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ORIGINAL RESEARCH

A mechanistic cohort study evaluating cognitive impairment in women treated for breast cancer

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Abstract Some women report cognitive impairment after adjuvant chemotherapy (CTh) for breast cancer. Here we explore cognitive function, and underlying mechanisms with blood tests and functional magnetic resonance imaging (fMRI). Women treated for early breast cancer were recruited to three groups based on self-reported cognitive symptoms (CS) using FACT-Cog scores. CTh + CS+ (n = 44) had received chemotherapy and self-reported cognitive symptoms; CTh + CS- (n = 52) had chemotherapy but did not report cognitive problems; CTh- (n = 30) had not received chemotherapy. Clinical and computer-based neuropsychological tests were performed. Blood tests included 10 cytokines, sex hormones, coagulation factors, and apolipoprotein-E genotype. fMRI (n = 101) was performed while subjects performed an n-back memory task. Participants had median age 50 (range: 29–60) years and were a median of 17 months post-diagnosis. On clinical neuropsychological tests 19% had cognitive impairment using Global Deficit Score, and 36% using International Cancer and Cognition Task Force criteria with no significant differences in cognitive impairment rates between groups. CTh + CS+ had significantly more fatigue, anxiety/depression and poorer quality-of-life than other groups. There was no association between FACT-Cog and neuropsychological scores. There were significant differences in frontal and parietal regions on fMRI scans: CTh- showed hyperactivation compared to chemotherapy-treated groups, CTh + CS+ had more frontal activation than CTh + CS-. Elevated IL-1, IL-2 were associated weakly and IL-8 more strongly with neuropsychological impairment (rho > 0.20). There were no differences in global cognitive impairment between groups. Cognitive symptoms were associated with fatigue and anxiety/depression, but not with objective cognitive impairment. fMRI scans differed among the three groups.

Keywords Cognitive impairment · fMRI · Breast cancer · Cancer survivors · Cognitive symptoms · Quality of life

Institute where work performed: Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada

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Introduction

Adjuvant chemotherapy has improved the survival of women with breast cancer. However, it may affect the quality of life (QOL) of survivors, by inducing fatigue(Bower et al. 2000; Servaes et al. 2007) and cognitive decline.(J. S. Wefel et al. 2011) The mechanisms by which chemotherapy causes cognitive dysfunction are unknown, but might include direct neurotoxicity, alteration of cytokine levels, hormonal changes, and thrombosis of small cerebral vessels.(Ahles and Saykin 2007) One study suggests greater cognitive impairment after

chemotherapy in people with the apolipoprotein ϵ4 allele (*APOE ϵ4*), also associated with increased risk of dementia and cognitive changes after trauma.(Ahles et al. 2003).

Previous studies suggest that cognitive impairment after chemotherapy is associated with structural or functional changes in the brain that can be detected by magnetic resonance imaging (MRI).(de Ruiter et al. 2011; Inagaki et al. 2007) Functional MRI (fMRI) detects changes in brain activation and is a non-invasive means of monitoring neurophysiological changes.

A limitation of longitudinal cognitive studies following chemotherapy is that only a small proportion of participants develop cognitive impairment, making mechanistic studies difficult. Therefore we designed this study to include women with breast cancer who either did/did not self-report cognitive symptoms (CS) following adjuvant chemotherapy, together with a non-chemotherapy control group. We characterized cognitive function using comprehensive neuropsychological tests and explored potential causes of cognitive impairment with laboratory and fMRI studies. Our primary hypotheses were that women who received chemotherapy (CTh+) would have more cognitive symptoms than those who did not (CTh-), and those who received chemotherapy and reported cognitive symptoms (CTh + CS+) would have more impairment on neuropsychological tests and differences in activation on fMRI. Secondary hypotheses were that cognitive impairment would be associated with increased inflammatory cytokines and *APOE ϵ4*. Our goal was to characterize the cognitive deficits in those patients who self-report them, and to identify potential mechanisms for future in-depth evaluation.

Patients and methods

Women were recruited from Princess Margaret Hospital clinics and patient support groups in Toronto. Participants were ≤60 years of age, and free of disease after invasive breast cancer in the previous five years. They completed the Functional Assessment of Cancer Therapy-Cognition version 2 (FACT-Cog) questionnaire, which assesses perceived cognitive function.(Wagner et al. 2009) Using the FACT-Cog summary score, excluding questions assessing impact on QOL, women were assigned to one of four groups: CTh + CS+ received adjuvant or neo-adjuvant chemotherapy and had perceived cognitive impairment (FACT-Cog ≤85/168); CTh + CS- completed adjuvant or neo-adjuvant chemotherapy and self-reported normal cognitive function (FACT-Cog ≥100); CTh- did not require chemotherapy (irrespective of FACT-Cog score); women with breast cancer who received chemotherapy with FACT-Cog score of 86–99 had no further assessment. The cut off score of ≤85/168 for the FACT-Cog was based on previous work.(J. Vardy et al. 2006) The reason for excluding those with intermediate FACT-Cog scores was to provide greater separation between those who had received chemotherapy and did and did not report cognitive symptoms.

Patients were excluded for the following reasons: psychiatric illness, alcohol abuse or co-morbidity that might interfere with neuropsychological function; use of psychotropic medication (apart from sedative benzodiazepines or serotonin reuptake inhibitors for hot flashes); non-fluency in English; or contraindication to MRI.

Participants underwent neuropsychological tests, fMRI, completed questionnaires and provided blood for laboratory testing. The neuropsychological tests and questionnaires were administered over ~2.5 h by a trained research assistant.

The Research Ethics Boards of the University Health Network approved the study. All participants gave written informed consent.

Objective neuropsychological tests

Cognitive tests were selected for psychometric properties and availability of demographically corrected normative data. We included: (i) clinical neuropsychological tests; (ii) the Cambridge Neuropsychological Test Automated Battery (CANTAB) completed on a touch-screen computer, and (iii) the modified Six Elements Test (SET). (Appendix, Table 1A).

Subjective tests of cognitive function

Participants completed two questionnaires assessing perception of cognitive function: (i) the 50-item FACT-Cog v2(Wagner et al. 2009) and (ii) the 34-item Patient's Assessment of Own Functioning Inventory (PAFI).(Chelune et al. 1986).

Self-report questionnaires

Fatigue was assessed by the FACT-F fatigue subscale,(Yellen et al. 1997) in conjunction with the FACT-G, which assesses health-related QOL.(D. F. Cella et al. 1993) The 12-item General Health Questionnaire assessed anxiety/depression.(Goldberg 1991).

Laboratory tests

Participants provided a non-fasting blood sample when they attended for study assessments, to evaluate hemoglobin, electrolytes, creatinine, liver function tests, sex hormones (estradiol, FSH, LH), cytokines (interleukin (IL)-1β,-2,-4,-6,-8,-10,-12, tumour necrosis factor-alpha (TNF), IFN-γ, and GM-CSF), homocysteine, blood coagulation markers (thrombin-antithrombin, prothrombin fragments 1 & 2, d-dimers), and *APOE* genotype. Cytokine levels were compared between groups, and to a convenience sample of women without

cancer, aged 23–60 years, taken from two prior studies. (J. Vardy et al. 2014; J. L. Vardy et al. 2007).

Functional MRI and data processing

Imaging was performed on a GE Healthcare Signa 1.5 T HD MRI scanner. A sagittal T1-weighted spoiled gradient echo scan of 120 slices was acquired (TR/TE = 12.3/5.3 ms, FOV 256x256mm). For the fMRI runs, three T2*-weighted echo planar images (EPIs) sensitive to the blood oxygen level dependent (BOLD) signal were obtained, each containing 25 axial slices (TR = 2 s, FOV 64x64mm).

Imaging data were processed and analyzed using SPM8 (Statistical Parametric Mapping; Wellcome Trust Centre for Neuroimaging, London). After manual reorientation to the anterior commissure, EPIs containing 224 volumes were slice-timed, realigned to the first volume. Next, individual T1 scans were segmented based on gray matter, white matter, and cerebrospinal fluid. The co-registered EPI and T1 scans were then normalized to the Montreal Neurologic Institute reference brain using segmentation parameters. EPIs were spatially smoothed with an 8 mm full-width-half-maximum (FWHM) kernel.

fMRI paradigm

During the three fMRI scans each subject performed three sessions of a working memory n-back task, each session with a different order of task conditions. Task stimuli were projected on a screen and subjects pressed a button when a letter was the same as that presented n-times earlier. There were 3 conditions: 1-back, 2-back and 3-back, in a blocked design. Each fMRI scan lasted 7.5 min, comprising five 30-s trial blocks of each condition, 15 task blocks in total per scan.

Statistical analysis:

This study was expanded from a pilot study with a planned sample size of 60 (20/group) to 125 women with evaluable data. Our primary aim was to assess potential associations between neuropsychological evaluations (clinical tests, CANTAB, and FACT-Cog) with laboratory tests and fMRI in the three groups. Prior to reviewing data, the protocol was modified to also compare the two chemotherapy (CTh+) groups to the non-chemotherapy (CTh-) group.

All outcomes and patient characteristics were summarized using standard descriptive statistics for each group. Neuropsychological tests were organized into cognitive domains using principal components analysis (Varimax with Kaiser normalisation). (Appendix Table 1A). For clinical tests and CANTAB, raw scores were demographically corrected to T or Z-scores respectively, and converted to a deficit rating, ranging from 0 (T-score $\geq$ 40 or Z-score > −1 = no impairment) to 5 (T-score < 20 or Z-score $\leq$ −3 = severe impairment). Deficit domain scores were derived and averaged to create a global deficit score (GDS) for each subject.(Carey et al. 2004) Impairment on individual cognitive tests was defined as a T-score < 40 or Z-score $\leq$ −1.

Global cognitive impairment was defined in two ways: (i) a priori by a GDS >0.5, and (ii) in accordance with subsequent International Cognition and Cancer Task Force (ICCTF) recommendations of 2SD below normative means on at least one cognitive test or greater than 1.5SD below on two or more tests.(J. S. Wefel et al. 2011) Ingraham and Aiken (1996) probability equations were used to determine if cognitive impairment exceeded expectation with use of multiple measures. For the SET, a profile score was calculated from 0 to 4, with 4 indicating good functioning.(Burgess et al. 1998).

Fatigue was defined as a standardized FACT-F score of <68/100.(D. Cella et al. 2002) Subjects were classified as having symptoms of anxiety/depression if the GHQ was <6/12.(Goldberg 1991).

The FACT-Cog v2 symptom summary score was reported. We derived cognitive domain scores from this, based on the 35-items and domains described in FACT-Cog v3.

Differences between the three groups were evaluated using the Kruskal-Wallis test for continuous variables and χ^2 test for categorical tests. Differences comparing two groups used a Wilcoxon rank sum test. The association between outcomes was evaluated using Spearman rank sum correlation. All *P*-values are uncorrected for multiplicity. All analyses, except fMRI, was performed using SAS v9.0 (Cary, NC).

From the fMRI time series, the three separate runs were merged into one 4D file and subsequently contrast images were created for each participant, which were subsequently used in random effects analyses. Three contrasts were modeled: 3-back versus 1-back, 2-back versus 1-back and 2 + 3-back versus 1-back. To identify voxels where brain activation differed between groups ANOVA and independent t-tests were used. Main task effects are reported at $P < .05$, whole-brain FDR corrected. Group interactions were masked with the appropriate main effect at $P < .05$ to reduce the search volume to those voxels showing a main effect of task, and are reported at $P < .001$ with a cluster size threshold of 10 voxels. Age and current anti-cancer hormone use (yes/no) were covariates in all analyses.

Results

In total, 155 women consented to participate, and completed the FACT-Cog. Nine chemotherapy subjects had FACT-Cog scores between 86 and 99 and were excluded from the study. Four subjects were ineligible (psychiatric history, poor visual acuity, progressive disease), two withdrew, and 14 could not be scheduled for assessments. A total of 126 women were

included for analysis: CTh + CS+ (chemotherapy and cognitive symptoms) 44, CTh + CS- (chemotherapy and no cognitive symptoms) 52, and CTh- (no chemotherapy) 30. Figure 1. Two patients assigned to CTh + CS+ were 3–4 months outside the 60 months post surgery. We included these patients in the analysis as assessments were completed prior to identification of the protocol violations.

Demographic information is presented in Table 1. Participants had a median age of 50 years (range 29–60), and were a median of 17 months after surgery (range 1.4–64). There were no statistically significant differences between the groups for any baseline demographic variable, except women who received chemotherapy (CTh+) were younger than those who did not (CTh-) (48.25 versus 52.3 years, $P = .008$).

Self-report of cognitive function:

Women who received chemotherapy (CTh+) reported greater cognitive symptoms than those who did not receive chemotherapy (CTh-): with a summary FACT-Cog score of 107 vs 131 ($P = .003$), and significant differences in each domain. Table 2. There was a strong association between the two questionnaires (FACT-Cog, PAFI) that evaluate cognitive symptoms (rho = .76, $P < .001$).

Objective neuropsychological testing:

No differences between the groups were seen in IQ scores. Clinical neuropsychological testing revealed cognitive impairment (GDS >0.5) in 19% of women: 26% of CTh + CS+, 14% CTh + CS- and 18.5% CTh-. Impairment based on ICCTF criteria was 35.5%: 39.5% CTh + CS+, 30% CTh + CS- and 41% CTh-. The CANTAB battery found cognitive impairment in 12% of women based on GDS: CTh + CS+ 14%, CTh + CS- 6% and CTh- 20%. Impairment on CANTAB was 67%, 56% and 44% in each group respectively based on ICCTF criteria. Impairment on the SET was 9%, 10.5% and 15% respectively. None of the differences in global cognitive impairment reached the level of statistical significance. Table 3.

Women who received chemotherapy [CTh+] had greater impairment on visual motor co-ordination ($P = .018$) (assessed by the grooved pegboard test) than non-chemotherapy survivors (dominant hand 43.95 versus 48.1, $P = .055$; non-dominant hand 45.0 versus 49.8, $P = .012$). Those who did not receive chemotherapy (CTh-) scored lower on verbal learning and memory ($P = .054$), and auditory attention/working memory ($P = .019$) domains.

There was only a weak association between clinical and CANTAB GDS scores (rho = 0.29, $P = .002$). There was no association between FACT-Cog or PAFI total scores and the clinical GDS, but a weak association between PAFI and CANTAB GDS. A weak association was found between the clinical GDS and FACT-Cog perceived cognitive abilities domain (rho −0.22, $P = .02$) and between CANTAB GDS and the PAFI cognitive domain (rho −0.27, $P = .005$). Appendix Table 3A.

Psychosocial self-report questionnaires

Overall 62% of participants reported fatigue: 88% in CTh + CS+, 46% CTh + CS-, and 52% CTh- ($P = <.001$). (Table 2). There was a significant association between cognitive symptoms (FACT-Cog) and fatigue ($r = 0.42$, $P < .001$), but no association between fatigue and the GDS for clinical

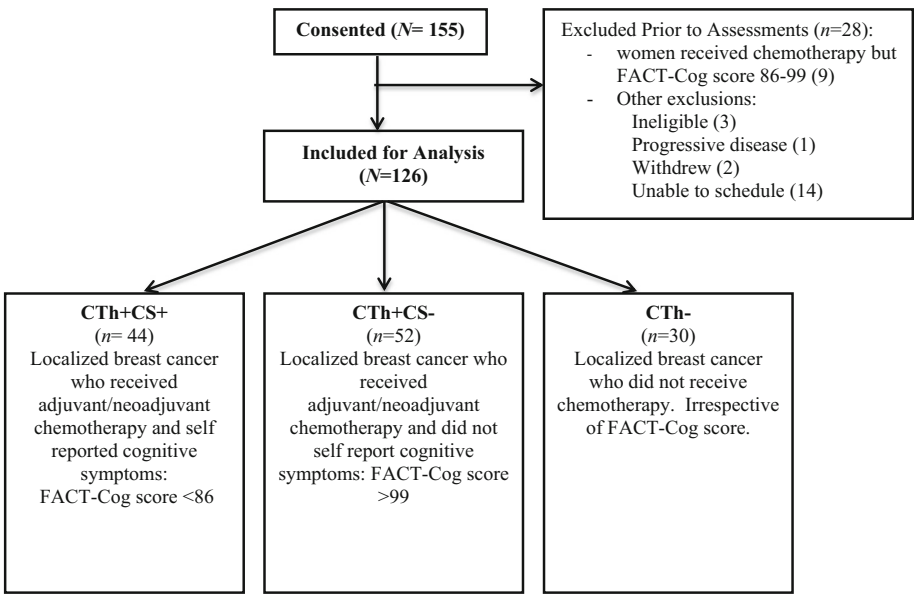

Fig. 1 CONSORT figure

CTh= chemotherapy; CS = cognitive symptoms

Table 1 Demographics of study participants

	CTh + CS + *N* = 44	CTh + CS - *N* = 52	CTh - *N* = 30	*P*-value#
Median age –years (Range)	48.39 (30–60)	48.39 (29–60)	54.10 (30–59)	.028
Time since surgery – Mean months (range)	21.16 *(4–64)	24.31 *(1.4–57)	20.08 (4–56)	.513
Type of surgery:				
Lumpectomy	27 (61%)	24 (47%)	21 (72%)	.075
Mastectomy	17 (39%)	26 (51%)	8 (28%)	
Unknown	0	1	1	
Received radiotherapy	34 (77%)	40 (78%)	22 (76%)	.962
Unknown	0	1	1	
Received adjuvant hormonal treatment	26 (59%)	34 (67%)	22 (73%)	.460
Unknown	0	1	0	
Taking adjuvant hormonal therapy at time of testing	21 (48%)	31 (58.5%)	20 (66.7%)	.210
Premenopausal at time of testing	6/39 (15%)	5/49 (10%)	7/29 (24%)	.266
Menstrual status unknown	5	3	1	
Mean Years of education (range)	15.5 (9–20)	15.1 (8–20)	15.37 (12–20)	.826
Status unknown	1	2	3	
WRAT3 score Mean (SD)	100.40 (10.1)	101.57 (11.4)	96.85 (11.1)	.151
English as a second language	9 (24%)	8 (17%)	7 (28%)	.530
Unknown	6	5	5	
Smoking status:				
Never	30 (75%)	38 (77.5%)	16 (61%)	.323^
Ex	9 (22.5%)	8 (16%)	8 (31%)	
Current	1 (2.5%)	3 (6%)	2 (8%)	
Unknown	4	3	4	
Alcohol				
Nil	19 (63%)	23 (57.5%)	13 (52%)	.699#
1–2 glasses	8 (27%)	17 (42.5%)	10 (40%)	
3+ glasses	3 (10%)	0 (0%)	2 (8%)	
Unknown	14	12	5	

CTh chemotherapy, *CS* cognitive symptoms

*Includes some patients who had neoadjuvant chemotherapy

P-value: CTh + CS+ vs CTh + CS- vs CTh-

^ Never smoked versus ex and current smokers. # Non-drinker versus drinker

neuropsychological tests ($r = 0.10$, $P > .30$). Overall 15% had symptoms of anxiety/depression: 28% in CTh + CS+, 10% CTh + CS- and 4% CTh-. Women reporting cognitive symptoms had lower QOL ($r = 0.53$, $P < .001$) and greater symptoms of anxiety/depression ($r = -0.43$, $P < .001$), but no association was found between these symptoms and objective neuropsychological results. There was an association between fatigue and symptoms of anxiety/depression ($r = 0.57$, $P < .001$).

Laboratory results

All groups had elevated cytokine levels compared to healthy volunteers ($n = 56$)(J. Vardy et al. 2014; J. L. Vardy et al. 2007) (Appendix Table 4A), but there was no significant difference in cytokine levels between the three breast cancer groups. Elevated levels of interleukin-8 were associated with more cognitive impairment on the clinical GDS (rho = 0.27, $p = .03$), and there was a weak association with elevated interleukin-1 and -2. (Appendix Table 5A). There was no association between cytokine levels and fatigue.

There were no clinically important differences between the groups in the remainder of the blood parameters, except that women who had not received chemotherapy had lower levels of prothrombin fragments 1 and 2 than those who had chemotherapy. Due to a freezer malfunction some blood samples were lost, in particular the prothrombin fragments and d-dimers, so the clinical significance of these results is unknown.

Apolipoprotein genotyping was available for 59 participants: 10 were heterozygotes for the *APOE ε4* allele (5/17 (29%) in CTh + CS+, 4/31 (13%) in CTh + CS- and 1/11

Table 2 Self-Report questionnaire results

	CTh + CS + *N* = 44	CTh + CS - *N* = 52	CTh - *N* = 30	*P*-value#	*P*-value CTh + vs CTh-
FACT-Cog Mean Summary Score (range)	69.2 (29–95)	138.75 (106–167)	130.8 (30–164)	<.001	.003
Domains:					
Perceived cognitive impairment	26.7	57.25	55.9	<.001	<.001
Perceived cognitive abilities	12.2	21.6	20.7	<.001	.024
Comments from others	8.6	13.9	14.3	<.001	.001
Impact on quality of life	7.2	14.6	14.3	<.001	<.001
PAFI Mean Total Score (range)	100.0 (72–135)	131.5 (70–160)	130.8 (75–148)	<.001	.003
Domains:					
Memory	27.2	38.0	38.4	<.001	.003
Language	24.5	33.3	32.1	<.001	.040
Sensory	20.7	22.0	22.5	.081	.256
Cognitive	27.7	38.1	37.8	<.001	.011
Standardized FACT-F Mean (range)	60.6 (41–100)	70.7 (45–100)	72.5 (49–100)	<.001	.029
N (%) Fatigued (FACT-F < 68/100)	37/42 (88%)	23/50 (46%)	13/25 (52%)	<.001	.251
FACT-G Mean (range)	76.6 (48–100)	89.4 (58–106)	89.4 (78–101)	<.001	.272
Physical well-being	20.0	23.8	25.8	<.001	.003
Social well-being	21.0	23.8	21.8	.133	.438
Emotional well-being	17.3	19.4	19.5	.016	.257
Functional well-being	18.0	22.5	22.4	.001	.359
GHQ Mean (range)	4.5 (0–12)	1.9 (0–9)	1.6 (0–9)	<.001	.021
Symptoms of Anxiety/Depression	12/43 (28%)	5/49 (10%)	1/25 (4%)	.016	.116

CTh chemotherapy, *CS* cognitive symptoms

FACT-Cog = Functional Assessment of Cancer Therapy -Cognition (lower score denotes more symptoms)

PAFI = Patients Assessment of Own Functioning Inventory (lower score denotes more symptoms)

FACT-F = Functional Assessment of Cancer Therapy – Fatigue (lower score denotes more symptoms)

FACT-G = Functional Assessment of Cancer Therapy –General (lower score denotes worse functioning)

GHQ = General Health Questionnaire 12 (higher score denotes more symptoms)

P-value: CTh + CS+ vs CTh + CS- vs CTh-

(9%) in CTh-; between-group difference not significant); there were no homozygotes. There were no significant differences in GDS on clinical or CANTAB tests, rates of cognitive impairment or FACT-Cog scores between those with an *APOE ε4* allele and those without.

Functional MRI (fMRI):

Two participant's fMRI data were excluded from analyses due to movement artifacts. The final sample consisted of 34, 44 and 23 participants in CTh + CS+, CTh + CS- and CTh- respectively. Across groups, the fMRI results showed a significant main task-effect for all contrasts in frontal and parietal areas. (Appendix Table 6A, Fig. 2). Group comparison analyses revealed that CTh + CS+ showed hypoactivation compared to CTh- on n-back 2 + 3 > 1 in the right precentral gyrus, and n-back 2 > 1 in the bilateral putamen, left amygdala, left caudate, right precentral gyrus and right cerebellum. Compared to CTh-, CTh + CS- also showed hypoactivation on n-back 2 + 3 > 1 in the occipital lobe and on 2 > 1 in the left putamen, left amygdala and left cerebellum. Comparing the two chemotherapy-treated groups we found hyperactivation in CTh + CS+ versus CTh + CS- in the middle frontal gyrus on n-back 3 > 1.

Finally, compared to CTh-, CTh + showed hypoactivation on n-back 2 + 3 > 1 and 2 > 1 in the right precentral gyrus, left putamen and amygdala and bilateral cerebellum. No other group differences were found.

Discussion

Our large cognitive study in breast cancer survivors is novel in its use of comprehensive cognitive testing with the use of both traditional and computerized tests, analysis using different criteria for impairment, and the inclusion of fMRI imaging and correlative studies. We found 19% of breast cancer survivors had cognitive impairment on clinical neuropsychological

Table 3 Number (percent) of participants classified as impaired on neuropsychological (NP) clinical tests and CANTAB tests by study group

Neuropsychological Test and Impairment criteria	CTh + CS + *N* = 43^ N (%)	CTh + CS - *N* = 50 N (%)	CTh - *N* = 30 N (%)	*P*-value#	*P*-value CTh + vs CTh-
Clinical NP Tests					
Overall Cognitive Impairment on GDS criteria [a]	11/43 (26%)	7/50 (14%)	5/27 (18.5%)	.363	1.000
Overall Cognitive Impairment on ICCTF criteria[b]	17/43 (39.5%)	15/50 (30%)	11/27 (41%)	.539	0.649
N (%) of tests with deficits:					
No deficits:	9 (21%)	13 (26%)	3 (11%)		
1 test	9 (21%)	15 (30%)	5 (18.5%)		
2 tests	11 (26%)	5 (10%)	7 (26%)		
3–4 tests	5 (12%)	11 (22%)	5 (18.5%)		
5–6 tests	8 (19%)	3 (6%)	6 (22%)		
7–13 tests	1 (2%)	3 (6%)	1 (4%)		
CANTAB tests	*N* = 35–36	*N* = 49–50	*N* = 25		
Overall Cognitive Impairment on GDS criteria [c]	5 (14%)	3 (6%)	5 (20%)	.159	.170
Overall Cognitive Impairment on ICCTF criteria[c]	24 (67%)	28 (56%)	11 (44%)	.224	.172
N (%) of impaired tests:					
No deficits	3 (8%)	10 (20%)	4 (16%)		
1 test	7 (19%)	10 (20%)	1 (4%)		
2 tests	6 (17%)	6 (12%)	6 (24%)		
3–4 tests	11 (30%)	10 (20%)	7 (28%)		
5–6 tests	7 (19%)	7 (14%)	3 (12%)		
7–15 tests	2 (6%)	7 (14%)	4 (16%)		
Six Elements Test	*N* = 35	*N* = 38	*N* = 27		
Impairment	3 (9%)	4 (10.5%)	4 (15%)	.787	.482

CTh chemotherapy, *CS* cognitive symptoms

[a] Impairment defined as GDS > 0.5

[b] Impairment defined as T-score < 35 on 2 tests or T-score < 30 on 1 test

[c] Impairment defined as Z-score < −1.5 on 2 tests or Z-score < −2 on 1 test

P-value: CTh + CS+ vs CTh + CS- vs CTh-

^ Numbers vary between total neuropsychological tests completed due to missing data

tests based on our GDS criterion, with 35.5% on ICCTF criterion, but there were no significant differences between groups, despite differences in cognitive symptoms. The discrepancy in cognitive impairment rates between the GDS and ICCTF criteria, on both clinical neuropsychological tests and CANTAB, was also seen in our large, longitudinal cognitive study in colorectal cancer survivors(J. L. Vardy et al. 2015), with higher impairment rates on ICCTF criteria. This highlights the importance of having an a priori definition for cognitive impairment. There was only a weak-to-moderate association seen between the GDS on the clinical neuropsychological tests and CANTAB in both studies, suggesting they are measuring different constructs, despite CANTAB being based on traditional cognitive tests. Based on our results we recommend primary use of clinical neuropsychological tests for future studies.

The only cognitive domain in which patients who received chemotherapy scored consistently lower than those who did not receive chemotherapy was visual motor co-ordination. We did not assess for chemotherapy-induced peripheral neuropathy, but the majority of the CTh + survivors received a taxane chemotherapy, which is known to cause peripheral neuropathy, and might have influenced fine motor control on the grooved pegboard test. Survivors who received chemotherapy self–reported more cognitive impairment than those who did not require chemotherapy, but we found at best a weak association between perceived cognitive impairment and objective neuropsychological testing. These findings are consistent with our study in colorectal cancer survivors, (J. Vardy et al. 2014; J. L. Vardy et al. 2015) and much of the breast cancer literature. (Ahles et al. 2002; Castellon et al. 2004; Schagen et al. 1999; Tchen et al. 2003) Consistent with most other studies we found no statistical association of anxiety/depression, fatigue or QOL with objective neuropsychological impairment, but did observe a relationship between these symptoms and self-reported cognitive impairment,(Castellon et al. 2004;

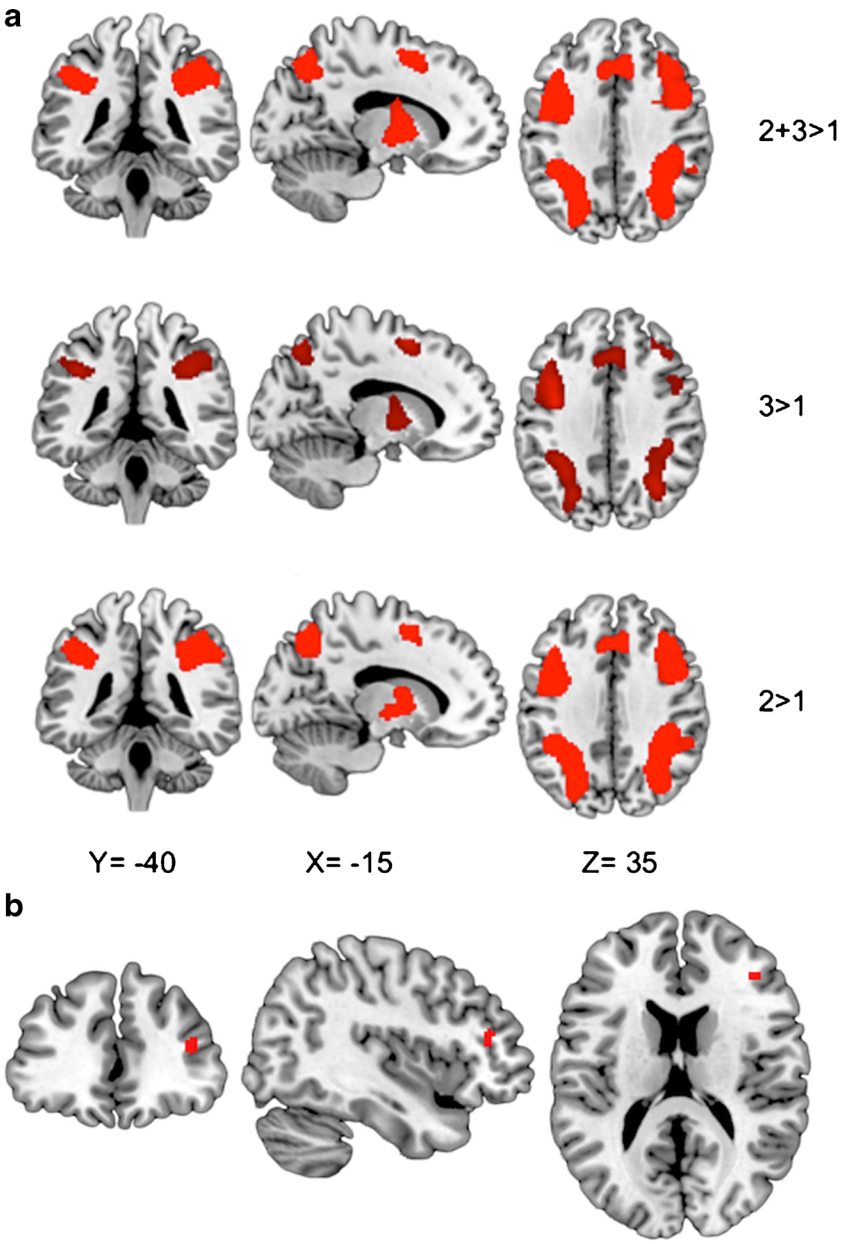

Fig. 2 fMRI Activation during n-back working memory task. **a** Main task effect across groups for all contrasts. **b** Chemotherapy with cognitive symptoms (CTh + CS+) compared to chemotherapy without symptoms (CTh + CTh-): CTh + with cognitive symptoms have more frontal activation than CTh + without symptoms on contrast 2 (n-back 3 > 1). **c** Chemotherapy with symptoms (CTh + CS+) compared to non-chemotherapy (CTh-): Non-CTh had more activation than CTh + with cognitive symptoms in frontal and parietal regions on contrast 1 (n-back 2 + 3 > 1, Fig. 2c-i). Non-CTh had more activation than CTh + with cognitive symptoms in temporal, frontal and parietal regions on contrast 3 (2 > 1, Fig. 2c–ii). **d** Chemotherapy without cognitive symptoms (CTh + CS-) compared to non-chemotherapy (CTh-): Non-chemotherapy have more activation than CTh + without symptoms on contrast 1 (n-back 2 + 3 > 1) in parietal region (Fig. 2d-i) and contrast 3 (n-back 2 > 1) in temporal region (Fig. 2d–ii)

Jenkins et al. 2006; Schagen et al. 2006; J. Vardy et al. 2014; J. L. Vardy et al. 2015; Jeffrey S. Wefel et al. 2010). This has implications for therapeutic and preventive strategies, and highlights the importance of assessing for anxiety/depression and fatigue in women reporting cognitive symptoms, and treating if present.

In healthy volunteers plasma cytokine levels are low, whereas elevated levels occurred in women with breast cancer up to 5 years after diagnosis. Because there were no significant differences between the breast cancer groups, it appears that the elevated cytokines are associated with cancer per se rather than chemotherapy. Three studies in women with breast cancer have shown elevated IL-6 and/or TNF to be associated with poorer cognitive function.(Ganz et al. 2013; S. Kesler et al. 2013; Patel et al. 2015) In our study, higher levels of IL-8, −1 and −2 were associated with a trend to greater impairment on the clinical

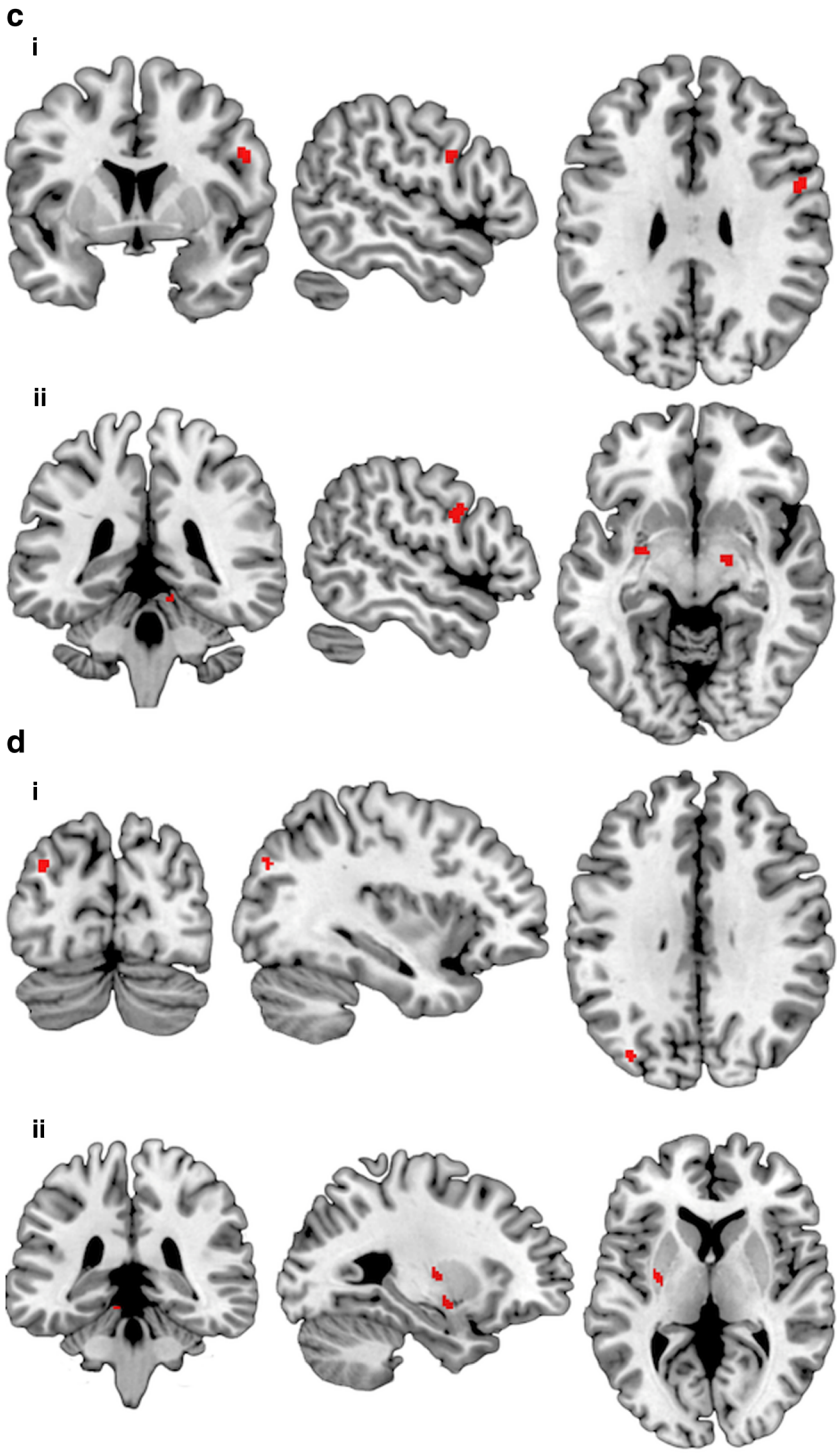

Fig. 2 (continued)

neuropsychological tests. This is in contrast to our larger colorectal cancer study in which we did not find an association between cytokine levels and global cognitive impairment, but did find elevated IL-8 to be associated with reduced information–processing speed.(J. L. Vardy et al. 2015).

The fMRI results are subtle but intriguing. All groups of breast cancer survivors had activation patterns similar to what would be expected in the general population, with increasing activation in the frontal and parietal areas as memory tasks become more difficult. Both groups of women who received chemotherapy had less intense patterns of activation. Women who had received chemotherapy who self-reported cognitive impairment had increased frontal activation compared to women who received chemotherapy, but did not report

cognitive impairment. However, this was only visible in the most difficult task-condition. These findings imply that chemotherapy has an effect on fMRI patterns in addition to any impact from cancer or other treatments.

Previous neuroimaging studies have generally had small sample sizes and many did not include comprehensive neuropsychological testing. A number of fMRI cross-sectional studies in breast cancer survivors have similarly found hypoactivation in prefrontal and parietal regions during tasks evaluating executive function 5–10 years after adjuvant chemotherapy,(Conroy et al. 2013; de Ruiter et al. 2012; de Ruiter et al. 2011; S. R. Kesler et al. 2011) with most not finding differences in task performance. de Ruiter evaluated fMRI and brain morphology with structural and diffusion MRI in breast cancer survivors 10 years after high-dose chemotherapy and found hypoactivity in the posterior parietal cortex, with reduction in gray matter volume and reduction in integrity in the adjacent white matter tracts(de Ruiter et al. 2012; de Ruiter et al. 2011) in comparison to patients who did not receive chemotherapy.(de Ruiter et al. 2011) Conroy et al. found greater decreases in activation with increasing time since chemotherapy, with some improvement in gray matter volume over time.(Conroy et al. 2013) A large study evaluating diffusion tensor imaging in women who were on average 20 years post-chemotherapy found time from treatment was associated with increased compromise of white matter integrity.(Koppelmans et al. 2014) The same group evaluated women with breast cancer prior to chemotherapy and found increasing prefrontal hyperactivation with increasing task difficulty for a planning but not a memory task, and decreased white matter integrity compared to non-cancer controls.(Menning et al. 2015).

A recent prospective longitudinal study reported that women with breast cancer had increased bifrontal and parietal activation, with decreased left parietal activation compared to non-cancer controls at baseline.(McDonald et al. 2012) One month after completing chemotherapy there was a decrease in prefrontal activation, that normalized at one year, and a decrease in frontal gray matter density. Another study from the same group confirmed the decrease in frontal gray matter density after chemotherapy, associated with changes in cognitive symptoms.(McDonald et al. 2013) Similar to many of the studies above, our fMRI results found hypoactivation in women who had received chemotherapy. We also found overall increased frontal activation on more difficult tasks in those who received chemotherapy and reported cognitive symptoms. Another study found differences in resting-state functional connectivity on MRI between women who had cognitive symptoms and those who did not after chemotherapy.(Piccirillo et al. 2015) Studies in the field of anxiety/depression symptomatology show that anxiety and depression can be related to increased,(Gotlib et al. 2005; Pannekoek et al. 2015) as well as decreased frontal activation.(Fitzgerald et al. 2008; Schlund et al. 2012) However, modulation of brain activation in relation to distress has been mainly investigated with resting state fMRI or task-fMRI with emotionally loaded stimuli. Although the fMRI task used in the present study did not comprise any emotional stimuli, we cannot completely rule out that the effects on the increased brain activation in the CTh + group with complaints, are confounded by the contributory role of distress.

Our study has limitations. Due to a technical problem we do not have response scores to the n-back task to correlate with brain activation patterns. As discussed, we did not examine the affect of distress, anxiety/depression or fatigue on the fMRI results, and therefore cannot exclude that these symptoms may impact on the differences we saw between the groups on fMRI. The study provides no information about cognitive function pre-chemotherapy or changes over time, and we did not include a non-cancer control group. The blood samples were not collected at standardized times of the day and some samples were lost leading to smaller sample numbers for the apolipoprotein and prothrombin tests. We acknowledge that we have not corrected for multiple comparisons and given the number of results some may be due to chance. Strengths of our study are the large sample size with functional brain imaging, use of both comprehensive clinical tests and computerized neuropsychological tests, with analysis according to two different criteria of impairment, inclusion of patient reported outcomes with comparison between those who received chemotherapy and then do and do not self-report cognitive symptoms, and laboratory correlates.

In summary, results of our study support our primary hypothesis that women who received adjuvant chemotherapy for breast cancer would report more cognitive impairment than women who did not receive chemotherapy. Formal neuropsychological test results did not support the hypothesis of objective differences in cognitive function between groups, but self-reported impairment was associated with fatigue, symptoms of anxiety/depression, and lower QOL. Elevated levels of IL-8 were associated with cognitive impairment on clinical tests. Functional imaging suggests that brain activation may be modified by chemotherapy with hypoactivation compared to women with breast cancer who had not received chemotherapy.

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Compliance with ethical standards

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Conflicts of interest None of the authors have any conflicts of interest to report.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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