CHEMOTHERAPY EFFECTS ON BRAIN GLUCOSE METABOLISM AT REST

NIPGI[§]

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ABSTRACT

Background: A growing number of studies reports that chemotherapy may impair brain functions inducing cognitive changes which can persist in a subset of cancer survivors.

Aims: To investigate the neural basis of the chemotherapy-induced neurobehavioral changes by means of metabolic imaging and voxel-based statistical parametric mapping analyses.

Methods: We studied the resting brain [18]FDG-PET/CT images of 43 adult cancer patients with solid (n=12, 28%) or hematologic malignancies (n=31, 72%); 12 patients were studied prior the chemotherapy (No chemotherapy) while treated patients were divided in two matched subgroups: Early High (<9 months after chemotherapy, >6 chemotherapy cycles, n=10), and Late Low (>9 months after chemotherapy, <6 chemotherapy cycles, n=21).

Findings: Compared to No chemotherapy, the Early High subgroup showed a significant bilateral ($p<0.05$) lower regional cerebral metabolic rate of glucose metabolism in both the prefrontal cortices and white matter, cerebellum, posterior medial cortices and limbic regions. A similar pattern emerged in the Early High versus Low Late comparison, while no significant result was obtained in the Low Late versus No chemotherapy comparison. The number of cycles and the post-chemotherapy time were negatively and positively correlated, respectively, with a set of these same brain regions.

Interpretation: The present study shows that chemotherapy induces significant transient changes in the glucose metabolism of multiple cerebral cortical and white matter regions with a prevailing involvement of the prefrontal cortex. The severity of these changes are significantly related with the number of chemotherapy cycles and a subset of brain regions seems to present longer lasting, but more subtle, metabolic changes.

INTRODUCTION

Background

Cognitive changes in cancer patients after adjuvant chemotherapy (CHT) treatments have been reported since the mid 1970s, with systematic research starting in the early 1990s. Since then most, but not all [12, 22, 28], neuropsychological studies on cancer survivors having received adjuvant CHT have reported cognitive impairments in multiple domains such as executive functions, learning, memory (especially working memory, while the retrieval of remote memories seems to be spared), attention, verbal fluency and speed of information processing [3, 4, 17, 36, 42, 49, 52]. Both prior meta-analyses [14, 21] and, more recently, longitudinal studies [9, 20, 41, 50] have consistently shown that the CHT-induced cognitive impairments are small to moderate, involving mostly the cognitive functions subserved by the frontal lobes.

Data are however less consistent regarding to the evolution in time of these changes and to the possible dependency on CHT dose. Findings emerging from controlled longitudinal studies [9, 15, 50, 51] indicate that the cognitive changes tend to fully resolve over time while cross-sectional studies suggest that they may persist for many years following completion of treatment at least in a significant subset of patients [2, 8]. The CHT dose-effects relationships among cancer survivors has been mostly investigated by comparing the neurobehavioral performance among breast cancer patients receiving high or standard dose adjuvant CHT. High-dose therapy was found to elevate the risk of cognitive dysfunctions in some [41, 49] but not in all studies [43]. In a study performed on a mixed population of long-term breast cancer and lymphoma survivors [2] a significant, but low, negative correlation was found between the number of cycles and the cognitive performance, indicating that more cycles of CHT was associated with a greater cognitive disturbance.

Recent structural Magnetic Resonance Imaging studies have provided consistent evidence that CHT can induce both gray and white matter changes which can however be, at least partially, reversible. A CHT-related reduction in the gray matter volume [19] of structures significantly correlated with

indices of attention/concentration and/or visual memory (such as the prefrontal, cingulate and parahippocampal cortex), and a loss of white matter integrity [6, 11, 16, 19, 47] has been shown by comparing CHT treated with untreated cancer patients. The reversibility, at least partial, of these changes was suggested by longitudinal examination of cancer patients finding a significant increase in white matter volume from 6 months to 1 year after CHT [7] and no more gray and white matter volume difference 3 years after completion of CHT [19]. Two neuroimaging studies suggest, however, that CHT can induce long lasting adverse effects on the brain functions. Using [15]O water PET in an activation short-term recall task, modulation of cerebral blood flow in specific regions of frontal cortex and cerebellum was significantly altered, compared to a control sample, in breast cancer women investigated 5-10 years after receiving CHT [45]. An fMRI memory task performed on a sample of breast cancer women recruited 3-5 years after completion of CHT [24] showed a lower prefrontal cortex activation and an altered cerebellar recruitment compared to controls.

In sum, neither behavioural nor neuroimaging data are conclusive about the relationships between CHT dosage/number of cycles and cognitive impairments, as well as about the reversibility of the effects. Besides, little is known about the mechanisms leading these changes and how the brain tries to adaptively react. The recruitment of compensatory mechanisms aimed at overcoming the CHT-induced structural and/or functional impairments has been suggested by a fMRI in pairs of 60-year-old identical twins discordant for breast cancer [16]. While performing an identical working memory task with increasing levels of difficulty, the CHT-treated twin showed, compared to the other, a greater spatial extent of activation in the fronto-parietal dorsal attentional network.

The mechanisms for CHT-induced cognitive changes are largely unknown; however, several candidate mechanisms have been proposed, including blood brain barrier alterations, cytokines and hormonal deregulation, as well as a direct neurotoxicity of chemotherapic agents [1].

Aim of the Study

The present study used brain resting state [18]FDG-PET, combined with neuropsycological tests to assess relations between regional cerebral metabolic glucose rate (rCMRglc), cognitive performances and oncologic/therapeutic variables.

MATERIALS AND METHODS

Patients

Cancer patients were enrolled among those who were planned to undergo a whole-body [18]FDG PET on a clinically routine basis for cancer staging or to monitor the disease after treatment.

Patients were considered eligible if they did not have symptoms of neurological and psychiatric disorders and medications that could potentially alter neuropsychological performances and/or brain metabolism. Eligible patients gave written informed consent to participate in the project, which was approved by the ethical committee of San Giovanni Battista University Hospital, Turin.

Of the 43 enrolled patients 31 (72%) were prior treated with systemic CHT and 12 patients (28%) were not treated (No CHT). Their demographic and clinical characteristics are shown in Table 1. The chemotherapy protocols used are presented in Supplementary Table 1. The number of cycles of CHT ranged from 2 to 16 cycles (39%, n=12, <5 cycles; 48%, n=15, 5-10 cycles and 13%, n=4, >10 cycles). The time elapsed from the end of the treatment ranged from 1 week to 51 months (29%, n=9, <1 month; 35%, n=11, 2-9 months; 13%, n=4, 12-24 months; 16%, n=5, 2-3 years and 6 %, n=2, >3 years).

Since both animal [29, 40] and human studies suggest that CHT dose [41, 49] and number of CHT cycles [2], as well as the time elapsed since completion of the treatment [9, 15, 50, 51] can modulate the CHT effects on neurobehavior and brain metabolism, we divided the CHT group in two subgroups on the basis of both number of cycles (C) and post-CHT time (T). The first subgroup (subgroup EH), comprised 10 cancer patients observed after a delay <9 months (Early) and received more than 6 CHT cycles (High). The second subgroup (subgroup LL) comprised the remaining 21 cancer patients, observed after a delay >9 months (Late) or received less than 6 CHT cycles (Low). The characteristics of subgroups are shown in Table 1. The two CHT subgroups were balanced for age, gender and education level between themselves and also compared to the No CHT subgroup. They showed a significant difference (p=0.006) in the red blood cell count (RBC), the EH subgroup

showing a greater degree of anemia. This latter finding was somewhat expected since anemia frequently occur in cancer patients and its incidence and severity increases with CHT [34]. Finally, within the Late cancer patients, we selected, for further subanalysis, a subgroup (LH) comprising only those patients (n=9) investigated after a delay >9 months (Late) and received ≥6 CHT cycles (High).

Neuropsychological examination

The assessment battery of psychological and neuropsychological tests included: Mini Mental State Examination (MMSE), Trail Making Test B (TMT-B), Phonemic Fluency, Short Story Test, Hospital Anxiety and Depression Scale (HADS), Montgomery-Asberg Depression Rating Scale (MADRS), State and Trait Anxiety Inventory (STAI).

PET Scanning

In a quiet waiting room participants, lying in a supine position, were asked to refrain from moving and instructed "to keep their eyes closed, to not engage in any structured mental activity such as counting, rehearsing, etc., and to avoid to fall asleep". They were then blindfolded and ear plugged and received intravenously about 4.5–5.5 MBq kg−1 of 2-deoxy-2 [18F]fluoro-D-glucose (FDG). About 30 minutes later PET/CT scan was performed by a Philips Gemini scanner (Philips Medical System, Cleveland, Ohio, USA). The brain scan acquisition time was of 20 minutes. Reconstructed brain images had a dimension of 128 x 128 x 90 voxels (2 x 2 x 2 mm^3). After the planned whole body FDG PET/CT examination was performed, the coronal, sagittal and transverse data sets were reconstructed using an 3D iterative technique (row action maximum likelihood algorithm, RAMLA-3D) and corrected with single scatter simulation (SSS).

Statistical parametric mapping analysis

[18]FDG-PET brain images were preprocessed and voxel-based statistical analyses were performed using SPM2 (www.fil.ion.ucl.ac.uk/spm) running on MATLAB 6.5 software. All images were non linearly spatially normalized into the Montreal Neurological Institute (MNI) space and smoothed with an isotropic Gaussian kernel with 12 mm FWHM. Confounding effects of global activities

differences were removed by normalizing the count of each voxel to the mean count of a standardized pontine region of interest (ROI) in order to avoid a biased normalization [5].

The hypothesis of a negative linear association between the rCMRglc and C was tested on the whole CHT group (n=21) on a voxel-by-voxel basis using the SPM2 single-subject covariate only option. Age, T (months) and hormonal status (see supplementary materials for its definition) were entered in the general linear model as nuisance factors.

The hypothesis of a positive linear association between the rCMRglc and the time elapsed since the end of the treatment was tested with the same SPM option with age, gender and C entered as nuisance factors.

Between subgroups (EH versus LL; EH versus No CHT; LL versus No CHT; No CHT versus CHT) comparison analyses were performed using the ANCOVA model using age, hormonal status and education level as covariate of no interest.

Results were thresholded at $p<0.005$ uncorrected for multiple comparisons, with an extent threshold cluster extent (Ke) of 20 voxels. Statistical inferences were performed by applying the Random Field Theory. Clusters with $p\leq0.05$ corrected for multiple comparisons were considered as significant.

For subanalyses, we examined the correlations between neuropsychological scores and regional glucose metabolism of the voxels of interest. Patient characteristic data were analyzed with Kruskal-Wallis test for continuous variables and $\chi2$ test for categorical variables. Pearson's correlations was used to analyze rCMRglc data resulting from SMP2 analysis. SPSS 13.0 was used for statistical analysis, $p<0.05$ was considered significant. To compare correlations between subgroups in our sample we used the Fisher's Z-test.

Supplementary Material

The supplementary material contains a complete section of Material and Methods with all the steps of patients selection, all the neuropsychological tests descriptions, the detailed PET scanning and statistical procedures used.

RESULTS

Correlation between T and rCMRglc

A large cluster (9798 voxels) involving bilaterally the pregenual anterior cingulate cortex, the anterior thalamic nuclei, most of the right PFC, including the inferior (BA44, BA9), middle (BA9, BA46), superior (BA10) and medial frontal (Supplementary Motor Area, BA6) gyri as well as the left middle cingulate cortex showed a significant (Z=4.15) positive correlation between T and the rCMRglc (see Table 3, Supplementary Tables 2, 3 and Figure 1a, 2a).

Correlation between C and rCMRglc

A significant (Z=3.66) large cluster (4555 voxels) encompassing both the prefrontal cortices (PFC) and the left temporal pole was negatively correlated with C (see Table 3, Supplementary Tables 2, 3 and Figure 1b, 2a). The PFC involvement included the right dorsolateral PFC (DLPFC, BA9 and BA46), the right ventrolateral PFC (BA44), both the frontal poles (right BA10 and left BA11) and the left medial PFC (BA10). As it can be appreciated in the Figure 1, both the temporal poles were involved (BA38) even if the significant threshold was reached, as reported on the table, only on the left side. The body of Corpus Callosum and the right side of the posterior limb of the internal capsule were included in this cluster.

Comparison between No CHT (n=12) and CHT (n=31) subgroups

No significant clusters survived in a whole brain analysis thresholded at $p<0.005$. However, by applying a small volume correction (SVC), a left parahippocampus cluster (177 voxel) showed a significant (Z=3.13, p=0.039 corrected for multiple comparison) rCMRglc decrease in patients having received CHT compared to non treated ones (Table 3). The SVC was performed since several previous animal studies [44, 53] indicate this region as one of the most vulnerable to the adverse effects on brain functions induced by the CHT (see Table 3, Supplementary Table 4, 5 and Figure 3a).

Comparison between No CHT (n=12) and EH (n=10) subgroups

Compared to No CHT, the EH patients showed (see Table 3, Supplementary Table 4, 5 and Figure 1c, 2b) a bilateral lower rCMRglc in the DLPFC (Z=4.42), including the middle and medial frontal (BA9, BA10, BA46), in the precentral gyri (BA4), in the cunei (BA19) and in cerebellum; right-sided involvement was shown in the precuneus (BA7), in the lingual and in the parahippocampal gyri (BA19); left-sided involvement in the superior temporal gyrus (BA38) and in the posterior (BA30) and middle anterior cingulate cortex (BA32). Both the anterior and posterior left corona radiata and the right anterior corona radiata were included in this cluster.

Comparison between No CHT (n=12) and LL (n=21) subgroups

No suprathreshold cluster was revealed.

Comparison between EH (n=10) and LL (n=21) subgroups

Compared to LL, the EH patients showed a lower rCMRglc in two main large clusters involving the right and medial occipital cortex, the right temporal cortex and the prefrontal cortex bilaterally (see Table 3, Supplementary Table 4, 5 and Figures 1d, 2b). The most significant posterior cluster (Z=3.61) included, on the right hemisphere, the fusiform and middle occipital gyri (BA19), the inferior (BA37) and medial (BA39, BA19) temporal gyri, the precuneus (BA7) and, medially, both cunei (BA19). The anterior prefrontal cluster included the middle frontal gyrus (BA11) on both sides, the right middle and medial frontal gyri (BA47, BA10), the right superior frontal gyrus (BA10, BA11), the left medial frontal gyrus (BA25) an the right superior temporal gyrus (BA38).

Neuropsychological scores and rCMRglc

We did not find any significant correlation between rCMRglc and neuropsychological or psychological variables in the total sample, in the CHT and in the No CHT groups. In the CHT subgroups no cluster survived to a correction for multiple comparison.

We did not find any significant difference in the neuropsychological performances of our subgroups (Table 2).

Comparison between EH (n=10) and LH (n=9) subgroups

This comparison was performed by applying at p=0.0058 to have the same threshold t=2.90 as in the above EH<LL comparison (see Table 3, Supplementary Table 4, 5 and Figure 1e). Compared to LH, the EH subgroup showed a lower rCMRglc in both the DLPFC, including the middle (BA10) and superior frontal gyri (BA10), and in the middle cingulate cortex (BA32). The CMRglc was moreover asymmetrically reduced in the left pregenual cingulate cortex (BA32) and the right superior frontal gyrus (BA9). Only the left cluster (DLPFC, MCC, pgACC) survived the correction for multiple comparisons.

Comparison between No CHT (n=12) and LH (n=9) subgroups

No suprathreshold cluster was revealed.

DISCUSSION

Amongst the several competing heuristic models of CHT-induced brain damage (see Figure 3c, 3d, 3e) which can be proposed through the analysis of the available neuropsychological and neuroimaging data, conclusive or convincing evidence favouring the one or the other has still not been reached. To address these questions we performed a set of correlation analysis exploring the relationships between the rCMRglc and T or C. We then performed a set of comparisons intended to confirm and strengthen the former analysis. The correlation analysis between the rCMRglc and T uncovered a set of brain areas (including the right middle, inferior and superior frontal gyri along with other midline and subcortical brain regions, as well as white matter regions including the body of corpus callosum and the posterior limb of internal capsule) partially reproduced and confirmed by the comparison of the two subgroups of CHT-treated patients (EH<LL, see Table 3, Supplementary Table 4, 5 and Figure 1c, 2b). Moreover, partially overlapping results were obtained in the comparison of the EH subgroup with the No CHT subgroup. Interestingly, the peak of greatest rCMRglc decrease found in this latter comparison in the right middle frontal gyrus (see Table 3, Supplementary Table 4, 5 and Figure 1c, 2b) was very close (x, y, z = 38, 62, 6) to that reported (x, y, z = 30, 64, 4) by a previous cited structural study [19] in a comparison between treated and non treated cancer patients. Our data therefore strongly support a reversible model of CHT-induced brain damage (see Figure 3d, 3e) suggesting that the chemotherapy-induced cerebral glucose metabolic impairment could be transient and rapidly (~1 year) reversible over the time, paralleling or even preceding the structural recovery of the CHT targeted brain regions. These results are in agreement with findings emerging from controlled longitudinal neuropsychological studies [9, 15, 50, 51] indicating that the cognitive changes tend to fully or partially resolve over 1 year. These assumptions on the reversibility of brain metabolic impairment must however be taken with caution. Obviously in a cross-sectional study as the present work, the real dynamics of the metabolic changes triggered by the CHT cannot be adequately studied. Furthermore, we cannot

exclude that a subset of brain regions did not recover or recover only partially over the time as suggested by some neuropsychological [2, 8] and neuroimaging [24, 45] studies. Candidate regions which could have, in the present study, a longer lasting metabolic impairment are the precentral gyri (BA4), a set of midline cortical structures such as left medial frontal gyrus (BA9, BA6) and the right posterior cingulate cortex (BA30), as well as the left middle frontal gyrus (BA46, BA8) and the cerebellum. These regions indeed showed a lower rCMRglc in the EH<No CHT comparison (see Table 3, Supplementary Table 4 and Figure 1c, 2b), but not in the EH<LL and in EH<LH comparisons (see Table 3, Supplementary Table 4 and Figure 1d, 1e, 2b) and did not show any significant positive correlation with T (see Table 3, Supplementary Tables 2, 3 and Figure 1a, 2a). Since both the No CHT>LL and No CHT>LH comparisons did not give significant results we suppose that these areas only partially recovered over time. With respect to the cerebellum, the partial recovery hypothesis suggested by our data, is consistent with previous [O-15] water PET [45] and fMRI studies [24].

A further region showing an incomplete recovery over time was the left parahippocampus, which showed in the comparison of non treated with the whole group of treated patients (No CHT>CHT: see Table 3, Supplementary Table 4, 5 and Figure 3) a significantly lower rCMRglc. A subanalysis evidenced that, compared to non treated patients, the rCMRglc of this region was significantly ($p=0.006$) lower in patients investigated at a mean of more than 1 year after the completion of CHT and having received a C comparable to the EH subgroup (LH subgroup, see Figure 3b).

To gain more insights on the mechanisms underlying these chemotherapy-related adverse effects we looked for a relationship between C and the cerebral glucose metabolism. The voxel-based correlation analysis evidenced a set of brain areas (including the right superior, middle, inferior frontal gyri, the left middle frontal and superior temporal gyri) showing a significant negative association between C and the rCMRglc, which partially overlapped those uncovered by both the comparative and correlation analysis described above. These results therefore support a model in which the CHT-induced brain damage appears to be related to C (Figure 3d), in agreement with

previous neuropsychological data [2] reporting a negative relationship between C and the cognitive performance.

The pattern of CHT-induced brain metabolic impairment, markedly characterized by a disproportionate involvement of frontal lobes, is reminiscent of that seen in ageing [10, 23, 32, 48] and some age-related neurodegenerative processes [46]. However, a salient finding of our study was that the wide frontal metabolic impairment evidenced in the EH subgroup was not associated with any significant cognitive impairment (Table 2). Such mismatch between metabolic and cognitive data is well known in the field of Alzheimer's disease (AD) FDG-PET studies. In cognitively normal subjects at genetic risk of developing AD, such as apolipoprotein E (ApoE) E4 carriers, such studies showed a reduced glucose metabolism in the same regions of the brain as in patients with probable AD [37-39] such as the retrosplenial, the parietal and the frontal cortex. Moreover they evidenced that in these cognitively normal subjects this reduced glucose metabolism could be associated with perceived loss of memory ability [13] and subjective cognitive complaints [31]. This could be relevant since, as reported by previous studies, cancer survivors treated with CHT often self-report higher levels of cognitive problems but, as was seen in this work, perform normally on neuropsychological tests [16, 35]. Furthermore, recent functional and structural studies have shown that cognitively healthy people with a maternal family history of late onset AD (FHm), have a rCMRglc reductions [30] and less gray matter volume [18] in AD-vulnerable brain regions, including the frontal cortex, compared to subjects with no parental history of AD. Interestingly, compared to subjects with a negative history those with a FHm showed the lowest CMRglc in the right superior frontal gyrus at coordinates (x, y, z = 34, 61, 4), similar to those found by us (x, y, z = 38, 62, 6). Moreover, FHm subjects compared to subjects with a paternal familial history of late onset AD showed a lower CMRglc in the superior frontal gyrus at coordinates (x, y, z = 30, 63, 1). Hypometabolism in FHm is thought to be due to a combination of dysfunction of mitochondria [54] (maternally inherited), increased oxidative stress, and possible mitochondrial DNA mutations [25], leading to CMRglc changes in vulnerable brain regions [33]. Many of these processes have been

proposed as candidate mechanisms mediating the adverse effects of CHT on brain functions [1]. Oxidative DNA damage and decreased mitochondrial function are well established processes underlying brain aging changes [27], findings leading Maccormick [26] to hypothesize that adverse effects associated with CHT might be related to acceleration of the ageing process. Inspired by this hypothesis we performed a post-hoc analysis looking for differences in the correlations between age and the rCMRglc of regions showing a lower glucose metabolism in the EH<No CHT subgroups comparison. This analysis showed that, compared to non treated patients, the CMRglc of the medial posterior cortices in the EH patients had a more significant and negative association with age (Fisher's z test: $p<0.05$, see Supplementary Table 6 and Figure 4a, 4b), meaning that older subjects undergo an higher than expected CHT-induced metabolic impairment. Taken together, these findings lead us to speculate that CHT induces aging-like and/or potentiates aging-related processes such as oxidative stress, and decreased mitochondrial function which could lead to the rCMRglc reduction evidenced, in patients receiving CHT.

The supplementary materials contains an extended in depth discussion and the study limitations.

Conclusions

This study evidenced significant chemotherapy-related changes of glucose metabolism in multiple brain regions involving both the cortex and the white matter, with a prevailing involvement of the frontal lobes. Such metabolic changes appear to be positively related in many of these same areas with the time elapsed from the end of the treatment suggesting that they are transient and rapidly reversible. A subset of these areas undergoes a metabolic impairment proportional to the number of CHT cycles while a subset, including the cerebellum and midline cortical regions, present evidences of partial or delayed metabolic recovery.

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TABLES

Table 1. Clinical and demographic characteristics

Subgroups	No CHT (n=12) A	CHT (n=31) B	EH (n=10) C	LL (n=21) D	LH (n=9) E	Sign.
Age (y)	57.5(47-67.8)	47(36-59)	41.5(28-54.8)	48(41.5-61)	56(45-67.5)	NS
Gender (m/f)	9/3	16/15	5/5	11/10	3/6	NS§
Education (y)	13(8-18.8)	13(8-15)	13(8-16.5)	11(8-14)	8(8-13)	NS
BMI (kg/m^2)	24.5(23.3-28)	26(22-30)	26(20.8-31.8)	26(22.5-29)	28(23-29.5)	NS
Solid Tumors [n(%)]	6(50)	6(19.4)	0(0)	6(28.6)	3(33.3)	
Lymphoma [n(%)]	6(50)	25(80.6)	10(100)	15(71.4)	6(67)	NS§
HL	1(8.3)	6(19.4)	2(20)	4(19)	2(22)	
NHL	5(41.7)	19(61.3)	8(80)	11(52.4)	4(44)	
Age at onset (y)	57.5(47-65.5)	45(35-58)	40.5(27.8-53.8)	45(37-59.5)	51(35-62)	A>B* A>C*
Disease Duration (mos)	1.5(1-4.3)	15(9-33)	11.5(8.3-25)	16(8.5-45.5)	34(29-63.5)	A>B* A>C* A>D* C>E*
Surgery [n (%)]	1(8.3)	6(19.4)	1(10)	5(23.8)	4(44.4)	NS§
RT [n (%)]	0(0)	14(45.2)	5(100)	9(42.9)	4(44.4)	A>C*§ A>D*§
Post RT time (mos)		4(3-35.3)	4(3-4.5)	7(3-47)	30.5(0-76.5)	NS
Cycles Number		6(4-8)	8(6.8-10.5)	4(3-6.5)	7(6-8.5)	C>D*
Post CHT time (mos)		5(1-23)	4(3-4.5)	12(1-27)	26(20.5-33)	C>E*
T/C ratio		0.8(0.3-3)	0.4(0.1-0.6)	2(0.5-3.4)	3.5(2.8-4.7)	C<D* C<E*
Corticosteroids [n (%)]	0(0)	24(77)	9(90)	15(71)	6(66.6)	NS§
WBC (10^3/mL)	8.2(5.2-9.7)	4.8(3.8-8)	6.2(3.8-8.7)	4.7(3.7-8.8)	4.8(4-6.9)	NS
RBC (10^6/mL)	4.8(4.7-5.3)	4.3(3.4-4.7)	3.4(2.9-3.9)	4.3(4.1-4.9)	4.6(4.1-5)	A>B* A>C* C<D* C<E*
Hb (g/dL)	14(13.5-15.2)	12.8(10.2-13.9)	10.2(9.9-14.2)	12.9(12.4-13.9)	13.8(12.9-14)	NS
Hb <12 g/dL [n (%)]	0 (0)	10 (32)	6(60)	4(19)	1(11)	A>B*
s-Glucose (mg/dL)	103(93-109)	99(86-111)	96(88-114)	99(81-112)	106(84-116)	A>B*§ C<D*§
Cholesterol (mg/dL)	202(146-225)	197(172-215)	199(161-219)	197(180-216)	213(168-245)	NS

Values represent, when not otherwise specified, median (interquartile range).
Significance (Kruskal Wallis test): NS = not significant, *p<0.05. §Chi Square Test.
BMI = Body Mass Index; CHT = Chemoterapy; C= number of CHT cycles; f = females; Hb = Hemoglobin; HL = Hodgkin's Lymphoma; m = males; mos = months; NHL = non Hodgkin's Lymphoma; RBC = Red blood cells; RT = Radiation Therapy; T = time post CHT; WBC = white blood cells; y = years.
EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.

Table 2. Neurobehavioral characteristics

Subgroups	No CHT (n=12) A	CHT (n=31) B	EH (n=10) C	LL (n=21) D	LH (n=9) E	Sign.
MMSE	27.4(26.2-28.3)	26.6(25.6-27.6)	26.7(25.6-27.8)	26.5(25.5-27.5)	27.2(25.4-27.5)	NS
<23.8 n(%)	0(0)	2(6)	1(10)	1(5)	1(10)	
Verbal Fluency	34.2(31.7-39.4)	30.7(24.2-41.6)	29.1(22-36)	30.8(25.1-42.4)	30.8(27.4-42.8)	NS
<17.5 n(%)	0(0)	3(10)	1(10)	2(10)	0(0)	
Short Story	11.9(7.8-12.8)	11.7(8.9-12.9)	12.3(9.6-12.9)	11.6(8.9-13.3)	11.7(10-14.1)	NS
<7.25 n(%)	3(25)	2(6)	0(0)	2(10)	0(0)	
TMT-B (n=42)	86(63.8-116.8)	80(53.3-128.8)	116(73-140.3)	78(43-109.8)	78(25.3-82.3)	NS
>283'' n(%)	0(0)	0(0)	0(0)	0(0)	0(0)	
MADRS	9.5(5.3-12.8)	6.0(3-15)	11.5(3.8-17.5)	5.0(3-13.5)	4.0(1-6)	NS
>11 n(%)	5(41)	11(35)	5(50)	6(28)	0(0)	
HADS-D (n=40)	5(4-8)	5(1-9)	5(0.8-8.8)	4(1-9)	3(0-8.5)	NS
>8 n(%)	3(25)	8(26)	2(20)	6(28)	2(22)	
HADS-A (n=40)	5(2-8)	5(2-8)	5(3.8-7.8)	5(1-8)	1(0.5-7)	NS
>8 n(%)	6(50)	8(26)	3(30)	5(24)	2(22)	
HADS-T (n=40)	11(7-14)	10(5-17)	11(5.8-17.3)	9(3-18)	8(0.5-13.5)	NS
>13 n(%)	8(66)	7(23)	3(30)	4(19)	2(22)	
STAI-Y1 (n=37)	48(37.3-53)	41(36-53.5)	41(37.5-52)	41(35.2-54.5)	41(38-46.3)	NS
>50 n(%)	4(33)	8(26)	2(20)	6(28)	1(11)	
STAI-Y2 (n=37)	44(34-51)	44(34-58.8)	44(32.8-48.3)	45(36.3-63.3)	43(35.5-60.8)	NS
>50 n(%)	3(25)	9(29)	3(30)	6(28)	2(22)	

Significance (Kruskal Wallis test): NS = not significant.
HADS-D = Hospital Anxiety and Depression Scale: Depression; HADS-A = Hospital Anxiety and Depression Scale: Anxiety; HADS-T = Hospital Anxiety and Depression Scale: Total; MADRS = Montgomery-Asberg Depression Rating Scale; MMSE = Mini Mental State Examination; STAI = State Trait Anxiety Inventory; TMT = Trail Making Test.
CHT = Chemotherapy; EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.
Other annotations as in Table 1.

Table 3. Voxel-based analysis

Cerebral Region	BA	p corr.	Ke	z	Talairach Coordinates x	y	z
Between subgroups comparison analysis (n=43) No CHT (n=12) > CHT (n=31)							
(L) Parahippocampus (Uncus)	28	0.039 (SVC)	177	3.13	-24	4	-29
Between subgroups comparison analysis (n=22) No CHT (n=12) > EH (n=10)							
(R) Middle Frontal Gyrus	10	<0.001	7622	4.42	38	62	6
(R) Superior Frontal Gyrus	10			3.92	34	57	14
(L) Cuneus	19	<0.001	3657	4.41	-2	-82	37
(R) Cuneus	19			4.25	8	-78	33
(L) Medial Frontal Gyrus (SMA)	6	<0.001	6644	4.15	-6	-7	52
(L) Middle Frontal Gyrus	46			3.82	-48	34	26
(L) Cerebellum Tonsilla	-	0.001	2493	3.61	-36	-51	-40
(L) Cerebellum Tuber	-			3.06	-32	-64	-29
(R) Cerebellum Tonsilla	-	0.017	1447	3.50	36	-41	-35
(R) Cerebellum Culmen	-			3.07	42	-42	-23
(R) Anterior Corona Radiata	WM	3.56	12	34	-8	3.56	12
(L) Anterior Corona Radiata	WM	3.02	-14	34	-12	3.02	-14
Between subgroups comparison analysis (n=33) No CHT (n=12) > LL (n=21)							
No suprathreshold cluster							
Between subgroups comparison analysis (n=19) No CHT (n=10) > LH (n=9)							
No suprathreshold cluster							
Between subgroups comparison analysis (n=31) EH (n=10) < LL (n=21)							
(R) Fusiform Gyrus	19	0.025	2714	3.61	30	-65	-9
(R) Middle Occipital Gyrus	19			3.29	44	-80	2
(R) Middle Frontal Gyrus	10	0.001	5263	3.43	42	52	20
(L) Middle Frontal Gyrus	11			3.22	-22	30	-13
Between subgroups comparison analysis (n=31) EH (n=10) < LH (n=9)							
(R) Superior Frontal Gyrus	10	0.012**	1074	3.94	40	59	16
(R) Superior Frontal Gyrus	9			3.24	38	44	29
(L) Middle Frontal Gyrus	10	0.046	1557	3.86	-40	53	18
(L) Anterior MCC	32			3.02	-6	18	38
Negative correlations with the number of CHT cycles in the CHT subgroup (n=31)							
(L) Superior Temporal Gyrus	38	0.002	4555	3.66	-46	16	-19
(R) Middle Frontal Gyrus	9			3.37	48	6	37
Positive correlations with the post-CHT time in the CHT subgroup (n=31)							
(L) Subgenual ACC	25	<0.001	9798	4.15	-2	9	-9
(R) Middle Frontal Gyrus	46			3.50	55	30	24
(L) Body of Corpus Callosum	WM	<0.001	9798	3.67	10	7	27
(R) Posterior Limb of Internal Capsule	WM			2.93	18	-3	15

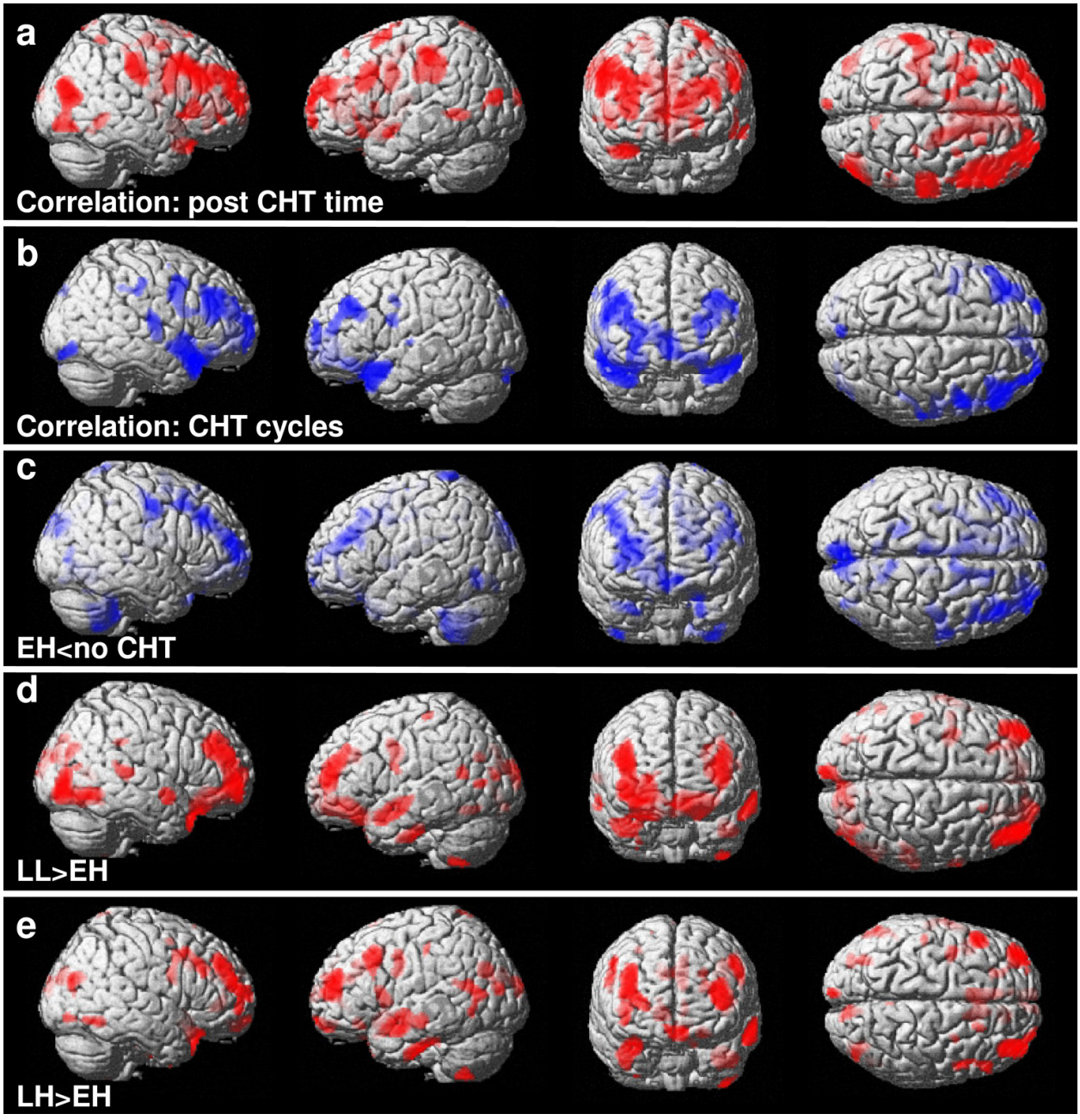
Height threshold p = 0.005 uncorrected for multiple correlations; p corr. = p corrected for multiple correlations; SVC = Small Volume Correction.

ACC = Anterior Cingulate Cortex; BA = Brodmann Area; CHT = chemotherapy; Ke = cluster extent; L = left; R = right; SMA = Supplementary Motor Area; WM = White Matter.

EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.

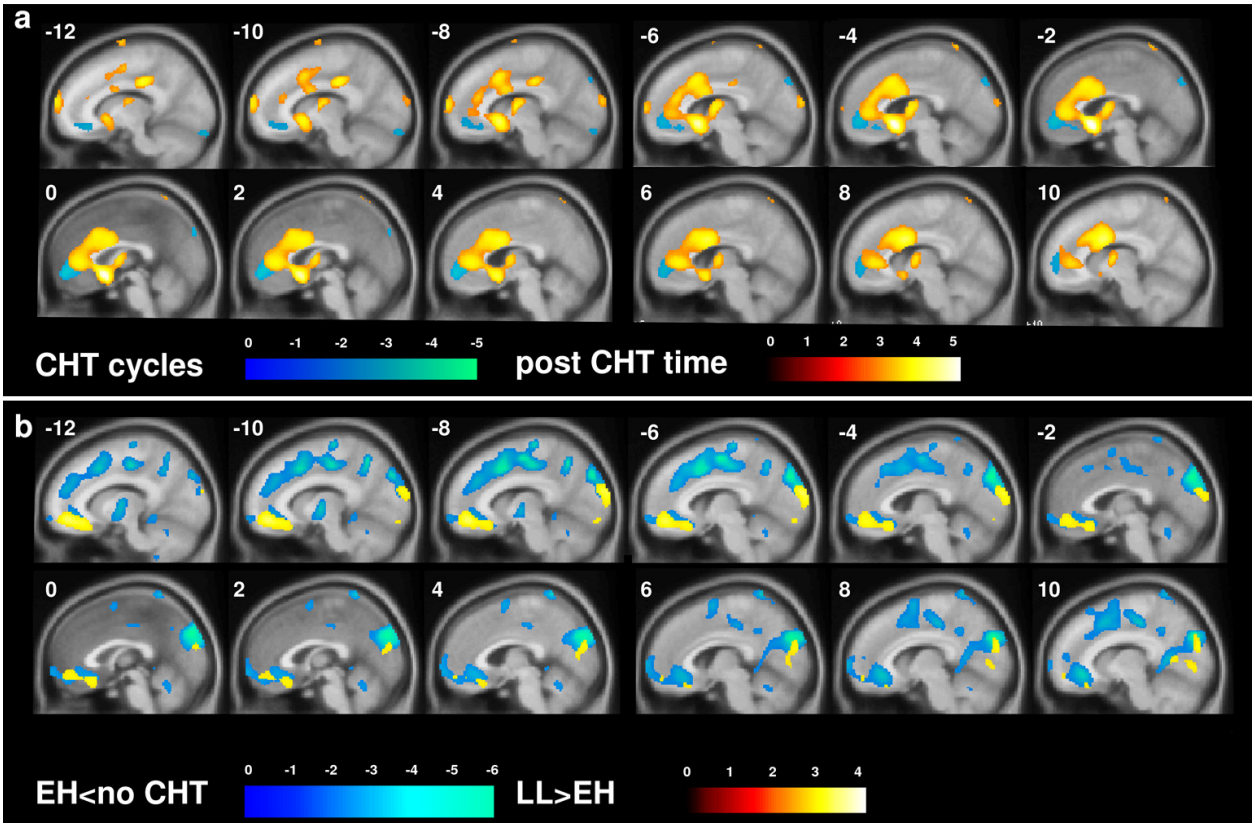
FIGURES

Figure 1. Patterns of CHT-induced rCMRglc changes



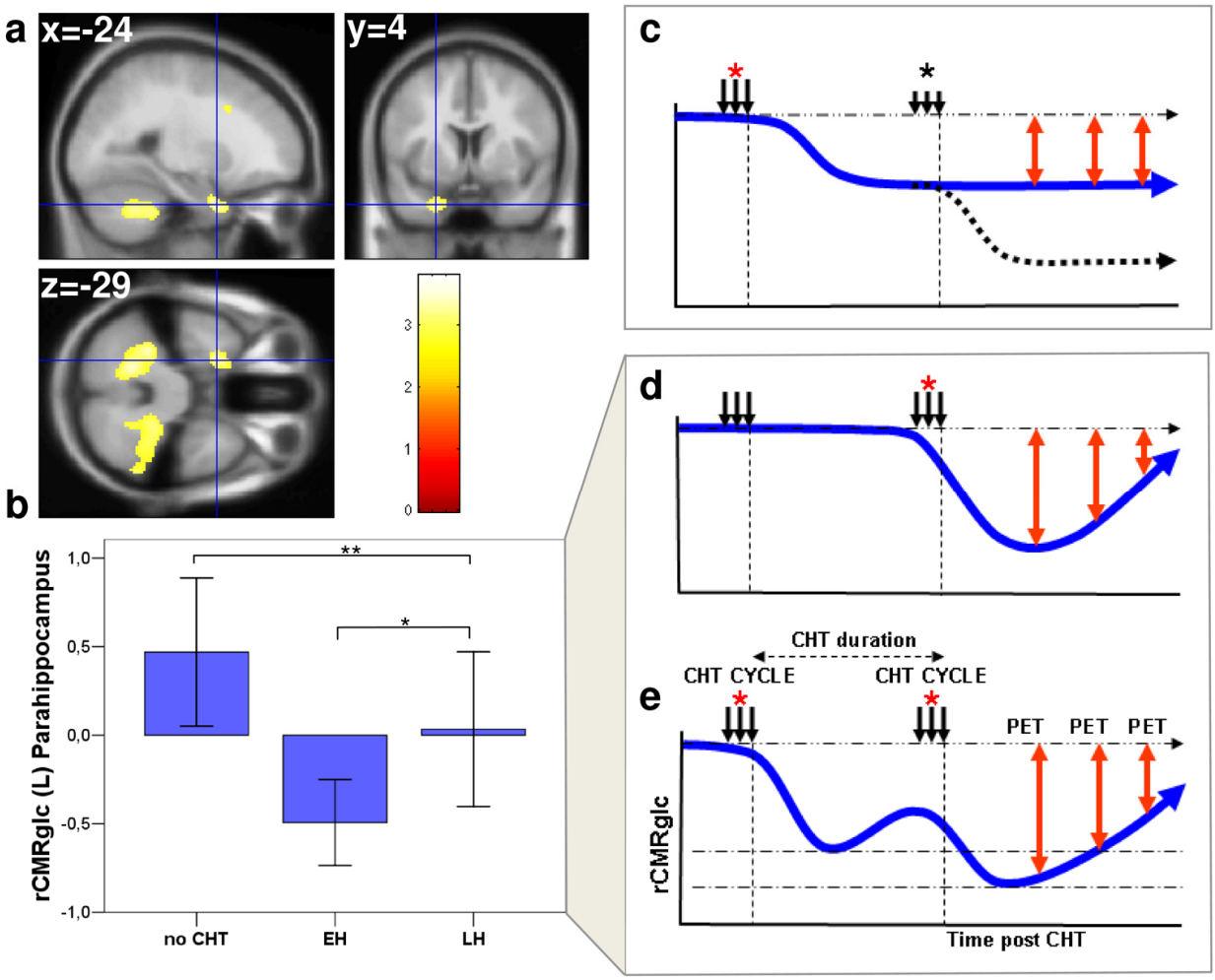
T-maps obtained by correlations (a-b) and comparisons (c-d) analysis overlaid on canonical 3-D brain templates. Red coloured maps show positive correlations (a) or comparisons (d); blue coloured maps show negative correlations (b) or comparisons (c). CHT = chemotherapy; EH = Early High subgroup; LL = Late Low subgroup. As it can be appreciated, at the cortical level a significant overlap exist among the four voxel-based analysis results.

Figure 2. Patterns of CHT-induced rCMRglc changes in midline cerebral structures



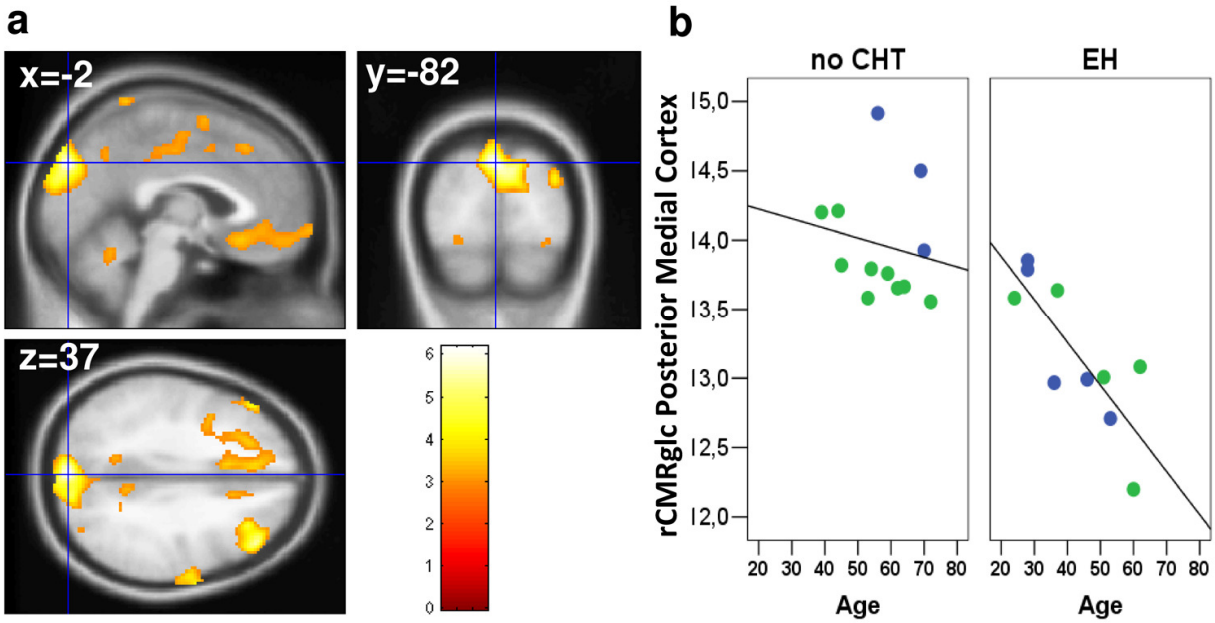
T-maps obtained by correlations (a) and comparisons (b) analysis overlaid on sagittal brain slices of a canonical brain template. Colour bars show the t values. Other annotations as in figure 1.
The time-related metabolic recovery in the anterior cingulate cortex, in the Corpus Callosum and in the thalamic Anterior Nuclei are clearly appreciable in the top (a). The midline cortical brain regions showing a lower rCMRglc in the EH subgroup compared to LL are evidenced in the bottom (b).

Figure 3. Comparison between the No CHT (n=12) and the CHT (n=21) groups



The whole group of patients having received chemotherapy compared to those non treated shows a significant lower rCMRglc in the left parahippocampus (a). Subanalysis indicates (b) the significant rCMRglc difference between the two subgroups (EH and LH) of CHT patients and the lack of a complete metabolic recovery in the LH subgroup (one asterisk=p<0.05, two asterisks=p<0.01). These findings are consistent with a time-dependent (d-e) against a non time-dependent (c, irreversible impairment) heuristic model of CHT-induced rCMRglc changes. The models take in account moreover the possible cumulative neurotoxic effects of the CHT cycles (asterisk).

Figure 4. Ageing in the No CHT (n=12) and EH (n=10) groups



Compared to non treated patients, the rCMRglc of the medial posterior cortices showed in the EH patients a more significant and negative association with age (a, b). Blue dots = females, green dots = males.

SUPPLEMENTARY MATERIALS

EXTENDED MATERIALS AND METHODS

Patients

Cancer patients were enrolled among those who were planned to undergo a whole-body [18]FDG PET on a clinically routine basis for cancer staging or to monitor the disease after treatment. The week before the scan session patients received a call from a researcher who initially described the aim and the procedure of the study and performed a brief standardized medical and neurobehavioral screening focused on signs and symptoms of neurological and psychiatric disorders and medications that could potentially alter neuropsychological performances and/or brain metabolism. On the day of the scan eligible patients completed session the medical assessment and gave written informed consent to participate in the project, which was approved by the ethical committee of our institution. Cancer patients were excluded from the study if they had signs or symptoms of CNS disease, had received intrathecal therapy or CNS radiation therapy, had an history of neurologic disorder, including head injury with loss of consciousness or had an axis I psychiatric disorder (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) such as drug abuse, mood, anxiety and psychotic-spectrum disorders.

Of the 45 eligible cancer patients who agreed to participate 2 were excluded once the FDG-PET scan was acquired since their brains showed metabolic patterns (clinically silent marked inter-hemispheric asymmetry, ventricular dilatation) that made them not suitable for the subsequent voxel-based analysis. Of the remaining 43 patients 31 (72%) were prior treated with systemic CHT and 12 patients (28%) were not treated (No CHT). Their demographic and clinical characteristics are shown in Table 1. Within the CHT patients the majority (n=19, 61%) had a non-Hodgkin's lymphoma (NHL), 6 (19%) a Hodgkin's lymphoma (HL) and 6 (19%) a solid tumor (sarcoma, n=1; breast cancer, n=3; bladder cancer, n=1; rectal cancer=1). Among them, 15 patients had not been

previously treated with surgery and radiation therapy (RT), 2 underwent surgery alone, 10 RT alone and 4 both surgery and RT. Within the 12 No CHT patients, 5 (41%) had a NHL, 1 (8%) a HL and 6 (50%) a solid tumor (bladder cancer, n=1; breast cancer=2; lung cancer, n=1; rectal cancer=2). Of these patients, only 1 underwent surgery while no one was subjected to radiotherapy.

The ABVD [Adriamycin (doxorubicin), bleomycin, vinblastine and dacarbazine] chemotherapy protocol (one of the most common CHT regimens for treating Hodgkin's Lymphoma) was used in 19% (n=6) of patients. The CHOP protocol [cyclophosphamide, hydroxydaunorubicin, Oncovin (vincristine), and prednisone/prednisolone] (widely employed in the treatment of NHL) was used in 39% (n=12) of patients, in all but one case associated with the monoclonal antibody rituximab, this latter being used in other combinations in more than 55% (n=17) of all cases. Corticosteroid therapy was used in about 77% (n=24) of patients. Other, less frequently used regimens, are presented in Supplementary Table 1. The number of cycles of CHT ranged from 2 to 16 cycles with 39% (n=12) of treatments consisting of less than 5 cycles, 48% (n=15) of less than 10 cycles and only 13% (n=4) in more than 10 cycles. The time elapsed from the end of the treatment ranged from 1 week (recorded as 0 months) to more than 4 years (51 months) with 29% (n=9) of cases observed after 1 month or less since the end of the treatment, 35% (n=11) between 2 and 9 months, 13% (n=4) between 12 months and two years, 16% (n=5) between 2 and 3 years and only 6 % (n=2) observed after more than 3 years from the end of the treatment.

Since both animal [32, 48] and human studies suggest that CHT dose [49, 56] and number of CHT cycles [3], as well as the time elapsed since completion of the treatment [13, 19, 59, 61] can modulate the CHT effects on neurobehavior and brain metabolism, we divided the CHT group in two subgroups on the basis of both number of cycles (C) and post-CHT time (T). We reasoned that patients having received an higher number of cycles and investigated in a shorter delay from the end of the CHT could have undergone greater neurobehavioral and metabolic disturbances if compared to patients after a longer delay or having received a lower number of cycles. To maximize the chance of finding these anticorrelated effects (that is a negative association between C and the

rCMRglc and a positive association between the T and the rCMRglc) we defined two cut-off scores (a C and a T cut-off), dividing the two subgroups on the basis of the following criteria: number of patients match for age, gender and education level, maximal C, T and T/C ratio differences between subgroups. We empirically found that, within our CHT group, the most parsimonious cut-offs satisfying these criteria were T=9 months and C=6 cycles. The first subgroup (subgroup EH), comprised 10 cancer patients observed after a delay <9 months (Early) and having received more than 6 CHT cycles (High). The second subgroup (subgroup LL) comprised the remaining 21 cancer patients, observed after a delay >9 months (Late) or having received less than 6 CHT cycles (Low). The characteristics of subgroups are shown in Table 1. The two CHT subgroups were balanced for age, gender and education level between themselves and also compared to the No CHT subgroup. They showed a significant difference (p=0.006) in the red blood cell count (RBC), the EH subgroup showing a greater degree of anemia. This latter finding was somewhat expected since anemia frequently occurs in cancer patients and its incidence and severity increases with CHT [42]. Finally, within the Late cancer patients we select, for further subanalysis, a subgroup (LH) comprising only those patients (n=9) investigated after a delay >9 months (Late) and received ≥6 CHT cycles (High) which were matched for age, gender and education level with all others subgroups (see Table 1 E), matched with the EH subgroup for number of cycles, and significantly different, compared to EH subgroup, with respect to post-CHT time (p<0.0001) and T/C ratio (p<0.0001).

Neuropsychological examination

The assessment battery of psychological and neuropsychological tests included: Mini Mental State Examination (MMSE), Trail Making Test B (TMT-B), Phonemic Fluency, Short Story Test, Hospital Anxiety and Depression Scale (HADS), Montgomery-Asberg Depression Rating Scale (MADRS), State and Trait Anxiety Inventory (STAI). The Italian standardization of the tests was used when available [54]. The neuropsychological battery was administered in a session of half an hour, following a standard protocol, in a quiet, private room in the Hospital before the PET scan. The psychological self-report scales were filled by the subjects after the neuropsychological

assessment. Mini Mental State Examination (MMSE), used to assess the presence of general cognitive impairment [21], is a brief 30-point questionnaire test commonly used to screen for dementia. It samples various functions including time and place orientation, repeating lists of words, arithmetic such as the serial sevens, language use and comprehension, copy of a geometric drawing and basic motor skills. MMSE was corrected for age and education and the cut-off for normality was 23.8. The Trail Making Test part B, used to test the visual sustained and alternating attention [22, 47], requires a subject to connect the dots of 25 consecutive targets on a sheet of paper in which the targets are letters and numbers that the subject alternates in crescent order (1, A, 2, B, 3, C, etc.). The goal of the subject is to finish the test as quickly as possible. The Trail Making Test is age and education corrected and the cut-off for normality was 283''. Phonemic Fluency, used to evaluate the retrieval of words with an uncommon searching trategy [41], requires to name as many F-, A- and S- beginning words as possible in consecutive 1-min time periods, exclusive of perseverations or out-of-category words. The score is the total number of words retrieved. This test was age and education corrected and the cut-off for normality was 17.35. Short Stories Test evaluates verbal logical memory with the immediate and delayed free recall of verbal information [41]. The examiner reads to the examinee a text containing 27 elements to remind. After the reading an immediate recall is administered, then a delayed recall after 10'. The score is the sum of the reminded logical concepts in the two trials, the maximum score per trial is 8, the concepts are scored on the basis of their relevance in the story. We used 7.25 as cut-off for normality for the total score. HADS [63] Italian version [14] was used to evaluate the psychological distress in a non-psychiatric setting. It is composed of two self-report scales of 14 items, 7 regarding anxiety and 7 regarding depression. The two scores can be calculated separately with a cut-off of 8 to detect the presence of anxiety and depression [9]. By calculating the sum of the two scales, it is possible to identify the presence of disturbance in adaptation (cut-off 13). No psychological distress is evidenced if the sum of the two scores totals <13. MADRS is a 10-item scale of depression severity that is based on patient report and clinical observation. This measure was designed to be sensitive to

symptom change in clinical trials [34]. Scores on the MADRS range from 0 to 60, with higher scores indicating greater severity of depressive symptomatology, we used a cut-off of 11 to identify a patient as depressed or not [64, 65]. The STAI consists of two self-evaluation scales designed to assess state-anxiety (form Y1) and trait-anxiety (form Y2) separately. The state-anxiety scale evaluates a transitory state-anxiety in which unpleasant feelings, tension, and intensity vary according to the situation. The trait-anxiety scale assesses a longer-term personality characteristic. Each scale contains 20 items, each of which is rated from 1 to 4. Clinically significant levels of state-anxiety or trait-anxiety were defined as scores ≥50 on the state-anxiety or trait-anxiety sub scale [52, 53].

PET Scanning

In a quiet waiting room participants, lying in a supine position, were asked to refrain from moving and instructed "to keep their eyes closed, to not engage in any structured mental activity such as counting, rehearsing, etc., and to avoid to fall asleep". They were then blindfolded and ear plugged and received intravenously about 4.5–5.5 MBq kg^{-1} of 2-deoxy-2 [18F]fluoro-D-glucose (FDG). Subjects were previously asked to fast for at least 6 h before PET. About 30 minutes later PET/CT scan was performed by a Philips Gemini scanner (Phillips Medical System, Cleveland, Ohio, USA). The Gemini comprises a Philips MX 8000D dual-slice CT scanner with a gadolinium oxyorthosilicate GSO-based Allegro PET scanner. The PET scanner has an axial field of view (FOV) of 18 cm, a transaxial FOV of 30 cm, a full width at half maximum (FWHM) axial resolution of 5 mm and a transaxial resolution of 4.8 mm. A low-powered CT scan (120 kV, tube current 30 mA) is performed to correct attenuation. To minimize head motion, the subject's head was placed in a thermoplastic head holder mounted on the scanner table. The brain scan acquisition time was of 20 minutes. Reconstructed DICOM (Digital Imaging and Communications in Medicine) brain images with a dimension of 128 x 128 x 90 voxel (voxel dimension = 2 x 2 x 2 mm^3) were converted in Analyze format using MRIConvert, a free software application (http://lcni.uoregon.edu/~jolinda/MRIConvert). After the planned whole body FDG PET/CT

examination was performed, the coronal, sagittal and transverse data sets were reconstructed using an 3D iterative technique (row action maximum likelihood algorithm, RAMLA-3D) and corrected with single scatter simulation (SSS).

Statistical parametric mapping analysis

[18]FDG PET brain images were preprocessed and voxel-based statistical analyses were performed using SPM2 (www.fil.ion.ucl.ac.uk/spm) running on MATLAB 6.5 software. All images were non linearly spatially normalized into the Montreal Neurological Institute (MNI) space and smoothed with an isotropic Gaussian kernel with 12 mm FWHM. Voxel size was set at 2 x 2 x 2 mm^3. Confounding effects of global activities differences were removed by normalizing the count of each voxel to the mean count of a standardized pontine region of interest (ROI) in order to avoid a biased normalization [5]. The pons was chosen on the basis of its relative stability an late involvement in neurodegenerative diseases such as Alzheimer disease [33], a finding leading other investigators to use it as reference region [11, 37, 57]. The ROI was a rectangular multislice region (x/x'=-8/8, y/y'=-32/-24, z/z'=-44/-34; MNI space) sampling 144 voxels on the central pontine region and manually drawn on the PET SPM template using the MRIcro application (http://www.sph.sc.edu/comd_/rorden/mricro.html). Both ROI coordinates and dimensions were chosen to avoid low-counts background voxel sampling and to minimize the random noise effect. A previous careful visual inspection of the pons was conducted on each spatially normalized but non smoothed brain scan in order to detect metabolic changes which could alter the ROI measure. The same ROI was then employed on each spatial normalized and smoothed brain image and the pons mean voxel values ($\bar{Y}_p$) sampled. Using the image calculation tool of SPM, the scaled voxel values (Y') of each brain was set at $Y' = (Y / \bar{Y}_p)$ were Y was the non scaled ("raw") voxel value. Only voxel values greater than 80% of the whole brain mean MRglc were included in the analysis. Two correlation analysis and four between groups comparisons were performed. The hypothesis of a negative linear associations between the regional cerebral metabolic rate of glucose (rCMRglc) and

the number of CHT cycles was tested on the whole CHT group (n=21) on a voxel-by-voxel basis using the SPM2 single-subject covariate only option. Age, post-CHT time (months) and a categorical variable that we label "hormonal status" [defined as follows: pre menopause females (n=5) =0, females with drug-induced amenorrhea (n=3) =1; post menopause females (n=7) =2; males (n=16) =3] were entered in the general linear model as nuisance factors. This variable was used as confounding factor in order to minimize the recognized confounding multiple effects of estrogens on brain functions [8, 25]. The hypothesis of a positive linear associations between the rCMRglc and the time elapsed since the end of the treatment was tested with the same SPM option with age, gender and number of CHT cycles entered as nuisance factors. comparisons between subgroups (EH versus LL; EH versus No CHT; LL versus No CHT; No CHT versus CHT) were performed using the ANCOVA model using age, hormonal status and education level as covariates of no interest.

The set of SPM t-statistics resulting from these analysis were transformed into SPM Z scores. Statistical inferences were performed by applying the Random Field Theory. Results were thresholded at $p<0.005$ uncorrected for multiple comparisons, with an extent threshold cluster extent (Ke) of 20 voxels. This threshold is the same than previously used in FDG-PET studies in neurodegenerative disease such as Alzheimer's disease or mild cognitive impairment and estimated to provide the best compromise, neither too liberal nor over-conservative with risk of type 2 errors.

The MNI peaks coordinates of the SPM t were converted into the Talairach coordinates using the Brett transformation (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach). Then the Talairach Daemon was used ([28], http://www.talairach.org) to label the transformed coordinates respect to hemisphere, lobe and gyrus. Only coordinates within 2 mm to a gray matter where classified. The cingulate cortex was defined according to the four division model proposed by Vogt et al. [58].

For the other coordinates we inspected whether they fell inside a white matter fasciculus overlapping the MNI peaks coordinates with the ICBM DTI-81 (http://www.loni.ucla.edu/Atlases/)

with the MRIcron viewer (http://www.cabiatl.com/mricro/mricron). This atlas includes the most relevant fasciculi in the MNI space extracted by the mean of 81 subjects' DTI.

Clusters with $p \leq 0.05$ corrected for multiple comparisons were considered as significant.

For subanalyses, we examined the correlations between neuropsychological scores and regional glucose metabolism of the voxels of interest: where cancer patients exposed to adjuvant CHT had a significantly lower metabolism and regions where a significant correlation was found. Mean regional CMRglc of the most significant clusters (see Table 3 and Supplementary Table 6) were calculated by using the volume of interest (VOI) function in the SPM2 software.

Patient characteristic data were analyzed with Kruskal-Wallis test for continuous variables and $\chi 2$ test for categorical variables. Pearson's correlations was used to analyze rCMRglc data resulting from SMP2 analysis. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, $p<0.05$ was considered significant. To compare the medians of two defined subgroups we used a Kruskal-Wallis non-parametric 2 independent samples test. To compare neurobehavioral characteristic of No CHT, EH, LL we used a Kruskal-Wallis non parametric 3 independent sample test. To compare correlations between subgroups in our sample we used the Fisher's Z-test. This is a simple Z test computed on the Fisher transformed correlation coefficients (so to become normally distributed).

EXTENDED DISCUSSION

Several neuroimaging studies have consistently shown that CHT induces structural [1, 6, 7, 16, 24] and functional [17, 20, 50] brain changes involving both the cortex and the white matter and with a prevailing involvement of the prefrontal cortex. However the time course of such changes are less well consistently defined with a study suggesting a complete recovery of the structural damage few years after the completion of the treatment [24] and others reporting a longer lasting functional impairment [27, 50]. Moreover, no previous neuroimaging study has investigated whether, in agreement with some neuropshychological studies, the CHT-induced brain damage could be related to the dose or the number of cycles of chemotherapic agents. Thus, neither conclusive nor convincing evidence has been reached favouring the one or the other among the several competing heuristic models of CHT-induced brain damage which can be proposed by means of the analysis of the available neuropsychological and neuroimaging data (see Figure 3c, 3d). To address these questions, we performed a set of correlation analyses exploring, in patients investigated at different times after the completion of treatment, the relationships between the rCMRglc and both these two variables, that is the time post-CHT and the number of cycles. We then performed a set of comparisons intended to confirm and strengthen the former analysis. The correlation analysis between the rCMRglc and the time post-CHT, in which the number of cycles was treated as confounding factor, uncovered a set of brain areas (including the right middle, inferior and superior frontal gyri along with others midline and subcortical brain regions as well as white matter regions including the body of corpus callosum and the posterior limb of internal capsule) showing a significant positive association between the rCMRglc and the time elapsed from treatment. These results were partially reproduced and confirmed by the comparison of the two subgroups of CHT-treated patients (EH<LL) (see Table 3, Supplementary Table 4, 5 and Figure 1c, 2b). Partially overlapping results were moreover obtained in the comparison of the EH subgroup with the non treated subgroup (EH<No CHT). Interestingly, the peak of greatest rCMRglc decrease found in this

latter comparison in the right middle frontal gyrus (see Table 3, Supplementary Table 4, 5 and Figure 1c, 2b) was very close (x, y, z = 38, 62, 6) to that reported (x, y, z = 30, 64, 4) by a previous cited structural study [24] in a comparison between treated and non treated cancer patients. Our data therefore strongly support a reversible model of CHT-induced brain damage (see Figure 3c, 3d) suggesting that the chemotherapy-induced cerebral glucose metabolic impairment could be transient and rapidly (~1 year) reversible over the time, paralleling or even preceding the structural recovery of the CHT targeted brain regions. These results are in agreement with findings emerging from controlled longitudinal neuropsychological studies [13, 19, 59, 61] indicating that the cognitive changes tend to fully or partially resolve over 1 year. These assumptions on the reversibility of brain metabolic impairment must however be taken with caution. In a cross-sectional study as the present work, the real dynamics of the metabolic changes triggered by the CHT obviously cannot be adequately studied. Furthermore, we cannot exclude that a subset of brain regions did not recover or recovered only partially over the time, as suggested by some neuropsychological [3, 10] and neuroimaging [27, 50] studies. Candidate regions which could have, in the present study, a longer lasting metabolic impairment are the precentral gyri (BA4), a set of midline cortical structures such as left medial frontal gyrus (BA9, BA6) and the right posterior cingulate cortex (BA30), as well as the left middle frontal gyrus (BA46, BA8) and the cerebellum. These regions indeed showed a lower rCMRglc in the EH<No CHT comparison (see Table 3, Supplementary Table 4 and Figure 1c, 2b), but not in the EH<LL and in EH<LH comparisons (see Table 3, Supplementary Table 4 and Figure 1d, 1e, 2b) and did not show any significant positive correlation with the post-CHT time (see Table 3, Supplementary Tables 2, 3 and Figure 1a, 2a). Since both the No CHT>LL and No CHT>LH comparisons did not give significant results we suppose that these areas have only partially recovered over time. With respect to the cerebellum, the partial recovery hypothesis suggested by our data, is consistent with previous [O-15] water PET [50] and fMRI studies [27] reporting an altered cerebellar recruitment during a memory task in breast cancer women investigated at 3-5 and 5–10 years, respectively, after completion of CHT and compared to

a control sample. If one considers the wide prefrontal metabolic impairment involving both the gray and the white matter the bilateral cerebellar metabolic lower glucose metabolism is not surprising. It could result from a disruption of the fronto-cortico-cerebellar networking, that is, from the disconnection of one of the afferent loops.

A further region showing an incomplete recovery over time was the left parahippocampus, which showed in the comparison of non treated with the whole group of treated patients (No CHT>CHT: see Table 3, Supplementary Table 4, 5 and Figure 3) a significantly lower rCMRglc. A subanalysis evidenced that, compared to non treated patients, the rCMRglc of this region was significantly ($p=0.006$) lower in patients investigated at a mean of more than 1 year after the completion of CHT and having received a number of CHT cycles comparable to the EH subgroup (LH subgroup) (see Figure 3b).

To gain more insights on the mechanisms underlying these chemotherapy-related adverse effects we looked for a relationship between the number of cycles and the cerebral glucose metabolism. The voxel-based correlation analysis evidenced a set of brain areas (including the right superior, middle, inferior frontal gyri, the left middle frontal and superior temporal gyri) showing a significant negative association between the number of CHT cycles and the rCMRglc, which partially overlapped those uncovered by both the comparative and correlation analysis described above. These results support therefore a model in which the CHT-induced brain damage appears to be related to the number of CHT cycles (Figure 3d), in agreement with previous neuropsychological data [3] reporting a negative relationship between the number of cycles and the cognitive performance. We were however unable to replicate in our sample of cognitively unimpaired cancer patients this latter finding.

The pattern of CHT-induced brain metabolic impairment, markedly characterized by a disproportionate involvement of frontal lobes, is reminiscent of that seen in ageing [15, 26, 37, 55] and some age-related neurodegenerative processes [51]. However, a salient finding of our study was that the wide frontal metabolic impairment evidenced in the EH subgroup was not associated with

any significant cognitive impairment (Table 2). Such mismatch between metabolic and cognitive data is well known in the field of Alzheimer's disease (AD) FDG-PET studies. In cognitively normal subjects at genetic risk of developing AD, such as apolipoprotein E (ApoE) E4 carriers, such studies showed a reduced glucose metabolism in the same regions of the brain as in patients with probable AD [44-46] such as the retrosplenial, the parietal and the frontal cortex. Moreover they evidenced that in these cognitively normal subjects this reduced glucose metabolism could be associated with perceived loss of memory ability [18] and subjective cognitive complaints [36]. For example, in cognitively normal ApoE4 carriers, the rCMRglc of parahippocampal gyrus was the most accurate predictor of the subjective cognitive complaints reported by participants [36]. The parahippocampal gyrus was, in our study, one of the brain region targeted by the CHT and not showing evidence of a complete metabolic recovery. This could be relevant since, as reported by previous studies, cancer survivors treated with CHT often self-report higher levels of cognitive problems but, as was seen in this work, perform normally on neuropsychological tests [20, 43]. Furthermore, recent functional and structural studies have showed that cognitively healthy people with a maternal family history of late onset AD (FHm), have a rCMRglc reductions [35] and less gray matter volume [23] in AD-vulnerable brain regions, including the frontal cortex, compared to subjects with no parental history of AD. Interestingly, compared to subjects with a negative history those with a FHm showed the lowest CMRglc in the right superior frontal gyrus at coordinates (x, y, z = 34, 61, 4), similar to those found by us (x, y, z = 38, 62, 6). Moreover, FHm subjects compared to subjects with a paternal familial history of late onset AD showed a lower CMRglc in the superior frontal gyrus at coordinates (x, y, z = 30, 63, 1). Hypometabolism in FHm is thought to be due to a combination of dysfunction of mitochondria [62] (maternally inherited), increased oxidative stress, and possible mitochondrial DNA (mtDNA) mutations [29], leading to CMRglc changes in vulnerable brain regions [38]. Many of these processes has been proposed as candidate mechanism mediating the adverse effects of CHT on brain functions [2]. For example, evidence for oxidative DNA damage was found in peripheral blood lymphocytes after CHT for breast cancer [4,

40] and a chemotherapy-induced increased number of point mutations in mitochondrial DNA was found in patients with various cancer [60]. Oxidative DNA damage, and decreased mitochondrial function are moreover well established processes underlying brain aging changes [31], a finding leading Maccormick [30] to hypothesize that adverse effects associated with CHT might be related to acceleration of the ageing process. Inspired by this hypothesis we performed a post-hoc analysis looking for differences in the correlations between age and the rCMRglc of regions showing a lower glucose metabolism in the EH<No CHT subgroups comparison. This analysis showed that, compared to non treated patients, the CMRglc of the medial posterior cortices showed in the EH patients a more significant and negative association with age (Fisher's z test: $p<0.05$) (see Supplementary Table 6 and Figure 4), meaning that older subjects undergo an higher than expected CHT-induced metabolic impairment. The inverse was true at the right cerebellar level were the No CHT patients showed a more significant and more negative correlation between rCMRglc and age ($p<0.05$) (Figure 4a, 4c). This latter finding could perhaps be interpreted taking in account the significant CMRglc cerebellar decrease even in younger treated patients (Figure 4b, 4d) which could prevent or slow further age-related CMRglc decline. Taken together, these findings lead us to speculate that CHT induces aging-like and/or potentiates aging-related processes such as oxidative stress, and decreased mitochondrial function which could lead to the rCMRglc reduction evidenced, in patients having received CHT, by the present study.

In summary, this study evidenced significant chemotherapy-related changes of glucose metabolism in multiple brain regions involving both the cortex and the white matter with a prevailing involvement of the frontal lobes. Such metabolic changes appear to be positively related in many of these same areas with the time elapsed from the end of the treatment suggesting that they are transient and rapidly reversible. A subset of these areas undergoes a metabolic impairment proportional to the number of CHT cycles while a subset, including the cerebellum and midline cortical regions, present evidences of a partial or delayed metabolic recovery.

Study limitations

Some limits in our study came by its cross-sectional design, as only a longitudinal study could definitely demonstrate the relationship between rCMRglc and post-CHT time or number of cycles that we observed. Another problem was the heterogeneity of our sample, that was formed by different kind of cancer patients who received many kind of different adjuvant CHT protocols. This could be a confounding in determining how treatments (radiotherapy, corticosteroids, different combination of drugs) can interact and contribute to the brain damaging mechanisms. In animals this type of interacting noxious effects was demonstrated [39], but we did not have the possibility of explore this topic.

For the same motivation the non commensurability of the doses of very different treatments received by patients prevent us to discriminate between a pure dose dependency, a pure CHT duration dependency or a mixed/interaction form of dependency. The number of CHT cycles, in fact, could be thought as a mere proxy of either the above aspects, since an higher number of cycles could correlate with an higher dose, but also with a longer time of exposition. We cannot exclude, however, that the number of cycles represents the number of times that the system came in contact with CHT and the complex cascade of events triggered by this exposition. So this repeated interaction per se could be a factor that guide the changes and the adaptation mechanisms activated as a response by the system.

Still the heterogeneity of our sample could be interpreted also as a strength of this work, since the observed reversibility remains very significant in spite of possible confounding factors underlying this heterogeneity.

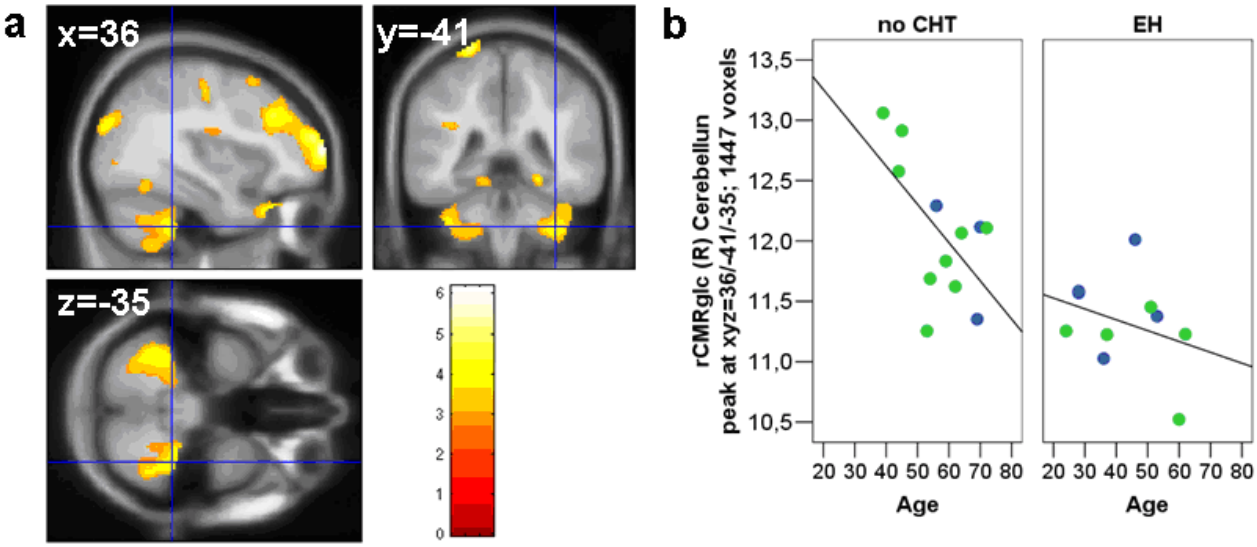
Lower RBC and Hb (chemotherapy-induced bone marrow suppression) constituted the main differences between the subgroups in our sample, so it could be worthy to better characterize the relationship between the brain's CHT-induced changes and these alterations (or the underlying mechanisms). Unfortunately we did not have enough elements to attend to this task, but we can suggest that future work should investigate in this direction. We can however affirm that the anaemia was not the sole or first causative factor of the metabolic lowering and its subsequent

recovery because, excluding from the sample the anaemic patient (Hb<12 g/dL), the correlation analysis showed similar results (not shown).

The subgroups did not statistically differ at any neuropsychological or psychological test. If it was not surprising that the psychological profile of cancer patients could be largely overlapping during diagnosis and treatment (with presence of depression, anxiety and stress), it was more concerning that, although we found an altered rCMRglc, comparing the subgroup we did not find any neuropsychological deficit. This could be due to the successful compensation of the brain that maintains its behavioral performance at cost of metabolic adaptation and changes (potentially stressfull in the long last period) or at an insufficient sensitivity of the neuropsychological tests (respect to our sample's size and CHT-induced changes). A further element that may have reduced the difference between the groups, was the use of the No CHT subgroup as control group, in fact, it has been found that the cancer patients also before the adjuvant CHT can show alterations in the brain activity and in behavioral tasks compared to healthy subjects [2, 12]. We cannot enrol healthy volunteers in our study due the Italian legislation that prohibit the prescription of a PET in persons not suffering from any disease.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Cerebellum in the No CHT (n=12) and EH (n=10) groups



Compared to non treated patients, the rCMRglc of the right cerebellar cortices showed in the EH patients a less significant association with age (a, b), but a general lower level of rCMRglc. In blue females, in green males.

SUPPLEMENTARY TABLES

Supplementary Table 1. Chemotherapy protocols and drugs

Cycles Number (patients)	Protocols	Drugs	Subgroups
2 (1)	MEC	Methotrexate, epirubicin, cisplatin	LL
2 (1)	R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone	LL
3 (3)	R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone	LL
3 (1)	Nigro	5-fluorouracil, mitomycin	LL
4 (2)	ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine	LL
4 (3)	R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone	LL/LH
4 (1)	EPIRUBICIN	Epirubicin	LL
6 (1)	R-CNOP	Rituximab, cyclophosphamide, mitoxantrone, vincristine, prednisone	LL/LH
6 (1)	CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisone	LL
6 (1)	ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine	LL/LH
6 (1)	FEC	Fluorouracil (5FU), epirubicin, cyclophosphamide	LL/LH
7 (1)	TXT/EPI	Docetaxel, epirubicin	LL/LH
8 (1)	CMF	Cyclophosphamide, methotrexate,5-fluorouracil	LL/LH
8 (1)	R-CVP	Rituximab, cyclophosphamide, vincristine, prednisolone	LL/LH
9 (1)	R-FND	Fludarabina, mitoxantrone, dexametasone, rituximab	LL/LH
12 (1)	ABVD+ DHAP+ EDX+ IGEV+ BEACOP-R+ GITMO GLOBAL	-Doxorubicin, bleomycin, vinblastine, decarbazine -Cisplatin, cytarabine, desametasone -Cyclophosphamide -Ifosfamide, gemcitabine, vinorelbine -Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone, rituximab -Melphalan, ThioTEPA, Cyclophosphamide	LL/LH
6 (1)	HD-AraC+ R-HDS+ R-EPOCH	-Cytarabine -Rituximab -Rituximab, Cyclophosphamide, etoposide, aletuzumab, doxorubicin, vincristine, prednisone	EH
6 (1)	R-CHOP+ MAD-R	-Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone -Cytarabine, mitoxantrone, rituximab	EH
7 (1)	VACOP	Etoposide, adriamycin,, cyclophosphamide, vincristine, prednisone	EH
7 (1)	VINCRISTINE + R-FC+ ZEVALIN	-Vincristine -Rituximab, fludarabine, cyclophosphamide -Zevalin	EH
8 (1)	R-CHOP+ RDHAP+ ZBEAM	-Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone -Rituximab, cisplatin, cytarabine, desametasone -Zevalin,	EH
8 (2)	R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone	EH
10 (1)	ABVD+ BEACOPP	-Doxorubicin, bleomycin, vinblastine, decarbazine -Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone	EH
12 (1)	R-VACOPB	Rituximab, VP16, adriamycin, cyclophosphamide, vincristine, prednisone, bleomycin	EH
16 (1)	ABVD+ BEACOPP+ IGEV+ DHAP	-Doxorubicin, bleomycin, vinblastine, decarbazine -Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone -Ifosfamide, gemcitabine, vinorelbine -Cisplatin, cytarabine, desametasone	EH

EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.

Supplementary Table 2. Grey matter rCMRglc correlation analysis in CHT subgroup (n=31)

Cerebral Region	BA	p corr.	Ke	Z	Talairach Coordinates		
					x	y	z
Negative correlations with the number of chemotherapy cycles							
(L) Superior Temporal Gyrus	38	0.002	4555	3.66	-46	16	-19
(R) Middle Frontal Gyrus	9			3.37	48	6	37
(R) Middle Frontal Gyrus	46			3.32	48	36	28
(R) Middle Frontal Gyrus	9			3.13	36	29	32
(L) Middle Frontal Gyrus	11			3.12	-22	32	-12
(L) Medial Frontal Gyrus	10			3.02	0	50	-7
(R) Middle Frontal Gyrus	10			2.97	30	63	10
(R) Superior Frontal Gyrus	10			2.90	34	54	23
(R) Inferior Frontal Gyrus	44			2.83	53	9	20
Positive correlations with the post chemotherapy time							
(L) Subgenual ACC	25	<0.001	9798	4.15	-2	9	-9
(R) Middle Frontal Gyrus	46			3.50	55	30	24
(R) Superior Frontal Gyrus	10			3.41	36	61	14
(R) Medial Frontal Gyrus (SMA)	6			3.37	16	-7	56
(R) Middle Frontal Gyrus	9			3.34	48	12	36
(R) Inferior Frontal Gyrus	44			3.33	51	7	20
(R) Pregenual ACC	32			3.32	2	39	2
(L) Thalamus AN	-			3.28	-6	-5	9
(R) Precentral Gyrus	9			3.28	38	25	34
(R) Inferior Frontal Gyrus	9			3.27	51	7	31
(L) Anterior MCC	32			3.27	-4	17	32
(R) Thalamus VAN	-			3.11	10	-3	8
(L) Posterior MCC	24			2.99	-14	-3	48
(L) Pregenual ACC	32			2.98	-6	36	17
(R) Superior Frontal Gyrus	9			2.81	18	58	27

Height threshold p = 0.005 uncorrected for multiple correlations; p corr. = p corrected for multiple correlations; ACC = Anterior Cingulate Cortex; AN = Anterior Nucleus; BA = Brodmann Area; CHT = chemotherapy; Ke = cluster extent; L = left; R = right; MCC = Middle Cingulate Cortex; SMA = Supplementary Motor Area; VAN = Ventral Anterior Nucleus.

Supplementary Table 3. White matter rCMRglc correlation analysis in CHT subgroup (n=31)

White Matter Fasciculus	p corr.	Ke	Z	Talairach Coordinates		
				x	y	z
Negative correlations with the number of chemotherapy cycles						
No suprathreshold cluster						
Positive correlations with the post chemotherapy time						
(L) Body of Corpus Callosum	<0.001	9798	3.67	10	7	27
(R) Posterior Limb of Internal Capsule			2.93	18	-3	15

Height threshold p = 0.005 uncorrected for multiple correlations; p corr. = p corrected for multiple correlations; BA = Brodmann Area; CHT = chemotherapy; Ke = cluster extent; L = left; R = right.

Supplementary Table 4. Grey matter rCMRglc, comparison between subgroups (n=43)

Cerebral Region	BA	p corr.	Ke	Z	Talairach Coordinates		
					x	y	z
No CHT (n=12) > CHT (n=31)							
(L) Parahippocampus (Uncus)	28	0.039 (SVC)	177	3.13	-24	4	-29
No CHT (n=12) > EH (n=10)							
(R) Middle Frontal Gyrus	10	<0.001	7622	4.42	38	62	6
(R) Superior Frontal Gyrus	10			3.92	34	57	14
(R) Medial Frontal Gyrus	10			3.91	22	51	12
(R) Precentral Gyrus	4			3.74	61	-6	39
(L) Superior Temporal Gyrus	38			3.26	-24	10	-32
(L) Precentral Gyrus	4			3.24	40	-13	49
(L) Cuneus	19	<0.001	3657	4.41	-2	-82	37
(R) Cuneus	19			4.25	8	-78	33
(R) Parahippocampal Gyrus	19			3.82	24	-47	-5
(R) Lingual Gyrus	19			3.68	24	-68	-2
(R) Dorsal PCC	30			3.49	16	-54	6
(R) Precuneus	7			3.27	20	-58	49
(L) Medial Frontal Gyrus (SMA)	6	<0.001	6644	4.15	-6	-7	52
(L) Middle Frontal Gyrus	46			3.82	-48	34	26
(L) Middle Frontal Gyrus	10			3.37	-32	63	8
(L) Medial Frontal Gyrus	9			3.27	-8	38	26
(R) Anterior MCC	32			3.24	12	18	40
(R) Medial Frontal Gyrus (SMA)	6			3.14	6	5	61
(L) Middle Frontal Gyrus	8			3.10	-40	29	43
(L) Cerebellum Tonsilla	-	0.001	2493	3.61	-36	-51	-40
(L) Cerebellum Tuber	-			3.06	-32	-64	-29
(R) Cerebellum Tonsilla	-	0.017	1447	3.50	36	-41	-35
(R) Cerebellum Culmen	-			3.07	42	-42	-23
No CHT (n=12) > LL (n=21)							
No suprathreshold cluster							
EH (n=10) < LL (n=21)							
(R) Fusiform Gyrus	19	0.025	2714	3.61	30	-65	-9
(R) Middle Occipital Gyrus	19			3.29	44	-80	2
(R) Lingual Gyrus	18			3.23	18	-80	0
(R) Inferior Temporal Gyrus	37			3.12	51	-55	-6
(L) Cuneus	19			3.11	-6	-94	23
(R) Medial Temporal Gyrus	39			3.06	38	-72	27
(R) Precuneus	7			2.93	16	-78	37
(R) Cuneus	18			2.87	10	-84	24
(R) Cuneus	19			2.74	18	-88	21
(R) Medial Temporal Gyrus	19			2.68	57	-69	14
(R) Middle Frontal Gyrus	10	0.001	5263	3.43	42	52	20
(L) Middle Frontal Gyrus	11			3.22	-22	30	-13
(R) Superior Frontal Gyrus	10			3.17	22	56	-6
(R) Middle Frontal Gyrus	11			3.17	22	30	-15

(R) Superior Frontal Gyrus	11			3.03	34	57	-14
(R) Middle Frontal Gyrus	10			2.97	42	54	-4
(L) Medial Frontal Gyrus	25			2.84	-4	24	-15
(R) Middle Frontal Gyrus	47			2.69	44	38	-8
(R) Superior Temporal Gyrus	38			2.65	30	15	-38
(R) Medial Frontal Gyrus	10			2.60	16	55	8
EH (n=10) < LH (n=9)							
(R) Superior Frontal Gyrus	10	0.012	1074	3.94	40	59	16
(R) Superior Frontal Gyrus	9			3.24	38	44	29
(R) Middle Frontal Gyrus	10			3.07	44	60	-3
(L) Middle Frontal Gyrus	10	0.046	1557	3.86	-40	53	18
(L) Anterior MCC	32			3.02	-6	18	38
(R) Anterior MCC	32			2.85	10	21	28
(L) pregenual ACC	32			2.80	-6	34	22
(L) Superior Frontal Gyrus	10			2.72	-26	59	23

Height threshold p = 0.005 uncorrected for multiple correlations; p corr. = p corrected for multiple correlations; SVC = Small Volume Correction.

ACC = Anterior Cingulate Cortex; BA = Brodmann Area; CHT = chemotherapy; Ke = cluster extent; L = left; R = right; MCC = Middle Cingulate Cortex; PCC = Posterior Cingulate Cortex; SMA = Supplementary Motor Area. EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.

Supplementary Table 5. White matter rCMRglc, comparison between subgroups (n=43)

Cerebral Region	p corr.	Ke	Z	Talairach Coordinates		
				x	y	z
No CHT (n=12) > CHT (n=31)						
No suprathreshold cluster						
No CHT (n=12) > EH (n=10)						
(R) Anterior Corona Radiata	<0.001	7622	3.56	12	34	-8
(L) Anterior Corona Radiata			3.02	-14	32	-12
(L) Posterior Corona Radiata	<0.001	6644	3.21	-24	27	26
No CHT (n=12) > LL (n=21)						
No suprathreshold cluster						
EH (n=10) < LL (n=21)						
No suprathreshold cluster						
EH (n=10) < LH (n=9)						
No suprathreshold cluster						

Height threshold p = 0.005 uncorrected for multiple correlations; p corr. = p corrected for multiple correlations; BA = Brodmann Area; CHT = chemotherapy; Ke = cluster extent; L = left; R = right.
EH = Early High CHT subgroup; LL = Late Low CHT subgroup; LH = Late High CHT subgroup.

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Supplementary Table 6 Correlations between Age and rCMRglc in No CHT and EH

No CHT (n=12) > EH (n=10)							
Cerebral Region	BA	No CHT (n=12)		EH (n=10)		Fisher's-Z test	
		r	p	r	p	Z	p
(R) Middle Frontal Gyrus	10	-0.388	0.213	-0.685	0.029	0.851	NS
(L) Cuneus	19	-0.181	0.573	-0.814	0.004	2.027	<0.05
(L) Medial Frontal Gyrus (SMA)	6	-0.297	0.349	-0.707	0.022	1.141	NS
(L) Cerebellum Tonsilla	-	-0.514	0.087	0.409	0.240	1.989	<0.05
(R) Cerebellum Tonsilla	-	-0.599	0.040	-0.317	0.372	2.024	<0.05

BA = Brodmann Area; p = Sig. (two-tailed)M; ; r = Pearson's correlation coefficient; z = Fisher's Z score.

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