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The association between pro-inflammatory cytokines, regional cerebral metabolism, and cognitive complaints following adjuvant chemotherapy for breast cancer

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Abstract To examine relationships following adjuvant chemotherapy between circulating pro-inflammatory cytokines, regional cerebral metabolism, and cognitive complaints in early stage breast cancer patients. 33 breast cancer patients who had completed initial treatment (surgery, ± radiation, 23 chemotherapy, 10 no chemotherapy) obtained resting (18)F-FDG PET/CT brain imaging at baseline and 1 year later. Pro-inflammatory cytokine markers (IL-1ra, sTNF-RII, CRP, and IL-6) and cognitive complaints were also assessed at both time points. At baseline, consistent correlations were seen between the left medial frontal and right inferior lateral anterior temporal cortices and inflammatory markers within the chemotherapy group, and not in the no chemotherapy group. After 1 year, correlations persisted in the medial frontal cortex and the temporal cortex, the latter shifting superiorly. Both of these regional correlations demonstrated the highest levels of significance when looking across the 1 year time frame (IL-1ra: peak voxel $p<0.0005$; cluster size $p<0.0005$, $p=0.001$ after correction (medial prefrontal), $p<0.0005$; cluster size $p=0.001$, $p=0.029$ corr. (anterior temporal), sTNF-RII: $p<0.0005$; cluster size $p=0.001$, $p=0.040$ corr. (medial prefrontal)). Positive correlations were also seen within the chemotherapy group between baseline memory complaints and the medial frontal ($p<0.0005$; cluster size $p<0.0005$, $p<0.0005$ corr.) and anterior temporal ($p<0.0005$; cluster size $p<0.0005$, $p=0.002$ corr.) cortices at baseline and 1 year later. Metabolism in the medial prefrontal cortex and anterior temporal cortex was found to correlate with both memory complaints and cytokine marker levels in chemotherapy patients.

Keywords Positron emission tomography · Brain imaging · Breast cancer · Cognitive complaints · Chemotherapy · Pro-inflammatory cytokines

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Introduction

Cancer is a leading cause of death worldwide, accounting for nearly 8 million deaths per year (WHO 2012). In the United States, the American Cancer Society estimates an incidence

of 1.6 million new cases of cancer annually. With advances in diagnosis and treatment, more people are surviving cancer and living long after initial diagnosis and therapy. For example, of the more than 0.2 million people who are diagnosed with breast cancer each year, 90 % survive at least 5 years (ACS 2011). This increased survival has shed light on a sizable portion of patients who complain of cognitive dysfunction following cancer treatment, sometimes for years or even decades, after treatment had been completed.

There has been a large increase in research on this subject over the past few years, focusing mainly on breast cancer patients. Most early studies were cross-sectional and examined breast cancer survivors (Ahles et al. 2002; Brezden et al. 2000; Castellon et al. 2004; Schagen et al. 1999; Tchen et al. 2003; van Dam et al. 1998). Many found lower neuropsychological performance in chemotherapy treated patients, demonstrating that the scope of neurocognitive alterations is wide and can include problems with attention, learning, retrieval, language, visuoperception, construction abilities, and motor skills (Argyriou et al. 2011). Executive function, processing speed, and memory seem to be especially affected (Correa and Ahles 2008; Dietrich et al. 2008; Wefel et al. 2004; Wefel and Schagen 2012; Wefel et al. 2008). Early imaging studies with functional magnetic resonance imaging (fMRI), structural MRI, and positron emission tomography (PET) also displayed alterations in frontal and cerebellar regions in chemotherapy treated patients (Ferguson et al. 2007; Silverman et al. 2007; McDonald et al. 2010). Recently, many prospective longitudinal studies have been conducted showing similar neuropsychological dysfunction and alterations in brain structure and function including both white and grey matter (Ahles et al. 2010; Biglia et al. 2012; Collins et al. 2009; Deprez et al. 2012; Deprez et al. 2011; Jansen et al. 2011; McDonald et al. 2012; Wefel et al. 2010; McDonald et al. 2010).

A possible mechanism for these changes seen in chemotherapy treated patients involves the association between pro-inflammatory cytokines and decline in cognitive function (Barber 2011; Fritz-French and Tyor 2012; Kuo et al. 2005; O'Bryant et al. 2010; Teunissen et al. 2003; Tilvis et al. 2004). Cytokines can cross the blood brain barrier through active transport, a possible humoral route, and in regions where the blood brain barrier is weaker, including circumventricular areas (Myers 2010). Both administration and production of cytokines has been associated with a type of sickness behavior that includes inability to concentrate and impaired learning (Myers 2010). In addition, animal studies have demonstrated that cytokines can produce specific neuropathologic changes and functional impairment (Lee et al. 2004). Data are also available in humans. In healthy individuals, an inverse relationship was found between circulating IL-6 levels and performance on executive function, attention and working memory, and auditory recognition memory tests (Marsland et al. 2006). Recent studies of breast cancer patients have also shown reduced memory performance and elevated IL-6 and TNFα concentrations in chemotherapy treated patients (Kesler et al. 2012), and we recently reported higher sTNF-RII concentrations associated with increased memory complaints in chemotherapy-exposed patients (Ganz et al. 2012). Additionally, studies have shown an association between chemotherapy and elevated inflammatory cytokines (Pusztai et al. 2004; Torres et al. 2013; Wang et al. 2012).

In order to further investigate the associations between cytokines, cognitive function, and chemotherapy, we began a prospective, longitudinal, observational cohort study in 2007 (the UCLA Mind Body Study [MBS]) of early stage, newly-diagnosed breast cancer patients, described in greater detail previously (Ganz et al. 2012). Participants were recruited after the completion of primary treatment (surgery, radiation treatment, adjuvant chemotherapy treatment), and before the start of endocrine treatment. With this design, we aimed to compare patients who underwent chemotherapy vs. those who did not undergo chemotherapy, with assessment both before and after endocrine treatment if indicated. We also wanted to investigate the potential role of reproductive factors in the presenting cognitive dysfunction.

The MBS evaluated self-report cognitive complaints, standardized neuropsychological test performance, brain metabolic data (a sub-study population), behavioral symptoms (fatigue, depression, insomnia), and immune alterations (pro-inflammatory cytokines), at baseline (T1) and 1 year later (T3) (All measures except brain imaging were measured at a 6-month interval as well (T2). They will not be reported in this paper focusing on the brain imaging). Additionally, findings concerning many of the variables measured in the large MBS study have been reported elsewhere (Bower et al. 2011; Bower et al. 2013; Ganz et al. 2012; Ganz et al. 2013). Over all, this larger study was designed to examine the possible effects of chemotherapy, endocrine therapy, radiation therapy, fatigue, depression, insomnia, levels of pro-inflammatory cytokines, and endogenous hormones on cognitive function.

In this report, we present data on the PET sub-study population and their relationships between cerebral metabolic data, a battery of four inflammatory markers, and self-reported cognitive complaints, with both cross-sectional analysis at baseline (post-chemotherapy, prior to endocrine therapy) and one year later, and evaluating correlations longitudinally. Based on the previous associations seen between cytokines and cognitive changes (Barber 2011; Fritz-French and Tyor 2012; Kuo et al. 2005; O'Bryant et al. 2010; Teunissen et al. 2003; Tilvis et al. 2004), we hypothesized to also see changes in regional brain metabolism associated with cytokine levels, and also with cognitive complaints. In the present paper, we report on correlations with self-reported cognitive complaints rather than standardized neuropsychological test performance due to more significant differences between the chemotherapy group and no chemotherapy group in self-reported complaints.

Methods

Patients

This observational cohort study recruited women with early stage breast cancer from the Los Angeles community. Women entering the study could have undergone chemotherapy prior to the study or not. The study inclusion criteria were: (1) women aged 21–65 years; (2) newly diagnosed with Stage 0, I, II, or IIIA breast cancer; (3) completion of primary treatment (surgery, radiation treatment, and/or adjuvant chemotherapy treatment); (4) prior to start of endocrine therapy if planned; (5) geographically accessible for 1-year follow-up; (6) English language proficient; (7) able to provide informed consent. Exclusion criteria included: (1) evidence of current or past disorder/disease of the central nervous system or any medical condition that might be expected to impact cognitive functioning (e.g. multiple sclerosis, thyroid dysfunction); (2) history of head trauma with loss of consciousness greater than 30 min; (3) epilepsy, dementia, or severe learning disability; (4) current psychotic-spectrum disorder (e.g. schizophrenia, bipolar disorder, major depressive disorder) or current substance abuse or dependence; (5) history of whole brain irradiation or surgery; (6) history of past cancer treatment with chemotherapy; (7) active diagnosis of autoimmune and/or inflammatory disorder (e.g., systemic lupus erythematosis, rheumatoid arthritis, vasculitis) or disorders that may influence inflammatory processes (e.g. uncontrolled allergic condition or asthma); (8) chronic use of oral steroid medication; (9) hormone therapy (estrogen, progestin compounds) other than vaginal estrogen. The medical conditions noted above and women older than 65 were excluded because of the known potential impact on cognitive function or inflammation. A total of 190 patients were recruited into the MBS, and 33 participated in the PET sub-study. Table 1 displays the demographics of this sub-population. One of the 33 participants was excluded from baseline analyses including the pro-inflammatory cytokines because of lack of inflammatory measures at baseline and 1 year later, 3 other participants did not return for the follow-up PET scan, and 2 additional participants did not have inflammatory measures at T3 because of technical difficulty with drawing or processing of blood samples. Specifics of recruitment have been previously described (Bower et al. 2011; Ganz et al. 2012).

Measures

Demographic and clinical information was obtained from self-report and medical record abstraction. Cognitive complaints were assessed with the Patient's Assessment of Own Functioning Inventory (PAOFI), where higher scores indicate more cognitive complaints (Chelune et al. 1986). PAOFI has four sub-scores: memory, high level cognition, sensory motor, and language/communication. Participants may answer on a scale of 1–6 for each question. The combined scores were tabulated in the direction that higher scores corresponded to more complaints and the total scores (Rourke et al. 1999) were used to examine the relationship between cognitive complaints and patterns in brain metabolism.

Blood samples for circulating inflammatory markers were collected by venipuncture into EDTA tubes, placed on ice, centrifuged for acquisition of plasma, and stored at −80 °C for subsequent batch testing. We evaluated four inflammatory markers that have been examined previously by our group, and also reported to be altered following cancer diagnosis and/or treatment: IL-1 receptor antagonist (IL-1ra) and soluble TNF receptor type II (sTNF-RII), surrogate markers for IL-1 and TNF-α activity, respectively, as well as IL-6 and C reactive protein (CRP) (Alexander et al. 2009; Bower et al. 2002; Bower et al. 2007; Bower et al. 2009; Collado-Hidalgo et al. 2006; Orre et al. 2009; Wang et al. 2010; Wang et al. 2012). Specifics for determining the plasma levels have been previously described (Ganz et al. 2012). Tables within the manuscript display raw plasma cytokine levels; however the statistical analyses were conducted and graphical presentations made using the natural log of the plasma values in order to produce a more statistically normal distribution.

Acquisition and analysis of PET scans, as well as the complete PET protocol have been detailed previously (Silverman et al. 2007; Ganz et al. 2012). Briefly, PET was performed with 3D acquisitions, using a 64-slice PET/CT scanner (Siemens). Low-dose CT scans were used for attenuation correction. FDG was used to assess regional cerebral metabolism during mental rest. Subjects were scanned in the supine position, 40 min following injection of 185 MBq FDG in a dimly lit room having low ambient noise, with eyes and ears unoccluded. Effects on resting metabolism were evaluated by both standardized volume of interest (sVOI) and statistical parametric methods. In sVOI analyses, mean activities in 47 standardized volumes of interest were quantified for each scan, and normalized to mean global activity, using a commercially available display-and-analysis software package (NeuroQ; Syntermed Inc.). For statistical parametric mapping (SPM) analyses, SPM8 software was used by methods previously described (Silverman et al. 2011). In brief, a $p<0.01$ (uncorrected) height threshold, a 0 voxel extent threshold, and both family wise error (FWE) and false discovery rate (FDR) multiple comparison corrections were used. Images were coregistered and reoriented into a standardized Talairach Tournoux based coordinate system (Talairach and Tournoux 1988) to the MNI template, using the nonlinear spatial transformation package in SPM8 (Friston et al. 2007), smoothed three-dimensionally at a full-width half-maximum of 8 mm, and normalized to mean global activity. Pooled data were then statistically assessed to identify the voxels which significantly

Table 1 Patient demographic and medical characteristics

	Total (n=33)		Chemo (n=23)		No Chemo (n=10)		p-value[a]
	Percent or Mean (SD)	N	Percent or Mean (SD)	N	Percent or Mean (SD)	N	
Age at baseline	52.2 (9.0)		50.7 (9.5)		55.7 (6.8)		.14
Race: White	70 %	23	61 %	14	90 %	9	.12
Marital status: Married	58 %	19	52 %	12	70 %	7	.46
Education							
Post college	45 %	15	52 %	12	30 %	3	.44
College	33 %	11	30 %	7	40 %	4	
Post high school	21 %	7	17 %	4	30 %	3	
WTAR	116.0 (7.8)		116.3 (7.8)		115.2 (8.0)		.71
Employment: full- or part-time	61 %	20	70 %	16	40 %	4	.14
Annual household income: >$100,000	66 %	21	61 %	14	78 %	7	.44
BMI	26.7 (5.0)		27.4 (5.2)		25.1 (4.5)		.24
Surgery							
Mastectomy	18 %	6	17 %	4	20 %	2	1.00
Lumpectomy	82 %	27	83 %	19	80 %	8	
Radiation: Yes	85 %	28	87 %	20	80 %	8	.63
History of hormone replacement therapy: Yes	30 %	10	26 %	6	40 %	4	.44
Change in period after dx/treatment							
Post-menopausal	52 %	17	48 %	11	60 %	6	.05
No change	9 %	3	4 %	1	20 %	2	
Became irregular	3 %	1	0 %	0	10 %	1	
Stopped but resumed	0 %	0	0 %	0	0 %	0	
Amenorrhea	36 %	12	48 %	11	10 %	1	
Chemotherapy regimen: Anthracycline containing	35 %	8	35 %	8	n/a	n/a	n/a
Received endocrine therapy after baseline	61 %	20	61 %	14	60 %	6	
Type of endocrine therapy if yes							
Tamoxifen	65 %	13	64 %	9	67 %	4	1.00
Aromatase inhibitor	35 %	7	36 %	5	33 %	2	
Stage at diagnosis							
0	9 %	3	0 %	0	30 %	3	.002[b]
1	38 %	12	27 %	6	60 %	6	
2	44 %	14	59 %	13	10 %	1	
3	9 %	3	14 %	3	0 %	0	

[a] P-values are the result of t-tests for continuous variables, or Fisher's Exact test for categorical variables

[b] This p-value reflects a comparison of stage 0/1 vs. stage 2/3. *WTAR* Wechsler Test of Adult Reading, *BMI* Body Mass Index

differed between treatment groups, or within treatment groups at different points of time, or which significantly correlated with a specified neuropsychologic parameter or peripheral cytokine measure. Results were reported in terms of locations of the most significant effects (regionally and/or in x, y, z Talairach style millimeter coordinates) with corresponding t and p values, along with the statistical significance of the size of the region in some cases.

Results

Between group analyses

Between baseline and 1 year later, neither the chemotherapy nor no chemotherapy groups differed significantly in regional resting brain metabolism or circulating inflammatory cytokine marker levels. At baseline, the chemotherapy group

demonstrated higher mean values of IL-1ra, sTNF-RII, and CRP, and at 1 year, had higher CRP compared to the no chemotherapy group. The groups did report significantly different levels of memory complaints at baseline (p=0.004) and 1 year later (p=0.01).

Relationships between inflammatory markers and brain metabolism at baseline

When correlating baseline cytokine marker levels to baseline cerebral metabolism (raw values displayed in Table 2), within the chemotherapy group (n=23), scattered correlations were seen most consistently in the left medial frontal and right inferior lateral anterior temporal cortices. In the chemotherapy group, positive correlations were seen with SPM analyses between the left medial prefrontal cortex and the four cytokine markers measured (CRP (t=5.30, p<0.0005, peak voxel [−10, 42, 34]), IL-1ra (t=4.82, p<0.0005, [−14, 42, 36]), IL-6 (t=4.65, p<0.0005, [−10, 40, 38]), sTNF-RII (t=4.02, p<0.0005, [−6, 60, 36]). Also in the chemotherapy group, positive correlations were seen in the inferior lateral anterior temporal cortex in three of the cytokine markers (CRP (t=3.76, p=0.001, peak voxel [44, 2, −30]), IL-6 (t=3.63, p=0.001, [40, 4, −36], sTNF-RII (t=3.60, p=0.001, [46, −6, −30])) Fig. 1. Consistent negative correlations of comparable statistical significance were not seen within the chemotherapy group. Additionally, these correlations were not seen for the non-chemotherapy group.

Relationships between 1 year inflammatory markers and 1 year brain metabolism

At the 1 year time point, the chemotherapy group (n=21) continued to demonstrate positive correlations in the medial frontal cortex and the right temporal cortex, the latter shifting superiorly. Positive correlations were seen with SPM analyses between the medial prefrontal cortex and inflammatory markers (sTNF-RII (t=3.85, p=0.001, [−4, 58, −16]), IL-1ra (t=3.20, p=0.002, [10, 60, 0]). Positive correlations were also seen in the anterior temporal cortex (IL-6 (left t=5.59, p<0.0005, [−46, 18, −30], right t=4.38, p<0.0005, [58, 12, −14]), sTNF-RII (t=5.01, p<0.0005, [54, 14, −12], CRP (t=4.48, p<0.0005, [46, −26, −14])) Fig. 2. Consistent negative correlations were not seen in the chemotherapy group. And again, these correlations were not seen in the non-chemotherapy group (n=6). Additionally, when comparing change in metabolism to change in cytokine levels over one year within the chemotherapy group, two regions significantly (P<0.01) correlated; including a positive correlation between CRP and medial prefrontal cortex, as well as between sTNF-RII and the left associative visual cortex.

Relationships between baseline inflammatory markers and brain metabolism one year later

Within the chemotherapy group (n=21), correlations in both the medial prefrontal cortex and anterior temporal cortex showed the highest levels of significance when looking across the one year time frame, comparing baseline inflammatory marker levels to one year regional metabolism. The most significant were between IL-1ra and sTNF-RII and the bilateral medial frontal cortex (GFd), and between IL-1ra and the anterior temporal cortex. A positive correlation was seen between both IL-1ra and sTNF-RII and the bilateral GFd (IL-1ra: sVOI: rGFd r=0.4853, t=2.4195, p=0.0257, lGFd r=0.4960, t=2.4902, p=0.0222, Fig. 3, SPM: t=5.69, p<0.0005, peak voxel [−22, 60, −4]; cluster size=2,246 voxels, p<0.0005, p=0.001 FDR and FWE corr., sTNF-RII: sVOI: rGFd r=0.4721, t=2.3343, p=0.0307, lGFd r=0.4345, t=2.1028, p=0.0490, Fig. 4, SPM: t=4.54,

Table 2 Circulating inflammatory cytokine level and FDG-PET sVOI levels in chemotherapy subjects at baseline and one year later

Circulating Inflammatory Markers					
	Baseline chemo (n=23)		One-year chemo (n=22)		p-Value
	Mean	SD	Mean	SD	
Il-1ra (pg/mL)	283	146	272	165	0.8231
IL-6 (pg/mL)	1.7	1.3	1.7	1.3	0.8110
CRP (mg/L)	4.1	7.4	2.6	3.7	0.4016
sTNF-RII (pg/mL)	2480	669	2040	580	0.0230
FDG-PET sVOI Values					
	Baseline chemo (n=23)		One-year chemo (n=22)		p-Value
	Mean	SD	Mean	SD	
rGFd	1.085	0.023	1.081	0.024	0.5883
lGFd	1.080	0.048	1.074	0.050	0.6808
riLAT	0.885	0.019	0.875	0.018	0.0761
liLAT	0.900	0.025	0.878	0.020	0.0023

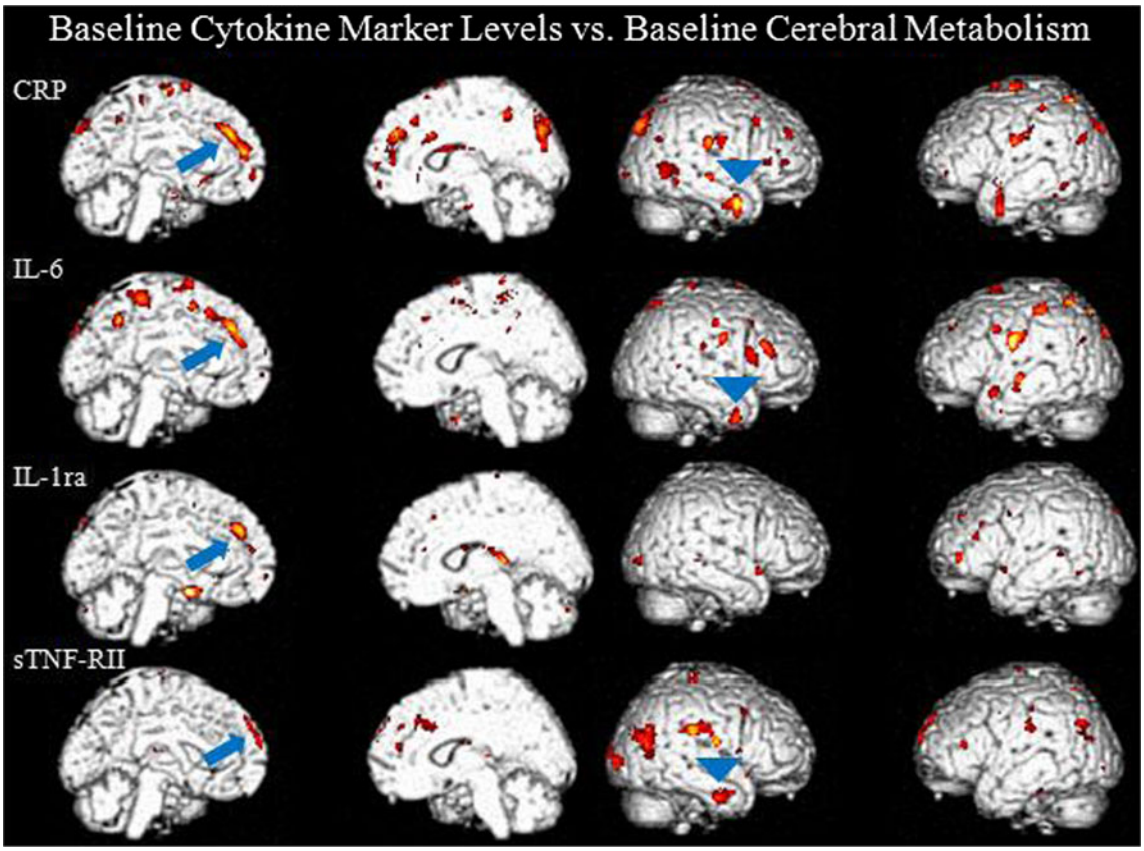


Fig. 1 Medial and lateral surface renders of correlations between baseline cytokine levels and baseline cerebral metabolism. The chemotherapy group demonstrates positive correlations in the left medial prefrontal cortex (*arrows*) and the right inferior lateral anterior temporal cortex (*arrow heads*). These correlations were absent in the no chemotherapy group. *For all figures: The *color scale* represents increasing significance in *yellow* (a higher t score) and decreasing significance in red (lower t score)

$p<0.0005$, peak voxel [−4, 58, −2]); cluster size=1,061 voxels, $p=0.001$, $p=0.040$ FDR corr., $p=0.046$ FWE corr. Fig. 5). Metabolism in the anterior temporal cortex correlated positively with IL-1ra ($t=4.93$, $p<0.0005$, peak voxel [60, 26, 8]; cluster size=1,034 voxels, $p=0.001$, $p=0.029$ FDR corr., $p=0.048$ FWE corr.). Comparable negative correlations were not present in the chemotherapy group. Additionally, these correlations were absent in the group who did not undergo chemotherapy. A comparison of the correlations described above is listed in Table 3.

Relationships between brain metabolism and memory complaints

Strong correlations in the anterior temporal cortex and the medial frontal cortex were also seen when comparing to memory complaints in the chemotherapy group ($n=23$ at T1, $n=21$ at T3). At baseline, the bilateral anterior temporal cortex and the bilateral medial frontal cortex correlated positively with total severity scores on the PAOFI memory subscale. The largest region to correlate was located in the bilateral medial frontal cortex and was corroborated by SPM and sVOI methods (sVOI: rGFd $r=0.5425$, $t=2.9595$, $p=0.0075$, lGFd $r=0.4278$, $t=2.1689$, $p=0.0417$, SPM: $t=5.98$, $p<0.0005$, peak voxel [−16, 44, 24]; cluster size=3,283 voxels $p<0.0005$, $p<0.0005$ FDR and FWE corr.). The bilateral anterior temporal cortex also positively correlated (right: $t=6.92$, $p<0.0005$, peak voxel [52, 12, −14]; cluster size=1,773 voxels, $p<0.0005$, $p=0.002$ FDR corr., $p=0.004$ FWE corr., left: $t=5.49$, $p<0.0005$, peak voxel [−46, −8, −14], cluster size=1,852 voxels, $p<0.0005$, $p=0.002$ FDR corr., $p=0.003$ FWE corr.). Interestingly, baseline total severity scores on the PAOFI memory subscale also positively correlated with baseline IL-6 values ($p=0.0287$), suggesting a link between cognitive complaints and plasma IL-6 levels, in addition to their correlation with specific regions of brain metabolism. When correlating baseline memory complaints to metabolism one year later the relationship persisted, however was less robust (anterior temporal: left $t=6.36$, $p<0.0005$, peak voxel [−46, 16, −28], $t=5.65$, $p<0.0005$, peak voxel [54, 12, −18], medial frontal: $t=5.42$, $p<0.0005$, peak voxel [−10, 32, 50]) Fig. 6. Additionally, a comparison of the correlations described above is listed in Table 4.

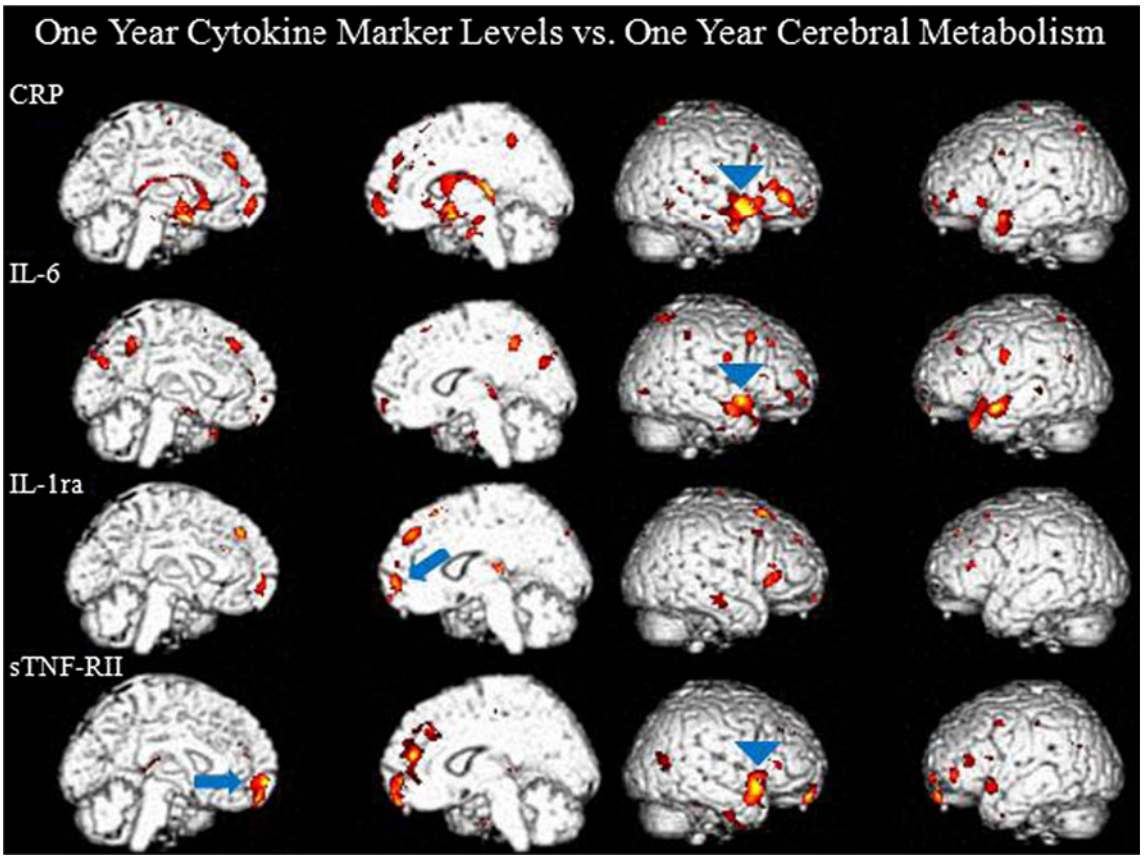


Fig. 2 Medial and lateral surface renders of correlations between 1-year cytokine levels and 1-year cerebral metabolism. The chemotherapy group demonstrates positive correlations in the medial prefrontal cortex and the anterior temporal cortex. These correlations were absent in the no chemotherapy group

Discussion

As described above, the most consistent correlations between cerebral metabolism and inflammatory markers were seen in the left medial frontal and right lateral anterior temporal cortices. Because this analysis was done on an exploratory basis, we currently have no *a priori* basis for why these regions may be more influenced by cytokines. We focus on these findings because both of these regional correlations showed the highest levels of significance when looking across the 1-year time frame, comparing baseline inflammatory marker levels to 1-year regional metabolism. This is consistent with the possibility that an initial inflammatory response could set up a cascade that has long-term impact on brain metabolism. Future studies may address the duration of this effect with long-term follow-up. The *positive*

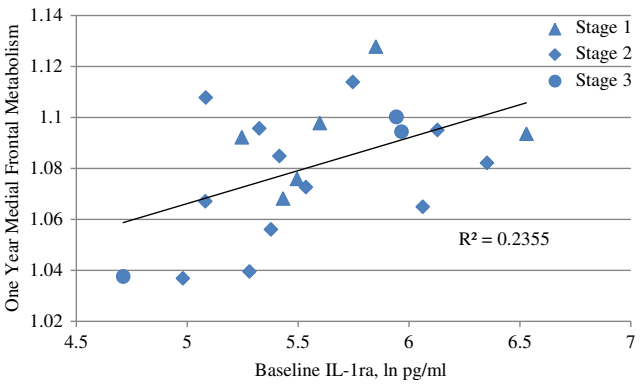


Fig. 3 Positive correlation between baseline IL-1ra and 1-year medial frontal resting metabolism in the chemotherapy group (n=21)

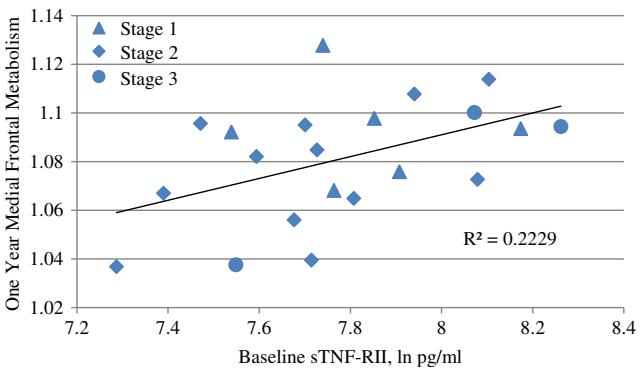


Fig. 4 Positive correlation between baseline sTNF-RII and 1-year medial frontal resting metabolism in the chemotherapy group (n=21)

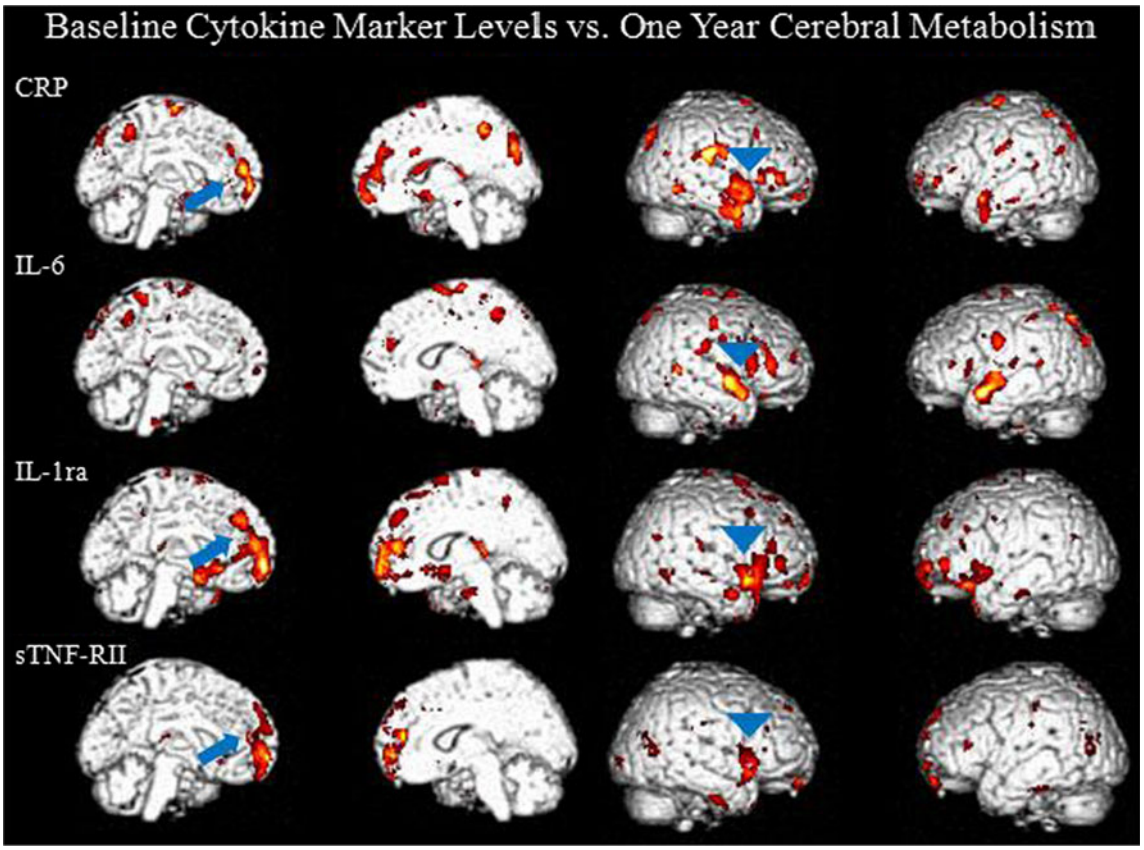


Fig. 5 Medial and lateral surface renders of correlations between baseline cytokine levels and 1-year cerebral metabolism. The chemotherapy group demonstrates positive correlations in the medial prefrontal cortex and the anterior temporal cortex. These correlations were absent in the no chemotherapy group

Table 3 Size and significance of cytokine marker vs. regional brain metabolism correlations

Correlation between	Peak voxel	No. of voxels	P-value	Z-value
Baseline CRP and baseline left medial prefrontal metabolism	[−10, 42, 34]	352	$p<0.0005$	4.18
Baseline IL-1ra and baseline left medial prefrontal metabolism	[−14, 42, 36]	276	$p<0.0005$	3.81
Baseline IL-6 and baseline left medial prefrontal metabolism	[−10, 40, 38]	276	$p<0.0005$	3.81
Baseline sTNF-RII and baseline left medial prefrontal metabolism	[−6, 60, 36]	73	$p<0.0005$	3.42
Baseline CRP and baseline inferior lateral anterior temporal metabolism	[44, 2, −30]	287	$p=0.001$	3.25
Baseline IL-6 and baseline inferior lateral anterior temporal metabolism	[40, 4, −36]	84	$p=0.001$	3.16
Baseline sTNF-RII and baseline inferior lateral anterior temporal metabolism	[46, −6, −30]	213	$p=0.001$	3.28
One-year IL-1ra and 1-year medial prefrontal metabolism	[10, 60, 0]	78	$p=0.002$	2.83
One-year sTNF-RII and 1-year medial prefrontal metabolism	[−4, 58, −16]	589	$p=0.001$	3.27
One-year CRP and 1-year right anterior temporal metabolism	[46, −26, −14]	1,458	$p<0.0005$	3.65
One-year IL-6 and 1-year right anterior temporal metabolism	[58, 12, −14]	563	$p<0.0005$	3.6
One-year IL-6 and 1-year left anterior temporal metabolism	[−46, 18, −30]	673	$p<0.0005$	4.25

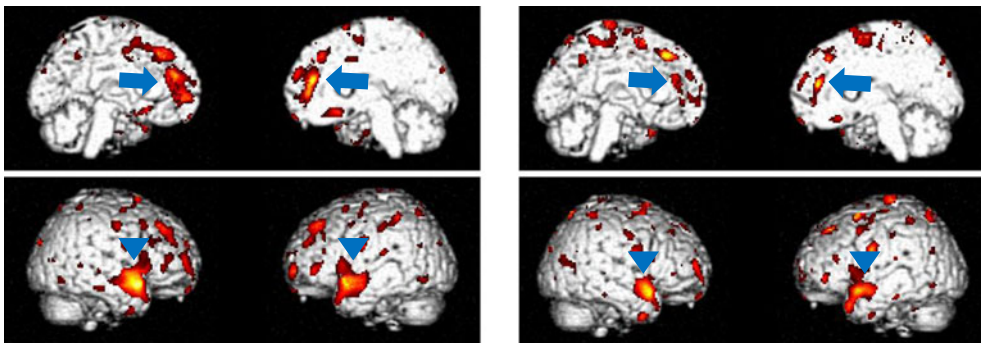
Table 3 (continued)

Correlation between	Peak voxel	No. of voxels	P-value	Z-value
One-year sTNF-RII and 1-year right anterior temporal metabolism	[54, 14, −12]	633	$p<0.0005$	3.95
Baseline IL-1ra and 1-year medial frontal metabolism	[−22, 60, −4]	2,246	$p<0.0005$ ($p=0.001$, FDR corr.)	4.29
Baseline sTNF-RII and 1-year medial frontal metabolism	[−4, 58, −2]	1,061	$p=0.001$ ($p=0.040$, FDR corr.)	3.69
Baseline IL-1ra and 1-year anterior temporal metabolism	[60, 26, 8]	1,034	$p=0.001$ ($p=0.029$, FDR corr.)	3.91

correlation between inflammatory cytokines and resting medial frontal and anterior temporal metabolism could represent increased resting metabolism as a compensatory mechanism for diminished function mediated by other areas. It may also represent a loss of diffuse inhibitory input into those regions, among other things. Interestingly, positive correlations were also seen within the chemotherapy group between baseline memory complaints and the medial frontal and anterior temporal cortices at baseline and 1 year later, which could represent a neurologic substrate for persistent sequelae of the acute treatment setting.

Perception of cognitive abilities and levels of circulating cytokines both vary widely across individuals before and after being diagnosed with cancer, as well as before and after undergoing chemotherapy. It is thus unsurprising to see no large or significant differences in these measures between chemotherapy-treated and untreated patients. In fact, it would be rather remarkable if group-based differences along these lines were detected in this investigation given the natural heterogeneity before and after the cancer diagnosis, let alone the further considerable inter-individual variability that may occur in response to chemotherapy, with respect to a wide array of clinical parameters, particularly as the longitudinal aspect of this study does not come into play until after initial therapy is complete. The most we might thus reasonably expect to observe (and which in fact was observed) is correlation between parameters that are biologically inter-related such as, putatively, responses to chemotherapy exposure with respect to levels of cytokines, cerebral metabolism, and perceived cognition. This is also in line with our original observations of brain metabolism patterns in adjuvant chemotherapy-treated breast cancer patients (Silverman et al. 2007), in which we did not report group-based differences in regional cerebral metabolism between chemotherapy-exposed and unexposed subjects, but rather a significant correlation between whatever level of cognitive impairment was present after chemotherapy and the degree of hypometabolism measured.

Similarly, in the present study, though when taken as groups, the chemotherapy-exposed and unexposed subjects do not significantly differ in regional resting brain metabolism or circulating inflammatory cytokine marker levels, the significance of the correlations described here nevertheless demonstrates that individuals within the chemotherapy group do possess metabolism in certain regions of the brain that co-vary with inflammatory cytokine marker levels, both concurrently and over time. The absence of such a relationship in the group unexposed to chemotherapy may reflect the absence of this common perturbing factor. The relationship that emerges in the chemotherapy-exposed group does not appear to be related to a special subset of patients, as both cytokine levels and regional metabolism levels are distributed relatively uniformly across their respective ranges, and throughout the scatter surrounding the line of regression relating them to each other. In fact, though there are no significant between-group differences in the cytokine markers described, there was a weak trend observed in the



Baseline Complaints vs. Baseline Cerebral Metabolism

Baseline Complaints vs. One Year Cerebral Metabolism

Fig. 6 Positive correlations between memory complaints and cerebral metabolism within the chemotherapy group

Table 4 Size and significance of memory complaints vs. regional brain metabolism correlations

Correlation between	Peak voxel	No. of voxels	P-value	Z-value
Baseline PAOFI memory subscale and baseline left medial frontal metabolism	[−16, 44, 24]	3,283	$p<0.0005$ ($p<0.0005$, FDR corr.)	4.52
Baseline PAOFI memory subscale and baseline right anterior temporal metabolism	[52, 12, −14]	1,773	$p<0.0005$ ($p=0.002$, FDR corr.)	4.94
Baseline PAOFI memory subscale and baseline left anterior temporal metabolism	[−46, −8, −14]	1,852	$p<0.0005$ ($p=0.002$, FDR corr.)	4.28

direction of the chemotherapy-treated group having higher levels of medial frontal metabolism at the 1 year time point, and when these patients with the higher levels were censored from the analysis, the significance of the cytokine correlation with metabolism became even stronger. In contrast, as stated above, memory complaint levels did differ between groups, with a substantially greater number of high complainers among those exposed to chemotherapy, and the significant correlation observed between complaint scores and medial frontal metabolism in the brains of chemotherapy-treated patients did depend upon inclusion of the high-complaining subgroup.

Cytokines have been discussed as a candidate mechanism for chemotherapy associated cognitive changes (Ganz et al. 2012). The rationale behind this includes the idea that cancer and chemotherapy can stimulate the release of peripheral cytokines that can cross the blood brain barrier. Cytokines are involved in neuronal and glial cell functioning, neuronal regeneration and neurodegeneration, and cholinergic and dopaminergic pathways. If chemotherapy deregulates cytokine levels, and in turn interferes with their important functions in the brain, this could lead to cognitive impairment (Ahles and Saykin 2007). Although the effects of cytokines on cognitive functioning in the breast cancer patient population is little explored, we recently found that among chemotherapy patients, higher levels of sTNF-RII were significantly correlated with greater memory complaints after controlling for age, BMI, radiation, depression, and time since last chemotherapy treatment. The memory complaints were assessed with the Squire Memory Questionnaire (Ganz et al. 2012).

Another recent study also found that an administration of endotoxin to nine healthy individuals was associated with increased levels of inflammatory cytokines (serum levels of tumor necrosis factor-α and interleukin-6 were measured) and higher normalized glucose metabolism as measured by FDG-PET in the insula and a trend toward lower normalized glucose metabolism in the cingulate (Hannestad et al. 2012). Because of the consistency of the metabolic changes seen across all subjects, the authors believe their data suggest that, "systemic inflammation induces fundamental physiologic changes in regional brain glucose metabolism." Additionally, alterations in fMRI have been associated with intravenous injection of low-dose endotoxin (Eisenberger et al. 2009). In a study conducted by Eisenberger and colleagues, 20 subjects received endotoxin while 16 received placebo. The subjects underwent fMRI while completing the Cyberball social exclusion task 2 h post endotoxin injection and IL-6 levels were measured hourly for 6 h. Within the group that received endotoxin, the subjects that had greater increases in IL-6 levels prior to scanning, demonstrated greater activity in the medial and dorsomedial prefrontal cortex, similar to findings in our study, as well as demonstrated greater activity in the posterior superior temporal cortex, the temporal pole, the posterior cingulate cortex, and the precuneus. Although these studies involve directly induced systemic inflammation and not chemotherapy, it is interesting to note changes seen in brain activity associated with serum cytokine levels.

As is generally the case for PET imaging studies of this kind, limitations to our study include the relatively small number of subjects. Secondly, lack of a healthy female comparison limits the study. Differences between the chemotherapy and non-chemotherapy groups potentially could be confounded by disease burden. It is also important to consider depression, fatigue, and menopausal status as possible confounds. To eliminate depression as a possible confound, patients with clinical depression were excluded from the study. As for fatigue and menopausal status, we found no relationship between menopausal status or fatigue and self-reported cognitive complaints within the full MBS sample (Ganz et al. 2013). Additionally, after controlling for menopausal status within our PET sub-population, the significance of the correlations discussed above did not change. It is also important to note that although we are identifying a chain of correlations that would be consistent with a plausible previously proposed mechanism for cognitive effects of chemotherapy; this cannot be concluded to demonstrate causality. The data presented here however, do provide a window through which these relationships have been able to be systematically examined for the first time in cancer patients who have been exposed to chemotherapy.

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Conflict of interest The authors declare that they have no conflict of interest.

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