):1905-13.

31. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 2007;109(1):146-56.

32. Abraham J, Haut MW, Moran MT, Filburn S, Lemiuex S, Kuwabara H. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 2008;8(1):88-91.

33. Jansen CE, Dodd MJ, Miaskowski CA, Dowling GA, Kramer J. Preliminary results of a longitudinal study of changes in cognitive function in breast

cancer patients undergoing chemotherapy with doxorubicin and cyclophosphamide. Psycho-oncology 2008;17(12):1189-95.

34. Stewart A, Collins B, Mackenzie J, Tomiak E, Verma S, Bielajew C. The cognitive effects of adjuvant chemotherapy in early stage breast cancer: a prospective study. Psycho-oncology 2008;17(2):122-30.

35. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. Psycho-oncology 2009;18(2):134-43.

36. Jim HS, Donovan KA, Small BJ, Andrykowski MA, Munster PN, Jacobsen PB. Cognitive functioning in breast cancer survivors: a controlled comparison. Cancer 2009;115(8):1776-83.

37. Kesler SR, Bennett FC, Mahaffey ML, Spiegel D. Regional brain activation during verbal declarative memory in metastatic breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15(21):6665-73.

38. Mehlsen M, Pedersen AD, Jensen AB, Zachariae R. No indications of cognitive side-effects in a prospective study of breast cancer patients receiving adjuvant chemotherapy. Psycho-oncology 2009;18(3):248-57.

39. Ouimet LA, Stewart A, Collins B, Schindler D, Bielajew C. Measuring neuropsychological change following breast cancer treatment: an analysis of statistical models. Journal of clinical and experimental neuropsychology 2009;31(1):73-89.

40. Quesnel C, Savard J, Ivers H. Cognitive impairments associated with breast cancer treatments: results from a longitudinal study. Breast cancer research and treatment 2009;116(1):113-23.

41. Vearncombe KJ, Rolfe M, Wright M, Pachana NA, Andrew B, Beadle G. Predictors of cognitive decline after chemotherapy in breast cancer patients. Journal of the International Neuropsychological Society : JINS 2009;15(6):951-62.

Chapter 1

42. Weis J, Poppelreuter M, Bartsch HH. Cognitive deficits as long-term side-effects of adjuvant therapy in breast cancer patients: 'subjective' complaints and 'objective' neuropsychological test results. Psycho-oncology 2009;18(7):775-82.

43. Ahles TA, Saykin AJ, McDonald BC, Li Y, Furstenberg CT, Hanscom BS, Mulrooney TJ, Schwartz GN, Kaufman PA. Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28(29):4434-40.

44. Debess J, Riis JO, Engebjerg MC, Ewertz M. Cognitive function after adjuvant treatment for early breast cancer: a population-based longitudinal study. Breast cancer research and treatment 2010;121(1):91-100.

45. Hermelink K, Kuchenhoff H, Untch M, Bauerfeind I, Lux MP, Buhner M, Manitz J, Fensterer V, Munzel K. Two different sides of 'chemobrain': determinants and nondeterminants of self-perceived cognitive dysfunction in a prospective, randomized, multicenter study. Psycho-oncology 2010;19(12):1321-8.

46. Reid-Arndt SA, Hsieh C, Perry MC. Neuropsychological functioning and quality of life during the first year after completing chemotherapy for breast cancer. Psycho-oncology 2010;19(5):535-44.

47. Tager FA, McKinley PS, Schnabel FR, El-Tamer M, Cheung YK, Fang Y, Golden CR, Frosch ME, Habif U, Mulligan MM, Chen IS, Hershman DL. The cognitive effects of chemotherapy in post-menopausal breast cancer patients: a controlled longitudinal study. Breast cancer research and treatment 2010;123(1):25-34.

48. Wefel JS, Saleeba AK, Buzdar AU, Meyers CA. Acute and late onset cognitive dysfunction associated with chemotherapy in women with breast cancer. Cancer 2010;116(14):3348-56.

49. Yamada TH, Denburg NL, Beglinger LJ, Schultz SK. Neuropsychological outcomes of older breast cancer survivors: cognitive features ten or more years after chemotherapy. The Journal of neuropsychiatry and clinical neurosciences 2010;22(1):48-54.

50. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, Nederveen AJ, Boven E, Schagen SB. Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Hum Brain Mapp 2011;32(8):1206-19.

51. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, Smeets A, Christiaens MR, Leemans A, Van Hecke W, Vandenberghe J, Vandenbulcke M, Sunaert S. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp 2011;32(3):480-93.

52. Jansen CE, Cooper BA, Dodd MJ, Miaskowski CA. A prospective longitudinal study of chemotherapy-induced cognitive changes in breast cancer patients. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer 2011;19(10):1647-56.

53. Kesler SR, Kent JS, O'Hara R. Prefrontal cortex and executive function impairments in primary breast cancer. Archives of neurology 2011;68(11):1447-53.

54. Falleti MG, Sanfilippo A, Maruff P, Weih L, Phillips KA. The nature and severity of cognitive impairment associated with adjuvant chemotherapy in women with breast cancer: a meta-analysis of the current literature. Brain and cognition 2005;59(1):60-70.

55. Pullens MJ, De Vries J, Roukema JA. Subjective cognitive dysfunction in breast cancer patients: a systematic review. Psycho-oncology 2010;19(11):1127-38.

56. Wefel JS, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. The lancet oncology 2011;12(7):703-8.

57. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neuroscience and

biobehavioral reviews 2011;35(3):729-41.

58. Reiriz AB, Reolon GK, Preissler T, Rosado JO, Henriques JA, Roesler R, Schwartsmann G. Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12(16):5000; author reply 5000-1.

59. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behav Brain Res 2008;186(2):168-75.

60. Seigers R, Schagen SB, Coppens CM, van der Most PJ, van Dam FS, Koolhaas JM, Buwalda B. Methotrexate decreases hippocampal cell proliferation and induces memory deficits in rats. Behavioural brain research 2009;201(2):279-84.

61. Dietrich J, Monje M, Wefel J, Meyers C. Clinical patterns and biological correlates of cognitive dysfunction associated with cancer therapy. The oncologist 2008;13(12):1285-95.

62. Froklage FE, Reijneveld JC, Heimans JJ. Central neurotoxicity in cancer chemotherapy: pharmacogenetic insights. Pharmacogenomics 2011;12(3):379-95.

63. Muldoon LL, Soussain C, Jahnke K, Johanson C, Siegal T, Smith QR, Hall WA, Hynynen K, Senter PD, Peereboom DM, Neuwelt EA. Chemotherapy delivery issues in central nervous system malignancy: a reality check. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25(16):2295-305.

64. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, Engelhardt B, Grammas P, Nedergaard M, Nutt J, Pardridge W, Rosenberg GA, Smith Q, Drewes LR. Strategies to advance translational research into brain barriers. Lancet neurology 2008;7(1):84-96.

65. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. J Biol 2008;7(4):12.

66. Meyers CA. How chemotherapy damages the central nervous system. Journal of biology 2008;7(4):11.

67. Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nature reviews. Cancer 2007;7(3):192-201.

68. Textbook of neuronal repair and rehabilitation; neuronal repair and plasticity. Cambridge: Cambridge Universtiy Press; 2006.

69. Nash M, Pribiag H, Fournier AE, Jacobson C. Central nervous system regeneration inhibitors and their intracellular substrates. Molecular neurobiology 2009;40(3):224-35.

70. Taki Y, Kinomura S, Sato K, Goto R, Wu K, Kawashima R, Fukuda H. Correlation between gray/white matter volume and cognition in healthy elderly people. Brain and cognition 2011;75(2):170-6.

71. de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Annals of neurology 2000;47(2):145-51.

72. Kantarci K, Senjem ML, Avula R, Zhang B, Samikoglu AR, Weigand SD, Przybelski SA, Edmonson HA, Vemuri P, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Petersen RC, Jack CR, Jr. Diffusion tensor imaging and cognitive function in older adults with no dementia. Neurology 2011;77(1):26-34.

73. Vernooij MW, Ikram MA, Vrooman HA, Wielopolski PA, Krestin GP, Hofman A, Niessen WJ, Van der Lugt A, Breteler MM. White matter microstructural integrity and cognitive function in a general elderly population. Arch Gen Psychiatry 2009;66(5):545-53.

74. Voineskos AN, Rajji TK, Lobaugh NJ, Miranda D, Shenton ME, Kennedy JL, Pollock BG, Mulsant BH.

Chapter 1

Age-related decline in white matter tract integrity and cognitive performance: A DTI tractography and structural equation modeling study. Neurobiology of aging 2010.

75. Brown MS, Simon JH, Stemmer SM, Stears JC, Scherzinger A, Cagnoni PJ, Jones RB. MR and proton spectroscopy of white matter disease induced by high-dose chemotherapy with bone marrow transplant in advanced breast carcinoma. AJNR Am J Neuroradiol 1995;16(10):2013-20.

76. Brown MS, Stemmer SM, Simon JH, Stears JC, Jones RB, Cagnoni PJ, Sheeder JL. White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. AJNR Am J Neuroradiol 1998;19(2):217-21.

77. Choi SM, Lee SH, Yang YS, Kim BC, Kim MK, Cho KH. 5-fluorouracil-induced leukoencephalopathy in patients with breast cancer. J Korean Med Sci 2001;16(3):328-34.

78. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast Cancer Res Treat 2010;123(3):819-28.

79. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, Douaud G, Lavini C, Linn SC, Boven E, van Dam FS, Schagen SB. Late Effects of High-Dose Adjuvant Chemotherapy on White and Gray Matter in Breast Cancer Survivors: Converging Results from Multimodal Magnetic Resonance Imaging. Hum Brain Mapp 2011;In Press.

80. Johnson C, Fitzsimmons D, Gilbert J, Arrarras JI, Hammerlid E, Bredart A, Ozmen M, Dilektasli E, Coolbrandt A, Kenis C, Young T, Chow E, Venkitaraman R, Howse F, George S, O'Connor S, Yadegarfar G. Development of the European Organisation for Research and Treatment of Cancer quality of life questionnaire module for older people with cancer: The EORTC QLQ-ELD15. European journal of cancer 2010;46(12):2242-52.

Chapter 1

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

Vincent Koppelmans
Monique M.B. Breteler
Willem Boogerd
Caroline Seynaeve
Sanne B. Schagen
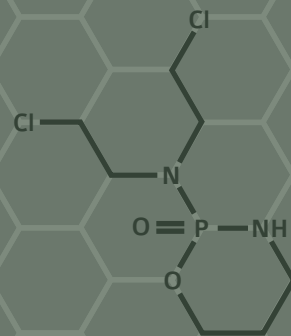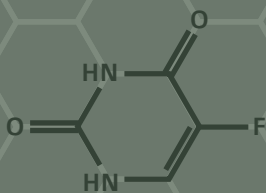
Supplementary material to Chapter 2:

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

Chapter 2

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

Vincent Koppelmans
Monique M.B. Breteler
Willem Boogerd
Caroline Seynaeve
Chad M. Gundy
Sanne B. Schagen

Chapter 3

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

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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

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Table 3. Neuropsychological test outcomes

Testoutcome	Chemotherapy-exposed breast cancer patients (N=196)			Reference group (N=1518)			p-value	difference of the Z-scores (95% c.i.)
	N	Mean	(Sd)	N	Mean	(Sd)		
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	

Forest plot axis: -0.5   -0.3   -0.1   0   0.1   0.3   0.5 — chemotherapy worse | chemotherapy better

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

References

1. Abraham J, Haut MW, Moran MT, Filburn S, Lemiuex S, Kuwabara H. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 2008;8(1):88-91.

2. Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, Whedon MB, Bivens S, Mitchell

T, Greenberg ER, Silberfarb PM. Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. J Clin Oncol 2002;20(2):485-93.

3. Ahles TA, Saykin AJ, McDonald BC, Li Y, Furstenberg CT, Hanscom BS, Mulrooney TJ, Schwartz GN, Kaufman PA. Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. J Clin Oncol 2010;28(29):4434-40.

4. Castellon SA, Silverman DH, Ganz PA. Breast cancer treatment and cognitive functioning: current status and future challenges in assessment. Breast Cancer Res Treat 2005;92(3):199-206.

5. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, Nederveen AJ, Boven E, Schagen SB. Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Human Brain Mapping 2011;32:1206-1219.

6. Debess J, Riis JO, Engebjerg MC, Ewertz M. Cognitive function after adjuvant treatment for early breast cancer: a population-based longitudinal study. Breast Cancer Res Treat 2010;121(1):91-100.

7. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, Smeets A, Christiaens MR, Leemans A, Van Hecke W, Vandenberghe J, Vandenbulcke M, Sunaert S. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp 2011;32(3):480-93.

8. Hermelink K, Untch M, Lux MP, Kreienberg R, Beck T, Bauerfeind I, Munzel K. Cognitive function during neoadjuvant chemotherapy for breast cancer: results of a prospective, multicenter, longitudinal study. Cancer 2007;109(9):1905-13.

9. Jansen CE, Cooper BA, Dodd MJ, Miaskowski CA. A prospective longitudinal study of chemotherapy-induced cognitive changes in breast cancer patients. Support Care Cancer 2011;19:1647-1656.

10. Jansen CE, Dodd MJ, Miaskowski CA, Dowling GA, Kramer J. Preliminary results of a longitudinal

study of changes in cognitive function in breast cancer patients undergoing chemotherapy with doxorubicin and cyclophosphamide. Psychooncology 2008;17(12):1189-95.

11. Jim HS, Donovan KA, Small BJ, Andrykowski MA, Munster PN, Jacobsen PB. Cognitive functioning in breast cancer survivors: a controlled comparison. Cancer 2009;115(8):1776-83.

12. Reid-Arndt SA, Hsieh C, Perry MC. Neuropsychological functioning and quality of life during the first year after completing chemotherapy for breast cancer. Psychooncology 2010;19(5):535-44.

13. Schagen SB, Muller MJ, Boogerd W, Mellenbergh GJ, van Dam FS. Change in cognitive function after chemotherapy: a prospective longitudinal study in breast cancer patients. Journal of the National Cancer Institute 2006;98(23):1742-5.

14. Scherwath A, Mehnert A, Schleimer B, Schirmer L, Fehlauer F, Kreienberg R, Metzner B, Thiel E, Zander AR, Schulz-Kindermann F, Koch U. Neuropsychological function in high-risk breast cancer survivors after stem-cell supported high-dose therapy versus standard-dose chemotherapy: evaluation of long-term treatment effects. Ann Oncol 2006;17(3):415-23.

15. Tager FA, McKinley PS, Schnabel FR, El-Tamer M, Cheung YK, Fang Y, Golden CR, Frosch ME, Habif U, Mulligan MM, Chen IS, Hershman DL. The cognitive effects of chemotherapy in post-menopausal breast cancer patients: a controlled longitudinal study. Breast Cancer Res Treat 2010;123(1):25-34.

16. van Dam FS, Schagen SB, Muller MJ, Boogerd W, vd Wall E, Droogleever Fortuyn ME, Rodenhuis S. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. Journal of the National Cancer Institute 1998;90(3):210-8.

17. Vearncombe KJ, Rolfe M, Wright M, Pachana NA, Andrew B, Beadle G. Predictors of cognitive decline after chemotherapy in breast cancer patients.

J Int Neuropsychol Soc 2009;15(6):951-62.

18. Wefel JS, Saleeba AK, Buzdar AU, Meyers CA. Acute and late onset cognitive dysfunction associated with chemotherapy in women with breast cancer. Cancer 2010;116(14):3348-56.

19. Weis J, Poppelreuter M, Bartsch HH. Cognitive deficits as long-term side-effects of adjuvant therapy in breast cancer patients: 'subjective' complaints and 'objective' neuropsychological test results. Psychooncology 2009;18(7):775-82.

20. Yamada TH, Denburg NL, Beglinger LJ, Schultz SK. Neuropsychological outcomes of older breast cancer survivors: cognitive features ten or more years after chemotherapy. J Neuropsychiatry Clin Neurosci 2010;22(1):48-54.

21. Wefel JS, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. The lancet oncology 2011.

22. Castellon SA, Ganz PA, Bower JE, Petersen L, Abraham L, Greendale GA. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. Journal of clinical and experimental neuropsychology 2004;26(7):955-69.

23. Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7(3):192-201.

24. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, Douaud G, Lavini C, Linn SC, Boven E, van Dam FS, Schagen SB. Late effects of high-dose adjuvant chemotherapy on white and gray matter in breast cancer survivors: Converging results from multimodal magnetic resonance imaging. Human Brain Mapping 2011.

25. Deprez S, Amant F, Smeets A, Peeters R, Leemans A, Van Hecke W, Verhoeven JS, Christiaens MR, Vandenberghe J, Vandenbulcke M, Sunaert S. Longitudinal Assessment of Chemotherapy-Induced Structural Changes in Cerebral White Matter and Its Correlation With Impaired Cognitive Functioning. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011.

26. Ferguson RJ, McDonald BC, Saykin AJ, Ahles TA. Brain structure and function differences in monozygotic twins: possible effects of breast cancer chemotherapy. J Clin Oncol 2007;25(25):3866-70.

27. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 2007;109(1):146-56.

28. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast Cancer Res Treat 2010;123(3):819-28.

29. Saykin AJ, Ahles TA, McDonald BC. Mechanisms of chemotherapy-induced cognitive disorders: neuropsychological, pathophysiological, and neuroimaging perspectives. Semin Clin Neuropsychiatry 2003;8(4):201-16.

30. Stemmer SM, Stears JC, Burton BS, Jones RB, Simon JH. White matter changes in patients with breast cancer treated with high-dose chemotherapy and autologous bone marrow support. AJNR Am J Neuroradiol 1994;15(7):1267-73.

31. Saykin AJ, McDonald BC, Ahles TA, Chesnut LA, Wang PJ, Furstenberg CT, Horrigan SA, Mamourian AC. Altered brain activation following systemic chemotherapy for breast cancer: Interim analysis from a prospective fMRI study. J Int Neuropsychol Soc 2006;12:1.

32. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neuroscience and biobehavioral reviews 2011;35(3):729-41.

33. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC. The

Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol 2009;24(9):553-72.

34. Euser SM, Schram MT, Hofman A, Westendorp RG, Breteler MM. Measuring cognitive function with age: the influence of selection by health and survival. Epidemiology 2008;19(3):440-7.

35. Tiffin J, Asher EJ. The Purdue pegboard; norms and studies of reliability and validity. J Appl Psychol 1948;32(3):234-47.

36. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-98.

37. Kalverboer AF, Deelman BG. De 15-woordentest A en B. In. Groningen: Academisch Ziekenhuis; 1986.

38. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc 1992;40(9):922-35.

39. Houx PJ, Jolles J, Vreeling FW. Stroop interference: aging effects assessed with the Stroop Color-Word Test. Exp Aging Res 1993;19(3):209-24.

40. Killgore WD, Glahn DC, Casasanto DJ. Development and Validation of the Design Organization Test (DOT): a rapid screening instrument for assessing visuospatial ability. J Clin Exp Neuropsychol 2005;27(4):449-59.

41. van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. J Int Neuropsychol Soc 2006;12(1):80-9.

42. van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. J Clin Exp Neuropsychol 2006;28(6):998-1009.

43. Knight RG, Williams S, McGee R, Olaman S. Psychometric properties of the Centre for Epidemiologic Studies Depression Scale (CES-D) in a sample of women in middle life. Behav Res Ther 1997;35(4):373-80.

44. Radloff LS. The CES-D scale: a self report depression scale for research in the genetal population. Applied Psychological Measurment 1977;1:385-401.

45. Dietrich J, Monje M, Wefel J, Meyers C. Clinical patterns and biological correlates of cognitive dysfunction associated with cancer therapy. Oncologist 2008;13(12):1285-95.

46. Mahalanobis PC. On the generalized distance in statistics. Proceedings of the National Institute of Sciences of India 1936;2(1):49–55.

47. Tabachnick BGF, L.S. Using multivariate statistics. 4th ed. Needham Heights, MA: Allyn & Bacon; 2001.

48. DeCarlo LT. On the meaning and use of kurtosis. Psychological Methods 1997;2(3):292-307.

49. Follmann DA. A simple multivariate test for one sided alternatives. Journal of the American Statistical Association 1996;91(434):854-861.

50. Wefel JS, Lenzi R, Theriault R, Buzdar AU, Cruickshank S, Meyers CA. 'Chemobrain' in breast carcinoma?: a prologue. Cancer 2004;101(3):466-75.

51. Ahles TA, Saykin AJ, McDonald BC, Furstenberg CT, Cole BF, Hanscom BS, Mulrooney TJ, Schwartz GN, Kaufman PA. Cognitive function in breast cancer patients prior to adjuvant treatment. Breast Cancer Res Treat 2008;110(1):143-52.

52. Ramaiah R, Lam AM. Postoperative cognitive dysfunction in the elderly. Anesthesiol Clin 2009;27(3):485-96, table of contents.

53. Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, Paraska KK, Ryan CM. Cognitive impairment associated with adjuvant therapy in breast cancer. Psycho-oncology 2006;15(5):422-30.

Chapter 3

54. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. Psycho-oncology 2009;18(2):134-43.

55. Quesnel C, Savard J, Ivers H. Cognitive impairments associated with breast cancer treatments: results from a longitudinal study. Breast cancer research and treatment 2009;116(1):113-23.

56. Stewart A, Collins B, Mackenzie J, Tomiak E, Verma S, Bielajew C. The cognitive effects of adjuvant chemotherapy in early stage breast cancer: a prospective study. Psychooncology 2008;17(2):122-30.

57. de Jong-van den Berg LT, Faber A, van den Berg PB. HRT use in 2001 and 2004 in The Netherlands: a world of difference. Maturitas 2006;54(2):193-7.

58. Castellon S, Ganz PA. Neuropsychological studies in breast cancer: in search of chemobrain. Breast Cancer Res Treat 2009;116(1):125-7.

59. Correa DD, Ahles TA. Neurocognitive changes in cancer survivors. Cancer J 2008;14(6):396-400.

60. Jenkins V, Shilling V, Deutsch G, Bloomfield D, Morris R, Allan S, Bishop H, Hodson N, Mitra S, Sadler G, Shah E, Stein R, Whitehead S, Winstanley J. A 3-year prospective study of the effects of adjuvant treatments on cognition in women with early stage breast cancer. Br J Cancer 2006;94(6):828-34.

61. Schagen SB, Vardy J, Steering Committee of the International C, Cancer Task F. Cognitive dysfunction in people with cancer. Lancet Oncol 2007;8(10):852-3.

62. Stewart A, Bielajew C, Collins B, Parkinson M, Tomiak E. A meta-analysis of the neuropsychological effects of adjuvant chemotherapy treatment in women treated for breast cancer. Clin Neuropsychol 2006;20(1):76-89.

63. Vardy J, Rourke S, Tannock IF. Evaluation of cognitive function associated with chemotherapy: a review of published studies and recommendations for future research. J Clin Oncol 2007;25(17):2455-63.

64. Vardy J, Wefel JS, Ahles T, Tannock IF, Schagen SB. Cancer and cancer-therapy related cognitive dysfunction: an international perspective from the Venice cognitive workshop. Ann Oncol 2008;19(4):623-9.

65. Wefel JS, Witgert ME, Meyers CA. Neuropsychological sequelae of non-central nervous system cancer and cancer therapy. Neuropsychol Rev 2008;18(2):121-31.

66. Dugdale AE, Chandler D, Jeffery H. Rapid repeated finger tapping. Aust Paediatr J 1980;16(3):175-6.

67. Leslie SC, Davidson RJ, Batey OB. Purdue pegboard performance of disabled and normal readers: unimanual versus bimanual differences. Brain Lang 1985;24(2):359-69.

68. Ouimet LA, Stewart A, Collins B, Schindler D, Bielajew C. Measuring neuropsychological change following breast cancer treatment: an analysis of statistical models. J Clin Exp Neuropsychol 2009;31(1):73-89.

69. Schagen SB, Muller MJ, Boogerd W, Rosenbrand RM, van Rhijn D, Rodenhuis S, van Dam FS. Late effects of adjuvant chemotherapy on cognitive function: a follow-up study in breast cancer patients. Ann Oncol 2002;13(9):1387-97.

70. Schagen SB, van Dam FS, Muller MJ, Boogerd W, Lindeboom J, Bruning PF. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer 1999;85(3):640-50.

71. Kreukels BP, van Dam FS, Ridderinkhof KR, Boogerd W, Schagen SB. Persistent neurocognitive problems after adjuvant chemotherapy for breast cancer. Clin Breast Cancer 2008;8(1):80-7.

72. Briones TL, Woods J. Chemotherapy-induced cognitive impairment is associated with decreases in cell proliferation and histone modifications. BMC neuroscience 2011;12:124.

73. Reiriz AB, Reolon GK, Preissler T, Rosado JO, Henriques JA, Roesler R, Schwartsmann G.

Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice. Clin Cancer Res 2006;12(16):5000; author reply 5000-1.

74. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behav Brain Res 2008;186(2):168-75.

75. Seigers R, Schagen SB, Coppens CM, van der Most PJ, van Dam FS, Koolhaas JM, Buwalda B. Methotrexate decreases hippocampal cell proliferation and induces memory deficits in rats. Behav Brain Res 2009;201(2):279-84.

76. Winocur G, Vardy J, Binns MA, Kerr L, Tannock I. The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice. Pharmacol Biochem Behav 2006;85(1):66-75.

77. Early Breast Cancer Trialists' Collaborative Group (EBCTG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365(9472):1687-717.

78. Levine MN, Whelan T. Adjuvant chemotherapy for breast cancer: 30 years later. N Engl J Med 2006;355(18):1920-2.

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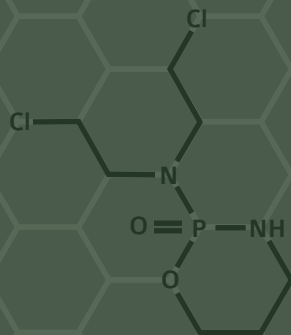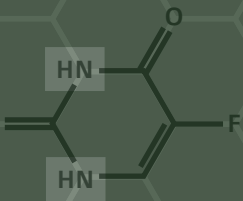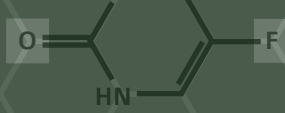
Late effects of chemotherapy on brain structure

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

Vincent Koppelmans
Michiel B. de Ruiter
Fedde van der Lijn
Willem Boogerd
Caroline Seynaeve
Aad van der Lugt
Henri Vrooman
Wiro J. Niessen
Monique M.B. Breteler
Sanne B. Schagen
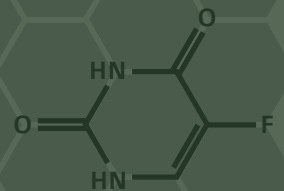
Abstract

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.

Chapter 4.1

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

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

Study	Number of subjects			Time since end of CT	White matter		Gray matter			Outcome
	CT⁺	CT⁻	HC	years (sd)	Measure	ROI	Measure	ROI		
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.

Chapter 4.1

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

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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.

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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)

Tissue / ROI	Chemotherapy-exposed breast cancer survivors (n=184)		Reference group (n=368)		β	95% CI for β	p
	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.

References

1. Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7(3):192-201.

2. Brown MS, Simon JH, Stemmer SM, Stears JC, Scherzinger A, Cagnoni PJ, Jones RB. MR and proton spectroscopy of white matter disease induced by high-dose chemotherapy with bone marrow transplant in advanced breast carcinoma. AJNR Am J Neuroradiol 1995;16(10):2013-20.

3. Choi SM, Lee SH, Yang YS, Kim BC, Kim MK, Cho KH. 5-fluorouracil-induced leukoencephalopathy in patients with breast cancer. J Korean Med Sci 2001;16(3):328-34.

4. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, Douaud G, Lavini C, Linn SC, Boven E, van Dam FS, Schagen SB. Late Effects of High-Dose Adjuvant Chemotherapy on White and Gray Matter in Breast Cancer Survivors: Converging Results from Multimodal Magnetic Resonance Imaging. Hum Brain Mapp 2011;In Press.

5. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, Smeets A, Christiaens MR, Leemans A, Van Hecke W, Vandenberghe J, Vandenbulcke M, Sunaert S. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp 2011;32(3):480-93.

6. Abraham J, Haut MW, Moran MT, Filburn S, Lemiuex S, Kuwabara H. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 2008;8(1):88-91.

7. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 2007;109(1):146-56.

8. Yoshikawa E, Matsuoka Y, Inagaki M, Nakano T, Akechi T, Kobayakawa M, Fujimori M, Nakaya

N, Akizuki N, Imoto S, Murakami K, Uchitomi Y. No adverse effects of adjuvant chemotherapy on hippocampal volume in Japanese breast cancer survivors. Breast Cancer Res Treat 2005;92(1):81-4.

9. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast Cancer Res Treat 2010;123(3):819-28.

10. Brown MS, Stemmer SM, Simon JH, Stears JC, Jones RB, Cagnoni PJ, Sheeder JL. White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. AJNR Am J Neuroradiol 1998;19(2):217-21.

11. Centers for Disease C, Prevention. Cancer survivors--United States, 2007. MMWR Morb Mortal Wkly Rep 2011;60(9):269-72.

12. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, Nederveen AJ, Boven E, Schagen SB. Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Hum Brain Mapp 2010.

13. Nash M, Pribiag H, Fournier AE, Jacobson C. Central nervous system regeneration inhibitors and their intracellular substrates. Mol Neurobiol 2009;40(3):224-35.

14. Koppelmans V, Schagen SB, Poels MM, Boogerd W, Seynaeve C, Lugt AV, Breteler MM. Incidental findings on brain Magnetic Resonance Imaging in long-term survivors of breast cancer treated with adjuvant chemotherapy. Eur J Cancer 2011.

15. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC. The Rotterdam Study: 2010 objectives and design update. European journal of epidemiology 2009;24(9):553-72.

16. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The Rotterdam Scan Study: design and update up to 2012. European journal of epidemiology 2011.

17. Ikram MA, Vrooman HA, Vernooij MW, van der Lijn F, Hofman A, van der Lugt A, Niessen WJ, Breteler MM. Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. Neurobiology of aging 2008;29(6):882-90.

18. Radloff LS. The CES-D scale: a self report depression scale for research in the genetal population. Applied Psychological Measurment 1977;1:385-401.

19. Klein A, Andersson J, Ardekani BA, Ashburner J, Avants B, Chiang MC, Christensen GE, Collins DL, Gee J, Hellier P, Song JH, Jenkinson M, Lepage C, Rueckert D, Thompson P, Vercauteren T, Woods RP, Mann JJ, Parsey RV. Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. Neuroimage 2009;46(3):786-802.

20. van Tol MJ, van der Wee NJ, van den Heuvel OA, Nielen MM, Demenescu LR, Aleman A, Renken R, van Buchem MA, Zitman FG, Veltman DJ. Regional brain volume in depression and anxiety disorders. Archives of general psychiatry 2010;67(10):1002-11.

21. van der Lijn F, den Heijer T, Breteler MM, Niessen WJ. Hippocampus segmentation in MR images using atlas registration, voxel classification, and graph cuts. Neuroimage 2008;43(4):708-20.

22. Coffey CE, Saxton JA, Ratcliff G, Bryan RN, Lucke JF. Relation of education to brain size in normal aging: implications for the reserve hypothesis. Neurology 1999;53(1):189-96.

23. Dotson VM, Davatzikos C, Kraut MA, Resnick SM. Depressive symptoms and brain volumes in older adults: a longitudinal magnetic resonance imaging study. J Psychiatry Neurosci 2009;34(5):367-75.

24. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. J Biol 2008;7(4):12.

25. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda

B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behavioural brain research 2008;186(2):168-75.

26. Koppelmans V, Breteler MMB, Boogerd W, Seynaeve C, Gundy C, Schagen SB. Neuropsychological performance in breast cancer survivors more than 20 years after adjuvant chemotherapy. Journal of Clinical Oncology 2011;accepted for publication.

27. Yamada TH, Denburg NL, Beglinger LJ, Schultz SK. Neuropsychological outcomes of older breast cancer survivors: cognitive features ten or more years after chemotherapy. The Journal of neuropsychiatry and clinical neurosciences 2010;22(1):48-54.

28. Dietrich J, Han R, Yang Y, Mayer-Proschel M, Noble M. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. J Biol 2006;5(7):22.

29. Joshi G, Sultana R, Tangpong J, Cole MP, St Clair DK, Vore M, Estus S, Butterfield DA. Free radical mediated oxidative stress and toxic side effects in brain induced by the anti cancer drug adriamycin: insight into chemobrain. Free radical research 2005;39(11):1147-54.
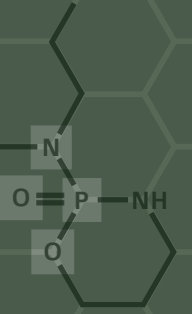
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Global and focal white matter integrity in breast cancer survivors 20 years after adjuvant chemotherapy
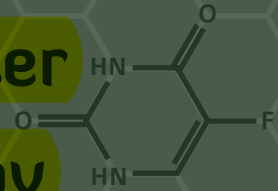
Vincent Koppelmans
Marius de Groot
Michiel B. de Ruiter
Willem Boogerd
Caroline Seynaeve
Meike W. Vernooij
Wiro J. Niessen
Sanne B. Schagen
Monique M.B. Breteler
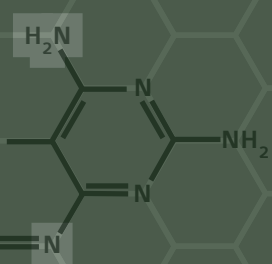
Long-term effects of adjuvant chemotherapy for breast cancer on carotid artery plaques, cerebral blood flow and cerebral perfusion

Vincent Koppelmans
Maryam Kavousi
Willem Boogerd
Caroline Seynaeve
Flora van Leeuwen
Meike W. Vernooij
Aad van der Lugt
Jacqueline C.M. Witteman
Sanne B. Schagen
Monique M.B. Breteler
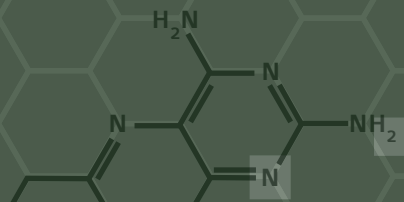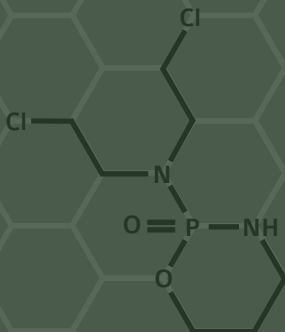
Chapter 5

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

Vincent Koppelmans
Sanne B. Schagen
Mariëlle M.F. Poels
Willem Boogerd
Caroline Seynaeve
Aad van der Lugt
Monique M.B. Breteler
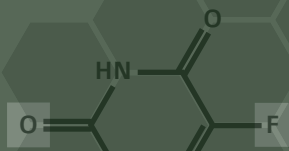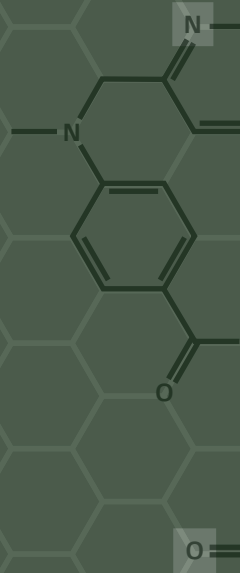
Abstract

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.

Chapter 5

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/m² on days 1-14, Methotrexate 40 mg/m² on days 1 and 8, 5-Fluorouracil 600 mg/m² 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

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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

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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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References

1. Abraham J, Haut MW, Moran MT, Filburn S, Lemiuex S, Kuwabara H. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 2008;8(1):88-91.

2. Cimprich B, Reuter-Lorenz P, Nelson J, Clark PM, Therrien B, Normolle D, Berman MG, Hayes DF, Noll DC, Peltier S, Welsh RC. Prechemotherapy alterations in brain function in women with breast cancer. J Clin Exp Neuropsychol 2010;32(3):324-31.

3. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, Nederveen AJ, Boven E, Schagen SB. Cerebral hyporesponsiveness and cognitive impairment ten years after chemotherapy for breast cancer. Human Brain Mapping 2010;00:000-000.

4. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Stock JVd, Smeets A, Christiaens M-R, Leemans A, Hecke WV, Vandenberghe J, Vandenbulcke M, Sunaert S. Chemotherapy-Induced Structural Changes in Cerebral White Matter and its Correlation With Impaired Cognitive Functioning in Breast Cancer Patients. Human Brain Mapping 2011;32:480-493.

5. Ferguson RJ, McDonald BC, Saykin AJ, Ahles TA. Brain structure and function differences in monozygotic twins: possible effects of breast cancer chemotherapy. J Clin Oncol 2007;25(25):3866-70.

6. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y,

Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 2007;109(1):146-56.

7. Kesler SR, Bennett FC, Mahaffey ML, Spiegel D. Regional brain activation during verbal declarative memory in metastatic breast cancer. Clin Cancer Res 2009;15(21):6665-73.

8. Robinson KE, Livesay KL, Campbell LK, Scaduto M, Cannistraci CJ, Anderson AW, Whitlock JA, Compas BE. Working memory in survivors of childhood acute lymphocytic leukemia: functional neuroimaging analyses. Pediatr Blood Cancer 2010;54(4):585-90.

9. Stemmer SM, Stears JC, Burton BS, Jones RB, Simon JH. White matter changes in patients with breast cancer treated with high-dose chemotherapy and autologous bone marrow support. AJNR Am J Neuroradiol 1994;15(7):1267-73.

10. Illes J, Kirschen MP, Edwards E, Stanford LR, Bandettini P, Cho MK, Ford PJ, Glover GH, Kulynych J, Macklin R, Michael DB, Wolf SM. Ethics. Incidental findings in brain imaging research. Science 2006;311(5762):783-4.

11. Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, Niessen WJ, Breteler MM, van der Lugt A. Incidental findings on brain MRI in the general population. N Engl J Med 2007;357(18):1821-8.

12. Bonadonna G, Rossi A, Valagussa P. Adjuvant CMF chemotherapy in operable breast cancer: ten years later. Lancet 1985;1(8435):976-7.

13. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC. The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol 2009;24(9):553-72.

14. Hemminki K, Jiang Y. Second primary neoplasms after 19281 endocrine gland tumours: aetiological links? Eur J Cancer 2001;37(15):1886-94.

15. Schoemaker MJ, Swerdlow AJ. Risk factors for pituitary tumors: a case-control study. Cancer Epidemiol Biomarkers Prev 2009;18(5):1492-500.

16. Goodwin PJ, Ennis M, Pritchard KI, Trudeau M, Hood N. Risk of menopause during the first year after breast cancer diagnosis. J Clin Oncol 1999;17(8):2365-70.

17. Ahsan H, Neugut AI, Bruce JN. Association of malignant brain tumors and cancers of other sites. J Clin Oncol 1995;13(12):2931-5.

18. Davis F, Tavelin B, Grutsch J, Malmer B. Second primary tumors following a diagnosis of meningioma in Sweden, 1958-1997. Neuroepidemiology 2007;29(1-2):101-6.

19. Helseth A, Mork SJ, Glattre E. Neoplasms of the central nervous system in Norway. V. Meningioma and cancer of other sites. An analysis of the occurrence of multiple primary neoplasms in meningioma patients in Norway from 1955 through 1986. APMIS 1989;97(8):738-44.

20. Malmer B, Tavelin B, Henriksson R, Gronberg H. Primary brain tumours as second primary: a novel association between meningioma and colorectal cancer. Int J Cancer 2000;85(1):78-81.

21. Rao G, Giordano SH, Liu J, McCutcheon IE. The association of breast cancer and meningioma in men and women. Neurosurgery 2009;65(3):483-9; discussion 489.

22. Schoenberg BS, Christine BW, Whisnant JP. Nervous system neoplasms and primary malignancies of other sites. The unique association between meningiomas and breast cancer. Neurology 1975;25(8):705-12.

23. Kubo M, Fukutomi T, Akashi-Tanaka S, Hasegawa T. Association of breast cancer with meningioma: report of a case and review of the literature. Jpn J Clin Oncol 2001;31(10):510-3.

24. Markopoulos C, Sampalis F, Givalos N, Gogas H. Association of breast cancer with meningioma. Eur J Surg Oncol 1998;24(4):332-4.

Chapter 5

25. Miller RE. Breast cancer and meningioma. J Surg Oncol 1986;31(3):182-3.

26. Clemons M, Goss P. Estrogen and the risk of breast cancer. N Engl J Med 2001;344(4):276-85.

27. Key TJ. Serum oestradiol and breast cancer risk. Endocr Relat Cancer 1999;6(2):175-80.

28. Moore JW, Clark GM, Bulbrook RD, Hayward JL, Murai JT, Hammond GL, Siiteri PK. Serum concentrations of total and non-protein-bound oestradiol in patients with breast cancer and in normal controls. Int J Cancer 1982;29(1):17-21.

29. Rosenberg CR, Pasternack BS, Shore RE, Koenig KL, Toniolo PG. Premenopausal estradiol levels and the risk of breast cancer: a new method of controlling for day of the menstrual cycle. Am J Epidemiol 1994;140(6):518-25.

30. Jay JR, MacLaughlin DT, Riley KR, Martuza RL. Modulation of meningioma cell growth by sex steroid hormones in vitro. J Neurosurg 1985;62(5):757-62.

31. Wigertz A, Lonn S, Hall P, Auvinen A, Christensen HC, Johansen C, Klaeboe L, Salminen T, Schoemaker MJ, Swerdlow AJ, Tynes T, Feychting M. Reproductive factors and risk of meningioma and glioma. Cancer Epidemiol Biomarkers Prev 2008;17(10):2663-70.

32. Merkle CJ, Moore IM, Penton BS, Torres BJ, Cueny RK, Schaeffer RC, Jr., Montgomery DW. Methotrexate causes apoptosis in postmitotic endothelial cells. Biol Res Nurs 2000;2(1):5-14.

33. Zeng L, Yan Z, Ding S, Xu K, Wang L. Endothelial injury, an intriguing effect of methotrexate and cyclophosphamide during hematopoietic stem cell transplantation in mice. Transplant Proc 2008;40(8):2670-3.

34. Tamura T, Jamous MA, Kitazato KT, Yagi K, Tada Y, Uno M, Nagahiro S. Endothelial damage due to impaired nitric oxide bioavailability triggers cerebral aneurysm formation in female rats. J Hypertens 2009;27(6):1284-92.

35. Sciubba DM, Gallia GL, Recinos P, Garonzik IM, Clatterbuck RE. Intracranial aneurysm following radiation therapy during childhood for a brain tumor. Case report and review of the literature. J Neurosurg 2006;105(2 Suppl):134-9.

36. Rheingold SR, Neugut AI, Meadows AT. Complications of Cancer and its Treatment. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E, editors. Cancer Medicine. 5th ed. Hamilton, Ontario, Canada: BC Decker; 2000. p. 2399-2406.

37. De Bruin ML, Huisbrink J, Hauptmann M, Kuenen MA, Ouwens GM, van't Veer MB, Aleman BM, van Leeuwen FE. Treatment-related risk factors for premature menopause following Hodgkin lymphoma. Blood 2008;111(1):101-8.

38. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. J Biol 2008;7(4):12.

39. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behavioural brain research 2008;186(2):168-75.

40. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neuroscience and biobehavioral reviews 2011;35(3):729-41.

41. Seigers R, Timmermans J, van der Horn HJ, de Vries EF, Dierckx RA, Visser L, Schagen SB, van Dam FS, Koolhaas JM, Buwalda B. Methotrexate reduces hippocampal blood vessel density and activates microglia in rats but does not elevate central cytokine release. Behavioural brain research 2010;207(2):265-72.

42. van der Lugt A. Incidental findings on brain magnetic resonance imaging. BMJ 2009;339:b3107.

43. Hartwigsen G, Siebner HR, Deuschl G, Jansen O, Ulmer S. Incidental findings are frequent in

young healthy individuals undergoing magnetic resonance imaging in brain research imaging studies: a prospective single-center study. J Comput Assist Tomogr 2010;34(4):596-600.

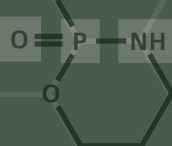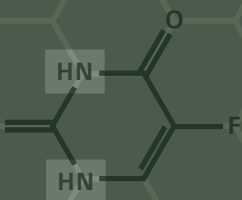**44**. Katzman GL, Dagher AP, Patronas NJ. Incidental findings on brain magnetic resonance

imaging from 1000 asymptomatic volunteers. JAMA 1999;282(1):36-9.

45. Weber F, Knopf H. Incidental findings in magnetic resonance imaging of the brains of healthy young men. J Neurol Sci 2006;240(1-2):81-4.

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

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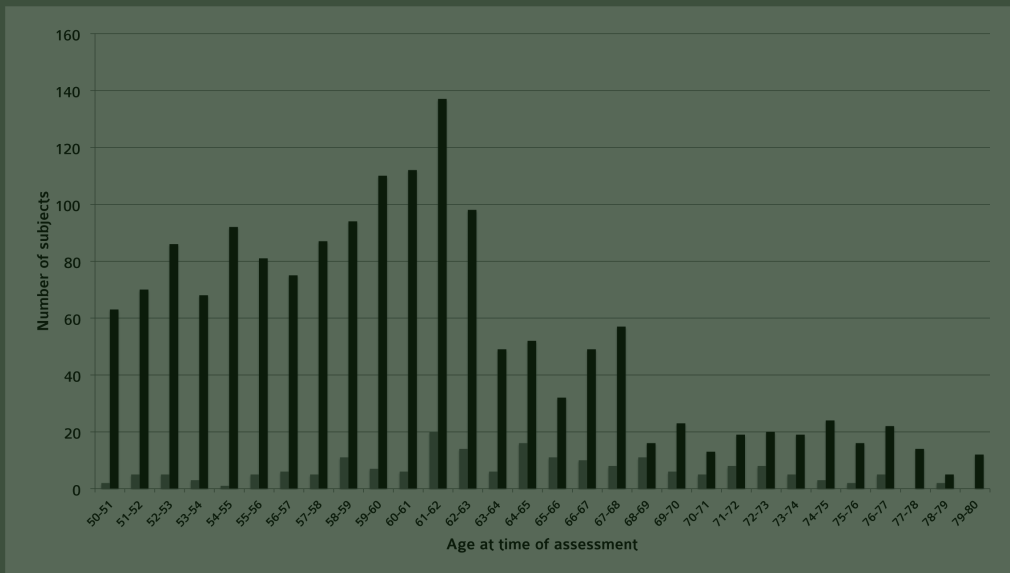
Figure 1. Number of subjects per age class for the chemotherapy-exposed breast cancer survivors and reference subjects. Gray bars indicate the number of chemotherapy-exposed breast cancer survivors; Black bars indicate the number of reference subjects available from the Rotterdam Scan Study

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

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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).

References

1. Sjostrom J. Predictive factors for response to chemotherapy in advanced breast cancer. Acta oncologica 2002;41(4):334-45.

2. Abraham J, Haut MW, Moran MT, Filburn S, Lemieux S, Kuwabara H. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 2008;8(1):88-91.

3. Choi SM, Lee SH, Yang YS, Kim BC, Kim MK, Cho KH. 5-fluorouracil-induced leukoencephalopathy in patients with breast cancer. J Korean Med Sci 2001;16(3):328-34.

4. Deprez S, Amant F, Smeets A, Peeters R, Leemans A, Van Hecke W, Verhoeven JS, Christiaens MR, Vandenberghe J, Vandenbulcke M, Sunaert S. Longitudinal Assessment of Chemotherapy-Induced Structural Changes in Cerebral White Matter and Its Correlation With Impaired Cognitive Functioning. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011.

5. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, Smeets A, Christiaens MR, Leemans A, Van Hecke W, Vandenberghe J, Vandenbulcke M, Sunaert S. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp 2011;32(3):480-93.

6. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 2007;109(1):146-56.

7. Vodermaier A. Breast cancer treatment and cognitive function: the current state of evidence, underlying mechanisms and potential treatments. Womens Health

(Lond Engl) 2009;5(5):503-16.

8. Mook S, Van 't Veer LJ, Rutgers EJ, Ravdin PM, van de Velde AO, van Leeuwen FE, Visser O, Schmidt MK. Independent Prognostic Value of Screen Detection in Invasive Breast Cancer. J Natl Cancer Inst 2011.

9. Paap E, Broeders MJ, van Schoor G, Otten JD, Verbeek AL. Large increase in a Dutch woman's lifetime risk of developing breast cancer. Eur J Cancer 2008;44(11):1485-7.

10. Porter PL. Global trends in breast cancer incidence and mortality. Salud Publica Mex 2009;51 Suppl 2:s141-6.

11. van Schoor G, Moss SM, Otten JD, Donders R, Paap E, den Heeten GJ, Holland R, Broeders MJ, Verbeek AL. Increasingly strong reduction in breast cancer mortality due to screening. Br J Cancer 2011;104(6):910-4.

12. Johnson C, Fitzsimmons D, Gilbert J, Arrarras JI, Hammerlid E, Bredart A, Ozmen M, Dilektasli E, Coolbrandt A, Kenis C, Young T, Chow E, Venkitaraman R, Howse F, George S, O'Connor S, Yadegarfar G. Development of the European Organisation for Research and Treatment of Cancer quality of life questionnaire module for older people with cancer: The EORTC QLQ-ELD15. European journal of cancer 2010;46(12):2242-52.

13. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, Uitterlinden AG, Vernooij MW, Witteman JC. The Rotterdam Study: 2012 objectives and design update. European journal of epidemiology 2011;26(8):657-86.

14. Gluck S. The expanding role of epirubicin in the treatment of breast cancer. Cancer control : journal of the Moffitt Cancer Center 2002;9(2 Suppl):16-27.

Chapter 6

15. DeCarlo LT. On the meaning and use of kurtosis. Psychological Methods 1997;2(3):15.

16. Follmann DA. A simple multivariate test for one sided alternatives. Journal of the American Statistical Association 1996;91(434):9.

17. Mahalanobis PC. On the generalized distance in statistics. J Asiat Soc Bengal 1936;2(1):8.

18. Tabachnick BGF, L.S. Using multivariate statistics. 4th ed. Needham Heights, MA: Allyn & Bacon; 2001.

19. Hermelink K, Untch M, Lux MP, Kreienberg R, Beck T, Bauerfeind I, Munzel K. Cognitive function during neoadjuvant chemotherapy for breast cancer: results of a prospective, multicenter, longitudinal study. Cancer 2007;109(9):1905-13.

20. Slavin MJ, Brodaty H, Kochan NA, Crawford JD, Trollor JN, Draper B, Sachdev PS. Prevalence and predictors of "subjective cognitive complaints" in the Sydney Memory and Ageing Study. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry 2010;18(8):701-10.

21. Svendsen AM, Kessing LV, Munkholm K, Vinberg M, Miskowiak KW. Is there an association between subjective and objective measures of cognitive function in patients with affective disorders? Nordic journal of psychiatry 2011.

22. Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7(3):192-201.

23. Deeken JF, Loscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. Clinical cancer research : an official journal of the American Association for Cancer Research 2007;13(6):1663-74.

24. de Vries NA, Beijnen JH, Boogerd W, van Tellingen O. Blood-brain barrier and chemotherapeutic treatment of brain tumors. Expert review of neurotherapeutics 2006;6(8):1199-209.

25. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neuroscience and biobehavioral reviews 2011;35(3):729-41.

26. Muldoon LL, Soussain C, Jahnke K, Johanson C, Siegal T, Smith QR, Hall WA, Hynynen K, Senter PD, Peereboom DM, Neuwelt EA. Chemotherapy delivery issues in central nervous system malignancy: a reality check. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25(16):2295-305.

27. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, Engelhardt B, Grammas P, Nedergaard M, Nutt J, Pardridge W, Rosenberg GA, Smith Q, Drewes LR. Strategies to advance translational research into brain barriers. Lancet neurology 2008;7(1):84-96.

28. Nash M, Pribiag H, Fournier AE, Jacobson C. Central nervous system regeneration inhibitors and their intracellular substrates. Molecular neurobiology 2009;40(3):224-35.

29. Brown MS, Simon JH, Stemmer SM, Stears JC, Scherzinger A, Cagnoni PJ, Jones RB. MR and proton spectroscopy of white matter disease induced by high-dose chemotherapy with bone marrow transplant in advanced breast carcinoma. AJNR Am J Neuroradiol 1995;16(10):2013-20.

30. Brown MS, Stemmer SM, Simon JH, Stears JC, Jones RB, Cagnoni PJ, Sheeder JL. White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. AJNR Am J Neuroradiol 1998;19(2):217-21.

31. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The Rotterdam Scan Study: design and update up to 2012. European journal of epidemiology 2011;26(10):811-24.

32. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. NeuroImage 2000;11(6 Pt 1):805-21.

33. van der Lijn F, den Heijer T, Breteler MM, Niessen WJ. Hippocampus segmentation in MR images using atlas registration, voxel classification, and graph cuts.

Neuroimage 2008;43(4):708-20.

34. Eijkenboom M, Van Der Staay FJ. Spatial learning deficits in rats after injection of vincristine into the dorsal hippocampus. Neuroscience 1999;91(4):1299–313.

35. Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. Neuron 2002;35(4):625-41.

36. Ystad MA, Lundervold AJ, Wehling E, Espeseth T, Rootwelt H, Westlye LT, Andersson M, Adolfsdottir S, Geitung JT, Fjell AM, Reinvang I, Lundervold A. Hippocampal volumes are important predictors for memory function in elderly women. BMC medical imaging 2009;9:17.

37. Wefel JS, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. The lancet oncology 2011.

38. Vernooij MW, Ikram MA, Vrooman HA, Wielopolski PA, Krestin GP, Hofman A, Niessen WJ, Van der Lugt A, Breteler MM. White matter microstructural integrity and cognitive function in a general elderly population. Archives of general psychiatry 2009;66(5):545-53.

39. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17(3):1429-36.

40. Song SK, Yoshino J, Le TQ, Lin SJ, Sun SW, Cross AH, Armstrong RC. Demyelination increases radial diffusivity in corpus callosum of mouse brain. Neuroimage 2005;26(1):132-40.

41. Wheeler-Kingshott CA, Cercignani M. About "axial" and "radial" diffusivities. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 2009;61(5):1255-60.

42. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TE. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006;31(4):1487-505.

43. Sprague BL, Trentham-Dietz A, Nichols HB, Hampton JM, Newcomb PA. Change in lifestyle behaviors and medication use after a diagnosis of ductal carcinoma in situ. Breast Cancer Res Treat 2010;124(2):487–95.

44. Velentzis LS, Keshtgar MR, Woodside JV, Leathem AJ, Titcomb A, Perkins KA, Mazurowska M, Anderson V, Wardell K, Cantwell MM. Significant changes in dietary intake and supplement use after breast cancer diagnosis in a UK multicentre study. Breast Cancer Res Treat 2011;128(2):473-82.

45. Liao Y, Tang J, Deng Q, Deng Y, Luo T, Wang X, Chen H, Liu T, Chen X, Brody AL, Hao W. Bilateral fronto-parietal integrity in young chronic cigarette smokers: a diffusion tensor imaging study. PloS one 2011;6(11):e26460.

46. Teipel SJ, Meindl T, Wagner M, Kohl T, Burger K, Reiser MF, Herpertz S, Moller HJ, Hampel H. White matter microstructure in relation to education in aging and Alzheimer's disease. J Alzheimers Dis 2009;17(3):571-83.

47. Xu J, Li Y, Lin H, Sinha R, Potenza MN. Body mass index correlates negatively with white matter integrity in the fornix and corpus callosum: A diffusion tensor imaging study. Human brain mapping 2011.

48. Muller M, van der Graaf Y, Visseren FL, Vlek AL, Mali WP, Geerlings MI. Blood pressure, cerebral blood flow, and brain volumes. The SMART-MR study. Journal of hypertension 2010;28(7):1498-505.

49. Jensen SA, Sorensen JB. 5-Fluorouracil-based therapy induces endovascular injury having potential significance to development of clinically overt cardiotoxicity. Cancer chemotherapy and pharmacology 2011.

50. Kalabova H, Melichar B, Ungermann L, Dolezal J, Krcmova L, Kasparova M, Plisek J, Hyspler R, Pecka M, Solichova D. Intima-media thickness, myocardial perfusion and laboratory risk factors of atherosclerosis in patients with breast cancer treated with anthracycline-based chemotherapy. Med Oncol 2010.

Chapter 6

51. Douglas AF, Christopher S, Amankulor N, Din R, Poullis M, Amin-Hanjani S, Ghogawala Z. Extracranial carotid plaque length and parent vessel diameter significantly affect baseline ipsilateral intracranial blood flow. Neurosurgery 2011;69(4):767-73.

52. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Vrooman HA, Hofman A, Krestin GP, Breteler MM. Total cerebral blood flow and total brain perfusion in the general population: the Rotterdam Scan Study. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2008;28(2):412-9.

53. Illes J, Kirschen MP, Edwards E, Stanford LR, Bandettini P, Cho MK, Ford PJ, Glover GH, Kulynych J, Macklin R, Michael DB, Wolf SM. Ethics. Incidental findings in brain imaging research. Science 2006;311(5762):783-4.

54. Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, Niessen WJ, Breteler MM, van der Lugt A. Incidental findings on brain MRI in the general population. The New England journal of medicine 2007;357(18):1821-8.

55. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, Douaud G, Lavini C, Linn SC, Boven E, van Dam FS, Schagen SB. Late Effects of High-Dose Adjuvant Chemotherapy on White and Gray Matter in Breast Cancer Survivors: Converging Results from Multimodal Magnetic Resonance Imaging. Hum Brain Mapp 2011;In Press.

56. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, Nederveen AJ, Boven E, Schagen SB. Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Hum Brain Mapp 2011;32(8):1206-19.

57. Ferguson RJ, McDonald BC, Saykin AJ, Ahles TA. Brain structure and function differences in monozygotic twins: possible effects of breast cancer chemotherapy. J Clin Oncol 2007;25(25):3866-70.

58. Kesler SR, Kent JS, O'Hara R. Prefrontal cortex and executive function impairments in primary breast cancer. Archives of neurology 2011;68(11):1447-53.

59. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast Cancer Res Treat 2010;123(3):819-28.

60. Yoshikawa E, Matsuoka Y, Inagaki M, Nakano T, Akechi T, Kobayakawa M, Fujimori M, Nakaya N, Akizuki N, Imoto S, Murakami K, Uchitomi Y. No adverse effects of adjuvant chemotherapy on hippocampal volume in Japanese breast cancer survivors. Breast cancer research and treatment 2005;92(1):81-4.

61. Ahsan H, Neugut AI, Bruce JN. Association of malignant brain tumors and cancers of other sites. J Clin Oncol 1995;13(12):2931-5.

62. Davis F, Tavelin B, Grutsch J, Malmer B. Second primary tumors following a diagnosis of meningioma in Sweden, 1958-1997. Neuroepidemiology 2007;29(1-2):101-6.

63. Helseth A, Mork SJ, Glattre E. Neoplasms of the central nervous system in Norway. V. Meningioma and cancer of other sites. An analysis of the occurrence of multiple primary neoplasms in meningioma patients in Norway from 1955 through 1986. APMIS 1989;97(8):738-44.

64. Malmer B, Tavelin B, Henriksson R, Gronberg H. Primary brain tumours as second primary: a novel association between meningioma and colorectal cancer. Int J Cancer 2000;85(1):78-81.

65. Rao G, Giordano SH, Liu J, McCutcheon IE. The association of breast cancer and meningioma in men and women. Neurosurgery 2009;65(3):483-9; discussion 489.

66. Schoenberg BS, Christine BW, Whisnant JP. Nervous system neoplasms and primary malignancies of other sites. The unique association between meningiomas and breast cancer. Neurology 1975;25(8):705-12.

67. Ikram MA, Vrooman HA, Vernooij MW, den Heijer T, Hofman A, Niessen WJ, van der Lugt A, Koudstaal PJ, Breteler MM. Brain tissue volumes in relation to cognitive function and risk of dementia. Neurobiology

of aging 2010;31(3):378-86.

68. Kesler SR, Bennett FC, Mahaffey ML, Spiegel D. Regional brain activation during verbal declarative memory in metastatic breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15(21):6665-73.

69. Silverman DH, Dy CJ, Castellon SA, Lai J, Pio BS, Abraham L, Waddell K, Petersen L, Phelps ME, Ganz PA. Altered frontocortical, cerebellar, and basal ganglia activity in adjuvant-treated breast cancer survivors 5-10 years after chemotherapy. Breast cancer research and treatment 2007;103(3):303-11.

70. Kreukels BP, Schagen SB, Ridderinkhof KR, Boogerd W, Hamburger HL, van Dam FS. Electrophysiological correlates of information processing in breast-cancer patients treated with adjuvant chemotherapy. Breast cancer research and treatment 2005;94(1):53-61.

71. Schagen SB, Hamburger HL, Muller MJ, Boogerd W, van Dam FS. Neurophysiological evaluation of late effects of adjuvant high-dose chemotherapy on cognitive function. Journal of neuro-oncology 2001;51(2):159-65.

72. Goodwin PJ, Ennis M, Pritchard KI, Trudeau M, Hood N. Risk of menopause during the first year after breast cancer diagnosis. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 1999;17(8):2365-70.

73. Henderson VW, Sherwin BB. Surgical versus natural menopause: cognitive issues. Menopause 2007;14(3 Pt 2):572-9.

74. Vearncombe KJ, Pachana NA. Is cognitive functioning detrimentally affected after early, induced menopause? Menopause 2009;16(1):188-98.

75. Cagney KA, Lauderdale DS. Education, wealth, and cognitive function in later life. The journals of gerontology. Series B, Psychological sciences and social sciences 2002;57(2):P163-72.

76. Coffey CE, Saxton JA, Ratcliff G, Bryan RN, Lucke JF. Relation of education to brain size in normal aging: implications for the reserve hypothesis. Neurology 1999;53(1):189-96.

77. Small GW, Silverman DH, Siddarth P, Ercoli LM, Miller KJ, Lavretsky H, Wright BC, Bookheimer SY, Barrio JR, Phelps ME. Effects of a 14-day healthy longevity lifestyle program on cognition and brain function. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry 2006;14(6):538-45.

78. Irving RA, Noakes TD, Buck R, van Zyl Smit R, Raine E, Godlonton J, Norman RJ. Evaluation of renal function and fluid homeostasis during recovery from exercise-induced hyponatremia. Journal of applied physiology 1991;70(1):342-8.

79. Mitchell AJ, Steven K, Benito-León J, Reuber M. The influence of cognitive impairment on health-related quality of life in neurological disease. Acta Neuropsychiatrica 2010;22:2-13.

80. NABON. MAMMACARCINOOM - Landelijke richtlijn met regionale toevoegingen - Versie 1.1. In; 2010.

81. Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, Whedon MB, Bivens S, Mitchell T, Greenberg ER, Silberfarb PM. Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2002;20(2):485-93.

82. Castellon SA, Ganz PA, Bower JE, Petersen L, Abraham L, Greendale GA. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. Journal of clinical and experimental neuropsychology 2004;26(7):955-69.

83. Scherwath A, Mehnert A, Schleimer B, Schirmer L, Fehlauer F, Kreienberg R, Metzner B, Thiel E, Zander AR, Schulz-Kindermann F, Koch U. Neuropsychological function in high-risk breast cancer survivors after stem-cell supported high-dose therapy versus standard-dose chemotherapy: evaluation of long-term treatment effects. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2006;17(3):415-23.

Chapter 6

84. Yamada TH, Denburg NL, Beglinger LJ, Schultz SK. Neuropsychological outcomes of older breast cancer survivors: cognitive features ten or more years after chemotherapy. The Journal of neuropsychiatry and clinical neurosciences 2010;22(1):48-54.

85. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. Journal of biology 2008;7(4):12.

86. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behavioural brain research 2008;186(2):168-75.

87. Seigers R, Schagen SB, Coppens CM, van der Most PJ, van Dam FS, Koolhaas JM, Buwalda B. Methotrexate decreases hippocampal cell proliferation and induces memory deficits in rats. Behavioural brain research 2009;201(2):279-84.

88. Seigers R, Timmermans J, van der Horn HJ, de Vries EF, Dierckx RA, Visser L, Schagen SB, van Dam FS, Koolhaas JM, Buwalda B. Methotrexate reduces hippocampal blood vessel density and activates microglia in rats but does not elevate central cytokine release. Behavioural brain research 2010;207(2):265-72.

89. Reiriz AB, Reolon GK, Preissler T, Rosado JO, Henriques JA, Roesler R, Schwartsmann G. Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12(16):5000; author reply 5000-1.

90. Winocur G, Vardy J, Binns MA, Kerr L, Tannock I. The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice. Pharmacology, biochemistry, and behavior 2006;85(1):66-75.

91. Levine MN, Whelan T. Adjuvant chemotherapy for breast cancer--30 years later. N Engl J Med 2006;355(18):1920-2.

92. Debess J, Riis JO, Engebjerg MC, Ewertz M. Cognitive function after adjuvant treatment for early breast cancer: a population-based longitudinal study. Breast cancer research and treatment 2010;121(1):91-100.

93. Jansen CE, Cooper BA, Dodd MJ, Miaskowski CA. A prospective longitudinal study of chemotherapy-induced cognitive changes in breast cancer patients. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer 2011;19(10):1647-56.

94. Quesnel C, Savard J, Ivers H. Cognitive impairments associated with breast cancer treatments: results from a longitudinal study. Breast cancer research and treatment 2009;116(1):113-23.

95. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. Psycho-oncology 2009;18(2):134-43.

96. Donovan KA, Small BJ, Andrykowski MA, Schmitt FA, Munster P, Jacobsen PB. Cognitive functioning after adjuvant chemotherapy and/or radiotherapy for early-stage breast carcinoma. Cancer 2005;104(11):2499-507.

97. Jim HS, Donovan KA, Small BJ, Andrykowski MA, Munster PN, Jacobsen PB. Cognitive functioning in breast cancer survivors: a controlled comparison. Cancer 2009;115(8):1776-83.

98. Schagen SB, van Dam FS, Muller MJ, Boogerd W, Lindeboom J, Bruning PF. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer 1999;85(3):640-50.

99. Ahles T, Saykin A, McDonald B, Harker Rhodes C, Moore J, Urbanowitz R, Tsongolis G, Tosteson T. Genetics of cognitive decline post cancer chemotherapy. In: ICCTF 2010. New York; 2010.

100. Small BJ, Sharp-Rawson K, Jim HS, Andrykowski MA, Jacobsen PB. Impact of Cathechol-O-Methyltransferase (COMT) Genotype on Cancer Treatment-Related Cognitive Deficits. In: ICCTF 2010; 2010; New York; 2010.

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101. Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, Paraska KK, Ryan CM. Cognitive impairment associated with adjuvant therapy in breast cancer. Psycho-oncology 2006;15(5):422-30.

102. Wefel JS, Saleeba AK, Buzdar AU, Meyers CA. Acute and late onset cognitive dysfunction associated with chemotherapy in women with breast cancer. Cancer 2010;116(14):3348-56.

103. Cheler F, Duyns J, Pardossi-Piquard R, Costa CAd. p53, a Molecular Bridge Between Alzheimer's Disease Pathology and Cancers? In: Curran T, Christen Y, editors. Two Faces of Evil: Cancer and Neurodegeneration, Research and Perspectives in Alzheimer's Disease. Berlin Heidelberg: Springer-Verlag; 2011. p. 95-101.

104. Kosik KS, Neveu P, Banerjee S. The miRNA System: Bifurcation Points of Cancer and Neurodegeneration. In: Curran T, Christen Y, editors. Two Faces of Evil: Cancer and Neurodegeneration, Research and Perspectives in Alzheimer's Disease. Berlin Heidelberg: Springer-Verlag; 2011. p. 133-142.

105. Devore EE, Prescott J, De Vivo I, Grodstein F. Relative telomere length and cognitive decline in the Nurses' Health Study. Neuroscience letters 2011;492(1):15-8.

106. Grodstein F, van Oijen M, Irizarry MC, Rosas HD, Hyman BT, Growdon JH, De Vivo I. Shorter telomeres may mark early risk of dementia: preliminary analysis of 62 participants from the nurses' health study. PloS one 2008;3(2):e1590.

107. Speroff L. Postmenopausal hormone therapy and primary prevention of cardiovascular disease -- Nurses' health study 20-year follow-up. Maturitas 2001;38(3):221-4.

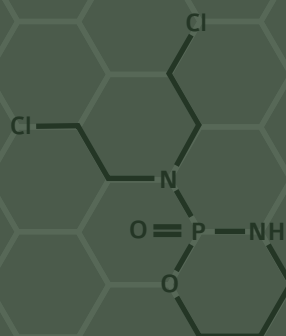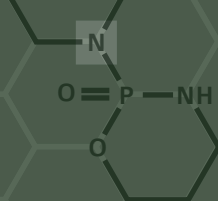**108**. Siemes C. New Insights into Genetic Variation and Cancer. Rotterdam: Erasmus University; 2007.

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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

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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.

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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

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Publicaţies

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.*

Koppelmans V, Breteler MMB, Boogerd W, Seynaeve C, Gundy C, Schagen SB. Neuropsychological performance in breast cancer survivors more than 20 years after adjuvant chemotherapy. *Journal of Clinical Oncology,* 2011; In Press.

Koppelmans V, de Ruiter MB, van der Lijn F, Boogerd W, Seynaeve C, van der Lugt A, Vrooman H, Niessen WJ, Breteler MM, Schagen SB. Global and focal brain volume in long-term breast cancer survivors exposed to adjuvant chemotherapy. *Breast Cancer Research and Treatment,* 2011; In Press.

Koppelmans V, Schagen SB. Effecten van chemotherapie op het cognitief functioneren van kankerpatiënten. *Oncologica* 2011;4:35-7.

Koppelmans V, Schagen SB, Poels MM, Boogerd W, Seynaeve C, Lugt A, Breteler MM. Incidental findings on brain Magnetic Resonance Imaging in long-term survivors of breast cancer treated with adjuvant chemotherapy. *European journal of cancer* 2011;47(17):2531-6.

Koppelmans V, Schoevers R, van Wijk CG, Mulder W, Hornbach A, Barkhof E, Klaassen A, van Egmond M, van Venrooij J, Bijpost Y, Nusselder H, van Herrewaarden M, Maksimovic I, Achilles A, Dekker J. The Amsterdam Studies of Acute Psychiatry - II (ASAP-II): a comparative study of psychiatric intensive care units in the Netherlands. *BMC public health* 2009;9:318.

Schoevers RA, Van HL, **Koppelmans V**, Kool S, Dekker JJ. Managing the patient with co-morbid depression and an anxiety disorder. *Drugs* 2008;68(12):1621-34.

van der Post L, Schoevers R, **Koppelmans V**, Visch I, Bernardt C, Mulder N, Beekman A, de Haan L, Dekker J. The Amsterdam Studies of Acute Psychiatry I (ASAP-I); a prospective cohort study of determinants and outcome of coercive versus voluntary treatment interventions in a metropolitan area. *BMC psychiatry* 2008;8:35.

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

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