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CLINICAL TRIAL

Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study

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Abstract Brain gray matter alterations have been reported in cross-sectional magnetic resonance imaging (MRI) studies of breast cancer patients after cancer treatment. Here we report the first prospective MRI study of women undergoing treatment for breast cancer, with or without chemotherapy, as well as healthy controls. We hypothesized that chemotherapy-associated changes in gray matter density would be detectable 1 month after treatment, with partial recovery 1 year later. Participants included breast cancer patients treated with (CTx+, $N = 17$) or without (CTx−, $N = 12$) chemotherapy and matched healthy controls ($N = 18$). MRI scans were acquired at baseline (after surgery but before radiation, chemotherapy, and/or anti-estrogen treatment), 1 month after completion of chemotherapy (M1), and 1 year later (Y1). Voxel-based morphometry (VBM) was used to evaluate gray matter density differences between groups and over time. There were no between-group gray matter differences at baseline. Group-by-time interactions showed declines from baseline to M1 in both cancer groups relative to controls. Within-group analyses indicated that at M1 relative to baseline the CTx+ group had decreased gray matter density in bilateral frontal, temporal, and cerebellar regions and right thalamus. Recovery was seen at Y1 in some regions, although persistent decreases were also apparent. No significant within-group changes were found in the CTx− or control groups. Findings were not attributable to recency of cancer surgery, disease stage, psychiatric symptoms, psychotropic medication use, or hormonal treatment status. This study is the first to use a prospective, longitudinal approach to document decreased brain gray matter density shortly after breast cancer chemotherapy and its course of recovery over time. These gray matter alterations appear primarily related to the effects of chemotherapy, rather than solely reflecting host factors, the cancer disease process, or effects of other cancer treatments.

Keywords Adjuvant chemotherapy · Brain · Breast cancer · Magnetic resonance imaging · Neuroimaging

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Introduction

Improvements in cancer screening and treatment have in turn increased long-term survivorship and attention to survivors' daily functioning and quality of life. Cognitive impairment related to breast cancer treatment has, therefore, become an important area of study. Studies have

typically shown decreased neuropsychological performance in breast cancer patients treated with chemotherapy, although such changes have not been apparent in some cohorts (for review and meta-analysis see [3, 10, 33]). Effects have been reported most prominently in working memory, executive functions, and processing speed, cognitive processes largely subserved by frontally mediated brain systems. Subjective symptoms are most often noted during chemotherapy and acutely post-treatment, but appear to resolve in most patients, with only a subset reporting persistent problems. These effects are observed independent of factors such as depression and anxiety, which can be related to both poor cognitive performance and cancer and its treatment.

Anti-estrogen (hormonal) therapies have shown detrimental cognitive effects distinct from chemotherapy in some studies [7, 9, 24, 30], although not others [15, 17]. A higher than expected incidence of impaired cognitive performance has also been found in breast cancer patients at baseline (before exposure to systemic treatment) [2, 36, 37], suggesting that some effects may be reflective of host factors and/or the disease process itself rather than treatment. A few prospective studies have documented cognitive changes differentially attributable to chemotherapy, radiation, and anti-estrogen treatment [9, 18, 25], further arguing for the investigation of the potentially additive and dissociable effects of cancer treatments and the disease process on cognition in vulnerable individuals [35].

The neural mechanisms underlying cognitive changes related to cancer and its treatment are poorly understood. Several possible biological pathways have been proposed to account for factors that increase risk for both cancer and cognitive impairment independently, as well as the potential interaction of these factors with cancer treatments [1]. Structural and functional neuroimaging techniques provide a unique opportunity to examine the neural substrate of cancer- and treatment-related cognitive changes. Voxel-based morphometry (VBM) is a method of quantitatively evaluating tissue changes on a voxel-by-voxel basis throughout the entire brain [4, 5, 13]. VBM is a fully automated procedure for examining tissue integrity that provides the ability to assess regional volume and density of brain tissue compartments, unlike morphological methods that involve manual segmentation of selected structures. As it evaluates every voxel within a tissue compartment relative to an a priori statistical threshold VBM provides an unbiased, comprehensive, and highly reliable assessment sensitive to local changes.

A small number of cross-sectional, retrospective studies have utilized VBM to examine gray matter changes after breast cancer treatment [14, 16, 21, 28, 42]. The few studies comparing gray matter between breast cancer patients who did and did not receive chemotherapy have demonstrated residual gray matter deficits in the chemotherapy-treated group, even several years after treatment completion [16, 21, 28]. Due to their retrospective design, these earlier studies are limited by the absence of a pre-treatment baseline against which to compare post-treatment changes. As yet, no study has prospectively examined these changes and their recovery over time. The purpose of this investigation was to assess gray matter alterations related to breast cancer and its treatment prospectively in patients treated with and without standard-dose systemic chemotherapy and demographically matched healthy controls. Prior cross-sectional studies in our lab [21, 28] and others [16] have shown persistent gray matter decreases in breast cancer survivors after chemotherapy. As this work was retrospective, with patients typically studied several years after chemotherapy completion, the acute effects of chemotherapy on gray matter density have not yet been established. We predicted that such changes would be detectable in the near-term (i.e., 1 month after chemotherapy completion) but would likely remit with time, given prior cognitive studies suggesting longitudinal improvement in brain function after chemotherapy and the typical clinical observation that patients generally evidence subjective symptomatic improvement with time [9, 36]. We therefore hypothesized that chemotherapy-related decreases in gray matter density would be detectable 1 month after treatment and show partial to complete recovery 1 year later.

Participants

Written informed consent was obtained from all participants according to the Declaration of Helsinki under a protocol approved by the Dartmouth College Committee for the Protection of Human Subjects.

Participants were female breast cancer patients treated with (CTx+, $N = 17$) and without (CTx−, $N = 12$) systemic chemotherapy and healthy controls ($N = 18$). There were no between-group demographic differences ($P > .05$). Patients had non-invasive (stage 0) or non-metastatic invasive (stages I, II, or IIIA) disease, and were treated with common standard-dose chemotherapy regimens (primarily AC-taxol; all received doxorubicin and cyclophosphamide; see Table 1 for demographic and treatment data). Exclusion criteria for all groups were: (1) prior treatment with cancer chemotherapy, CNS radiation, or intrathecal therapy; (2) current or past alcohol or drug dependence; and (3) neurobehavioral risk factors including neurologic, medical, or psychiatric conditions known to affect brain structure or function, except history of depression or anxiety in breast cancer patients. Potential participants for all groups were excluded for current diagnosis of any DSM-IV Axis I disorder or a history of any psychiatric disorder requiring hospitalization. Anxiety and depression symptoms were

Table 1 Sample demographics

	CTx+ ($N = 17$)	CTx− ($N = 12$)	Control ($N = 18$)
Age at baseline (years)	52.4 (8.5)	52.7 (7.2)	50.6 (6.5)
Education (years)	15.2 (2.6)	16.1 (2.3)	15.8 (1.8)
Estimated full scale IQ (Barona index [6])	112.0 (6.7)	114.7 (4.7)	113.7 (3.5)
Handedness (R, L/Amb)	15, 2	12, 0	16, 2
Days from baseline to M1 scan	170.0 (64.1)	191.8 (72.6)	182.6 (50.3)
Days from M1 to Y1 scan	352.3 (50.9)	303.9 (70.2)	324.9 (74.4)
Days from baseline to Y1 scan	522.3 (53.4)	495.8 (53.1)	507.5 (74.4)
Days from last cancer-related surgery to baseline scan*	29.9 (11.0)	49.9 (31.8)	
Days from baseline scan to initiation of adjuvant treatment	8.7 (6.1)	2.2 (22.9)	
Cancer stage: 0 (DCIS)	0	4	
I	4	6	
II	12	2	
IIIA	1	0	
Received radiotherapy	12	8	
Number on anti-estrogen therapy: baseline	0	1 TAM	
M1	3 TAM	6 TAM	
	1 ANA	1 TAM/GOS	
		2 ANA	
Y1	9 TAM	6 TAM	
	1 ANA	1 TAM/GOS	
	3 LET	2 ANA	
Number on psychotropic medication: baseline	4	5	
M1	5	4	
Y1	5	6	
Chemotherapy regimen[a]:			
Doxorubicin/cyclophosphamide/paclitaxel (AC-taxol)	12		
Docetaxel/doxorubicin/cyclophosphamide (TAC)	2		
Doxorubicin/cyclophosphamide (AC)	3		

Values are mean (SD)

* Significant between-group difference, $P < .05$

TAM tamoxifen, *ANA* anastrozole, *GOS* goserelin, *LET* letrozole

[a] One CTx+ patient was also treated with trastuzumab for 2 months after completion of chemotherapy

assessed at each study visit with the Center for Epidemiologic Studies-Depression Scale (CES-D) [26] and the State-Trait Anxiety Inventory-State subscale (STAI-S) [32] (Table 2).

Methods

All measures were completed at baseline (after surgery but before radiation, chemotherapy, and/or anti-estrogen treatment), 1 month following the completion of chemotherapy (M1), and 1 year later (Y1), or yoked intervals for the CTx− and control groups. One CTx− participant began tamoxifen 18 days prior to her baseline scan, and two CTx− participants had MammoSite® treatment prior to their baseline scans (3 days and 36 days prior to scanning, respectively). Data were analyzed with and without these subjects, without a change in the overall pattern of results.

Magnetic resonance imaging (MRI) scan acquisition

All scans were acquired on the same 1.5T Signa LX scanner (GE Medical Systems, Waukesha, WI) with echospeed gradients using the standard GE clinical RF head coil. A coronal T1-weighted three-dimensional spoiled gradient recalled acquisition in the steady state (SPGR) volume was used for VBM, with the following parameters: TR = 25 ms, TE = 3 ms, FOV = 24 cm, FA = 40°, NEX = 1, 124 1.5 mm thick coronal slices with no skip, 256 × 256 matrix, and in-plane resolution of .9375 mm^2. T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences were also acquired to rule out incidental pathology.

Image analysis

Locally developed MATLAB (Version 7, Mathworks, Inc., Natick, MA) scripts were used to implement optimized

Table 2 Mood and anxiety self-rating raw scores (mean (SD), number above threshold*)

Time-point	Measure	CTx+ ($N = 17$)	CTx− ($N = 12$)	Control ($N = 18$)
Baseline	CES-D	8.2 (8.2), 2	5.7 (6.6), 1	4.5 (4.5), 0
	STAI-S	30.2 (10.4), 2	28.8 (13.1), 1	27.1 (7.2), 0
M1	CES-D	9.9 (10.3), 3	6.1 (5.7), 1	4.4 (4.6), 0
	STAI-S	30.8 (13.7), 2	27.1 (9.2), 1	27.3 (8.2), 1
Y1	CES-D	6.8 (6.2), 3	7.5 (10.4), 1	4.7 (8.9), 1
	STAI-S	27.6 (8.8), 1	28.3 (11.3), 1	25.6 (7.2), 0

There were no significant between-group differences at any time-point or any group-by-time interactions ($P > .05$)

CES-D Center for Epidemiologic Studies-Depression Scale, *STAI-S* State-Trait Anxiety Inventory-State Scale

* Thresholds for significant symptomatology: CES-D raw score ≥16, STAI-S *T* score ≥65

VBM methods [4, 5, 13] using SPM (Version 5, Wellcome Department of Imaging Neuroscience, London, UK), similar to our prior studies [27, 29, 39]. Briefly, after reconstruction SPGR follow-up scans were registered to the baseline scan for each subject. Scans were then registered to the Montreal Neurological Institute (MNI) T1-weighted template and segmented into gray matter, white matter, and cerebrospinal fluid compartments using the MNI T1-weighted template and corresponding tissue probability maps. Gray matter maps were then spatially normalized to MNI space, resampled to 1 mm isotropic voxels, and smoothed using an isotropic Gaussian spatial filter (FHWM = 10 mm) to reduce residual inter-individual variability. The smoothed, normalized gray matter maps were subjected to statistical parametric mapping on a voxel-by-voxel basis using the general linear model as implemented in SPM5. The SPM5 prior probability gray matter template was used to restrict the statistical comparisons to the gray matter compartment. Random effects analyses were conducted using analysis of variance (ANOVA) to construct contrast maps of voxels in which local gray matter density differed between groups and over time. Comparisons were conducted within an omnibus group (three independent levels: CTx+, CTx−, and control) by time (three non-independent levels: baseline, M1, and Y1) ANOVA. The design matrix therefore included all time-points for all groups, accounting for the repeated measures factor of time (i.e., the matrix included nine columns, one for each group at baseline, M1, and Y1). The critical significance threshold (P_{crit}) was set to .001 with a minimum cluster extent (k) of 1,400 contiguous voxels. This k value was chosen to limit results only to regions that survived a whole-brain search cluster-level threshold of $P_{corrected} < .05$.

Within the omnibus SPM5 ANOVA design matrix, between-group comparisons were conducted using weighted contrast vectors. For example, pair-wise comparisons of gray matter density at baseline (CTx+ vs. CTx−, CTx+ vs. control, CTx− vs. control) were conducted by entering values of 1 and −1 in the appropriate columns in the matrix. In this manner, examination of regions where controls showed greater gray matter density than CTx+ at baseline would be conducted by entering 1 in the control baseline column and −1 in the CTx+ baseline column. Group-by-time interactions were conducted in a similar fashion. For example, to evaluate regions in which the control and CTx+ groups showed significant differences from baseline to M1, values of 1 would be entered in the CTx+ baseline and control M1 columns, and values of −1 would be entered in the CTx+ M1 and control baseline columns (and vice versa for the inverse interaction). Based on prior research and patient reports that cognitive changes are most apparent in the initial period after chemotherapy, with subsequent improvement over time, we hypothesized that in the CTx+ group decreases in gray matter density would be apparent at M1, with recovery at Y1. We also hypothesized that while some regions might recover fully, others might show persistent gray matter density changes at Y1. Two approaches were used to model these effects. Within the omnibus matrix described above, to examine decline from baseline to M1 followed by complete recovery at Y1 a vector of 1 −2 1 was entered in the baseline, M1, and Y1 columns for each group (CTx+, CTx−, and control) separately. To model regions where declines from baseline to M1 persisted at Y1 a vector of 2 −1 −1 was entered in the same fashion.

To address multiple testing issues significance levels are reported here for both voxel-level $P_{FWE\text{-}corrected}$ and cluster-level $P_{corrected}$. The voxel-level significance values can be interpreted as the chance (under the null hypothesis) of finding a voxel with as great or greater a height threshold (T or Z), with family-wise error correction for the whole-brain search volume (i.e., correction for multiple comparisons or "false positives"). Cluster-level significance in this context can be interpreted as the probability (under the null hypothesis) of finding a cluster with as great or greater

a number of voxels (cluster extent, k), corrected for the whole-brain search volume.

Results

For all VBM analyses MNI coordinates, cluster extent, P values, and region descriptions are presented in Table 3.

Between-group analyses

There were no between-group gray matter differences at baseline. Group-by-time interaction analyses showed reduced gray matter density in both cancer groups relative to controls at M1 relative to baseline. In CTx+ patients, reductions were apparent in bilateral middle frontal gyri and left cerebellum (Fig. 1a), whereas in the CTx− patients,

Table 3 Regional gray matter changes ($P_{crit} < .001$, $k = 1{,}400$)

MNI coordinates (x y z)	Cluster extent (k)	Cluster-level $P_{corrected}$	Voxel-level $P_{FWE\text{-}corrected}$	T	Region description (for cluster peak)
Between-group analyses					
Interaction control > CTx+ from baseline to M1					
−41 40 −7	2,204	.008	.01	5.36	L middle frontal gyrus (BA47)
−35 −63 −49	7,732	<.001	NS	4.85	L cerebellum
26 52 −11	1,952	.014	NS	4.62	R middle frontal gyrus (BA11)
Interaction control > CTx+ from baseline to Y1					
8 −80 −43	3,514	.001	NS	4.33	R cerebellum
−39 −53 −50	1,611	.032	NS	4.15	L cerebellum
Interaction control > CTx− from baseline to M1					
16 −75 −37	2,050	.011	NS	4.48	R cerebellum
Within-group (CTx+) analyses					
Gray matter decline from baseline to M1					
−44 39 −6	5,600	<.001	<.001	6.23	L middle frontal gyrus (BA47)
14 30 54	6,063	<.001	.011	5.34	R superior frontal gyrus (BA8)
52 −2 39	2,823	.002	.023	5.14	R precentral gyrus (BA6)
12 56 −16	3,895	<.001	.053	4.92	R superior frontal gyrus (BA11)
−37 −71 −33	4,915	<.001	NS	4.74	L cerebellum
−23 −23 −10	1,744	.023	NS	4.52	L parahippocampal gyrus (BA28)
−15 49 36	2,734	.003	NS	4.45	L superior frontal gyrus (BA9)
34 6 −45	2,568	.004	NS	4.35	R superior temporal gyrus (BA38)
24 −30 2	2,883	.002	NS	4.31	R thalamus
38 −74 −29	1,486	.043	NS	3.94	R cerebellum
Gray matter decline from baseline to M1 with recovery at Y1					
−42 39 −7	2,903	.002	.011	5.33	L middle frontal gyrus (BA47)
14 30 54	3,443	.001	.028	5.09	R superior frontal gyrus (BA8)
−12 49 37	1,753	.022	NS	4.76	L superior frontal gyrus (BA9)
33 9 −49	1,592	.033	NS	4.36	R superior temporal gyrus (BA38)
32 57 −4	1,906	.016	NS	4.35	R superior frontal gyrus (BA10)
9 −53 −27	1,537	.038	NS	4.23	R cerebellum
Gray matter decline from baseline to M1 persisting at Y1					
−44 39 −6	2,025	.012	.010	5.36	L middle frontal gyrus (BA47)
24 −30 2	2,776	.002	.025	5.12	R thalamus
16 17 59	2,460	.005	.030	5.07	R superior frontal gyrus (BA6)
−38 −58 −48	8,184	<.001	.035	5.03	L cerebellum
10 −78 −41	6,352	<.001	NS	4.86	R cerebellum
8 57 −17	1,836	.018	NS	4.78	R medial frontal gyrus (BA11)
35 −13 51	2,550	.004	NS	4.77	R precentral gyrus (BA6)
−33 29 41	1,849	.018	NS	4.16	L middle frontal gyrus (BA9)

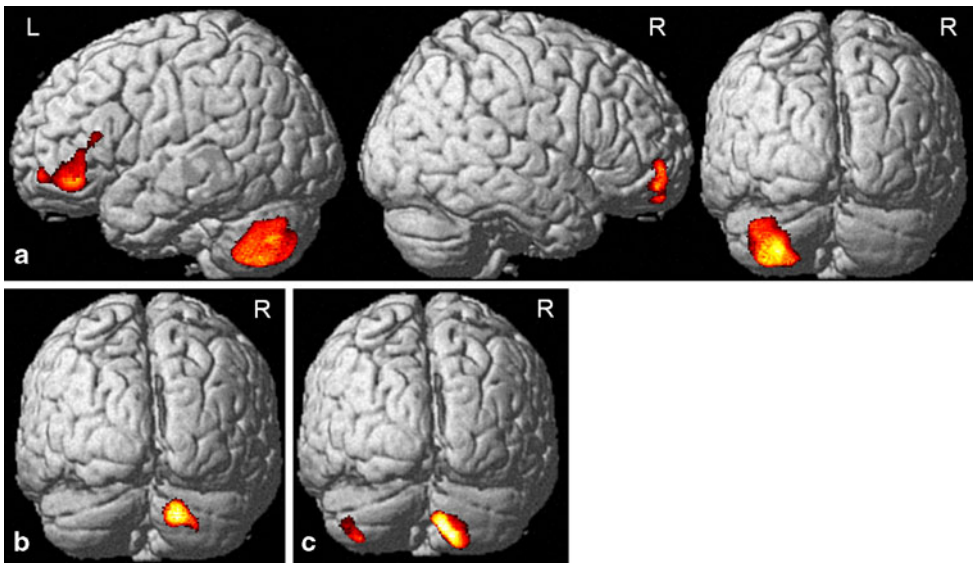

Fig. 1 Between-group interaction analyses of regional gray matter density declines in **a** chemotherapy-treated breast cancer patients relative to healthy controls from baseline to 1 month after chemotherapy; **b** breast cancer patients who did not receive chemotherapy relative to healthy controls over the same interval; and **c** chemotherapy-treated breast cancer patients relative to healthy controls from baseline to 1 year after chemotherapy ($P_{crit} < .001$, $k = 1{,}400$, see Table 3 for region descriptions)

group reductions were found only in right cerebellum (Fig. 1b). There were no regions where the control group showed lower gray matter than either cancer group at M1 relative to baseline, nor were there any regions where a significant interaction was found between the two cancer groups from baseline to M1. There were no significant group-by-time interactions from M1 to Y1 for any of the possible pair-wise comparisons. When group-by-time interactions from baseline to Y1 were examined the only significant difference was between the CTx+ and control groups, where gray matter density reductions were apparent in bilateral cerebellar regions in CTx+ participants relative to controls at Y1 relative to baseline (Fig. 1c).

Within-group analyses

At M1 relative to baseline the CTx+ group showed decreased gray matter density in bilateral frontal, temporal (including hippocampus and adjacent medial temporal structures), and cerebellar regions and right thalamus (Fig. 2). Recovery was seen from M1 to Y1 in some of these regions, including bilateral superior frontal, left middle frontal, and right superior temporal and cerebellar regions (Fig. 3a). Brain regions were also noted that did not demonstrate such recovery over time (where gray matter decreases from baseline to M1 persisted at Y1), including bilateral cerebellum, right thalamus and medial temporal lobe, left middle frontal gyrus, and right precentral, medial frontal, and superior frontal gyri (Fig. 3b). Within the control and CTx− groups no gray matter regions achieved statistical significance for any of these comparisons (decline from baseline to M1, recovery over time, or persistent decline).

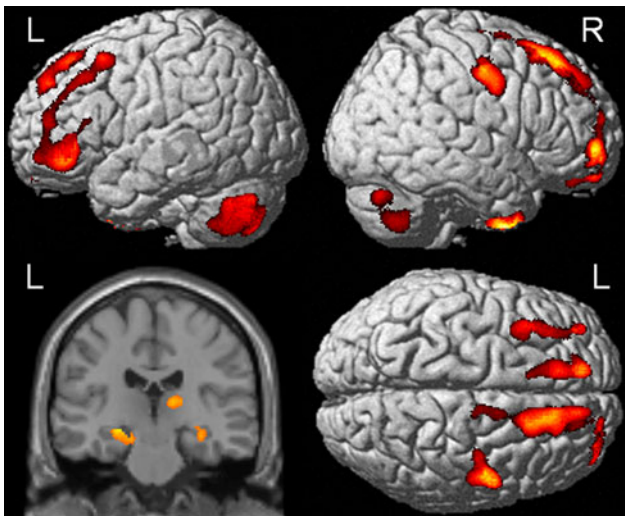

Fig. 2 Regional gray matter density declines in chemotherapy-treated breast cancer patients from baseline to 1 month after chemotherapy ($P_{crit} < .001$, $k = 1{,}400$, see Table 3 for region descriptions)

Covariate analyses

Inter-scan intervals did not differ between groups ($P > .05$, Table 1). Breast cancer patients started adjuvant cancer treatment (radiation, chemotherapy, or anti-estrogen therapy) within a week after the baseline scan on average. This interval did not differ between CTx+ and CTx− groups ($P > .05$, Table 1). The breast cancer groups did differ in the interval between last cancer-related surgery and baseline scan ($P < .05$, Table 1), with the CTx− group having a longer interval. This difference is attributable to the often tight time window between identification of an eligible patient scheduled to receive chemotherapy and planned

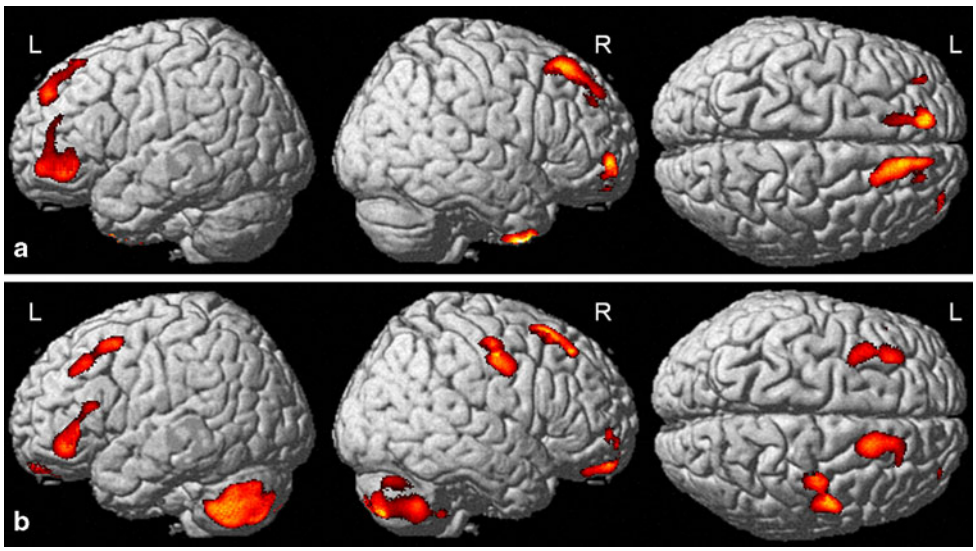

Fig. 3 Regional gray matter density changes over time in chemotherapy-treated breast cancer patients. Decline was apparent in these regions from baseline to 1 month after chemotherapy with **a** recovery 1 year later or **b** persistent decline 1 year later ($P_{crit} < .001$, $k = 1{,}400$, see Table 3 for region descriptions)

treatment initiation after surgery (i.e., typically chemotherapy was planned to be started sooner after surgery than either radiation or anti-estrogen treatment). As a result, for CTx− patients there was generally a larger time window before further treatment, allowing greater flexibility in scan scheduling. This practice pattern allowed matching either for baseline to treatment initiation interval or surgery to baseline interval, but not both. As the study was designed primarily to evaluate the cognitive effects of chemotherapy, the former was judged of greater import. To control for any effect of recency of surgery on imaging findings, group analyses were repeated with days between last cancer surgery and baseline scan included as a covariate (healthy controls were excluded from this analysis). While statistical power was somewhat attenuated with this model the overall pattern of findings was unchanged. This is consistent with our prior study which found no relationship between key surgical variables and baseline cognitive functioning [2]. Surgery to baseline interval was significantly correlated with disease stage ($P < .05$), which likewise differed between the CTx+ and CTx− groups, since disease stage is a major factor in treatment planning. Repeating the VBM statistical analyses with disease stage as a covariate did not affect the overall pattern of results.

We also examined the effect of several other potential contributory factors to verify that they did not account for the observed pattern of results. For self-reported symptoms of depression or anxiety there were no significant between-group differences at any time-point or any group-by-time interactions, and mean scores on both measures were well within normal limits for all groups at all time-points (Table 2). In addition, at all time-points only a very small number of subjects in any group exceeded commonly used clinical thresholds on either self-report measure. The overall pattern of imaging findings was unchanged after excluding these individuals or participants taking psychotropic medications from the design matrix. For anti-estrogen treatment the design matrix included a covariate modeling number of months on hormonal treatment at the time of each scan; again the overall pattern of findings remained the same.

Discussion

This is the first study to use a prospective, longitudinal approach to show decline in brain gray matter density shortly after chemotherapy and degree of recovery over time. No between-group gray matter differences were apparent at baseline (prior to chemotherapy, radiation, or anti-estrogen treatment), although declines were apparent from baseline to M1 in both cancer groups relative to controls. The within-group pattern of decline and partial recovery (i.e., the presence of regions where gray matter density fully recovered to baseline levels as well as regions where declines persisted from M1 to Y1) seen in chemotherapy-treated patients was not present in patients who did not receive chemotherapy or healthy controls. This suggests that these gray matter alterations are related to effects of chemotherapy, rather than solely reflecting host factors, the cancer disease process, or effects of other cancer treatments. Our results confirm findings from prior retrospective studies of changes in brain structure in long-term breast cancer survivors who were treated with chemotherapy [16, 21, 28]. There is notable overlap in the present findings with frontal and temporal regions where gray matter differences were detected in prior retrospective studies, consistent with current results that some gray matter changes do not return to baseline over time.

The present results were consistent with our hypothesis that CTx+ patients would demonstrate decreased gray matter in the acute post-treatment phase (M1), with subsequent partial recovery (normalization) after 1 year (Y1), and provide evidence for a structural neuroanatomic basis for the cognitive problems most commonly reported during and after chemotherapy, including impairment in episodic and working memory. Medial temporal lobe structures are critical in episodic memory, whereas attentional and executive functions such as working memory are subserved by frontal brain systems. Cerebellar regions are also increasingly recognized as important in higher cognitive functions [34]. The alterations in gray matter density observed in the CTx+ group are, therefore, consistent with the pattern of cognitive complaints and impairment found in neurocognitive studies. Such concerns tend to be most pronounced during and shortly after chemotherapy but remit in most patients over time, with only a subset reporting persistent cognitive problems. This temporal pattern is consistent with our finding that declines in gray matter density apparent at 1 month post-chemotherapy improve significantly over the following year, although they do not appear to normalize entirely. The presence of residual abnormalities is consistent with cognitive findings in long-term survivor cohorts [23, 38, 40] and the limited available neuroimaging data, including our preliminary survivor study [28]. Functional neuroimaging (functional MRI and positron emission tomography) studies have also shown abnormalities following chemotherapy corresponding to brain regions where we found decreased gray matter density, particularly in the frontal lobes and cerebellum [12, 31]. Our finding of decreased gray matter density in similar brain regions offers convergent data in support of a model of brain structural abnormality corresponding to functional changes after chemotherapy.

The specific etiology of these gray matter changes is unknown, although we and others have proposed possible mechanisms for chemotherapy-induced cognitive and brain changes, such as chemotherapy-induced DNA damage (directly or through increases in oxidative stress), individual variation in genes related to neural repair and/or plasticity, and chemotherapy-induced hormonal changes [1]. A limited literature in animal models has demonstrated neuronal changes following chemotherapy, including alterations in cytoskeletal and calcium regulating proteins and pyramidal cell dendritic retraction, although not frank neuronal loss [19, 20]. However, these studies focused on cytosine arabinoside (Ara-C), an agent with a different mechanism of action than that of the drugs with which our patients were treated. The finding of recovery in gray matter density over time, while consistent with our a priori hypothesis, also requires further exploration with regard to etiology. VBM studies have demonstrated increases in gray matter volume in healthy controls after lithium treatment [22], epilepsy patients following neurosurgery [41], and anorexic patients after nutritional recovery [8]. These increases were all seen after treatment and/or functional recovery, which is consistent with our hypothesized model of gray matter declines corresponding to chemotherapy-related cognitive impairment and later recovery relating to symptomatic improvement.

The findings of this study were statistically robust, as we used a stringent significance threshold for our imaging results. Some potential limitations must be considered, however. Group sizes are relatively small, although this is common in prospective studies of breast cancer patients, largely due to the challenges of recruiting and studying patients prior to adjuvant treatment. The cohort is racially and ethnically homogeneous (largely Caucasian, non-Hispanic), consistent with the rural northern New England population. In observational studies of this nature patients are not randomized to CTx+ and CTx− groups, with treatment practice patterns leading to between-group differences on disease-related variables. Our CTx− group tended to have earlier stage disease (predominantly stage 0–I) than the CTx+ group (mostly stage I–II), and a larger percentage of CTx− than CTx+ patients were on anti-estrogen treatment at M1, although comparable percentages were taking these medications at Y1. For both breast cancer groups, radiation and/or anti-estrogen treatment were administered to more than half of the participants. Although most patients were prescribed tamoxifen, other anti-estrogen agents were also used, potentially contributing to data variability. Several breast cancer patients were taking psychotropic medications (e.g., SSRIs for mood or menopause symptoms) at different points in the study. Although analyses covarying for medication use or excluding these individuals produced the same overall pattern as in the full sample, these agents may have independent effects which cannot be disambiguated from those of chemotherapy. This cohort was not powered to analyze other treatment effects fully or to clarify differential effects of chemotherapy regimens. In future studies it would be beneficial to include a larger, more diverse cohort, perhaps via multi-center collaborations, in order to assess individual contributions of specific cancer treatments and psychosocial variables.

Based on data from several groups, there is increased interest in abnormalities that may be present at baseline in breast cancer patients, as well as an emerging perspective that cancer or host factors may be associated with cognitive or anatomic changes over the course of treatment. The present finding of regional gray matter density reductions over time in patients who did not receive chemotherapy relative to controls suggests that the CTx− group also demonstrates gray matter changes related to cancer and/or

its treatment, but that these are lesser in severity and spatial extent than those seen after chemotherapy. Further study will be needed to directly address the effect of anti-estrogen treatment on brain structure and function, as previous studies suggest this may be a contributing factor [11, 31].

In summary, we report breast cancer chemotherapy-related reduction in gray matter density and its partial recovery over time prospectively in a single cohort. This pattern of gray matter changes was not observed in breast cancer patients not treated with chemotherapy or healthy controls, and was not attributable to potential confounds such as disease stage, time since last cancer-related surgery, mood, or medications. Gray matter changes were most prominent in frontal, temporal, and cerebellar regions, consistent with the profile of cognitive dysfunction and complaints observed in a subset of breast cancer patients after chemotherapy in previous research. Prior cross-sectional studies using other imaging approaches have shown structural and functional abnormalities in similar brain regions; our findings extend these earlier studies by demonstrating the evolution of these structural changes in the first 2 years after breast cancer diagnosis and treatment. While further investigation is needed to elucidate the underlying neural mechanism(s), these findings provide important new information to help guide future investigation of the temporal course and regional specificity of these changes.

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