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Brain Activity Associated With Attention Deficits Following Chemotherapy for Childhood Acute Lymphoblastic Leukemia

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Abstract

Background: The impact of contemporary chemotherapy treatment for childhood acute lymphoblastic leukemia on central nervous system activity is not fully appreciated.

Methods: Neurocognitive testing and functional magnetic resonance imaging (fMRI) were obtained in 165 survivors five or more years postdiagnosis (average age = 14.4 years, 7.7 years from diagnosis, 51.5% males). Chemotherapy exposure was measured as serum concentration of methotrexate following high-dose intravenous injection. Neurocognitive testing included measures of attention and executive function. fMRI was obtained during completion of two tasks, the continuous performance task (CPT) and the attention network task (ANT). Image analysis was performed using Statistical Parametric Mapping software, with contrasts targeting sustained attention, alerting, orienting, and conflict. All statistical tests were two-sided.

Results: Compared with population norms, survivors demonstrated impairment on number-letter switching ($P < .001$, a measure of cognitive flexibility), which was associated with treatment intensity ($P = .048$). Task performance during fMRI was associated with neurocognitive dysfunction across multiple tasks. Regional brain activation was lower in survivors diagnosed at younger ages for the CPT (bilateral parietal and temporal lobes) and the ANT (left parietal and right hippocampus). With higher serum methotrexate exposure, CPT activation decreased in the right temporal and bilateral frontal and parietal lobes, but ANT alerting activation increased in the ventral frontal, insula, caudate, and anterior cingulate.

Conclusions: Brain activation during attention and executive function tasks was associated with serum methotrexate exposure and age at diagnosis. These findings provide evidence for compromised and compensatory changes in regional brain function that may help clarify the neural substrates of cognitive deficits in acute lymphoblastic leukemia survivors.

Survival rates of childhood acute lymphoblastic leukemia (ALL) exceed 90% with current protocols (1,2), making ALL the largest diagnostic group of long-term survivors of childhood cancer (3). Higher survival is attributed to improved treatment including central nervous system-directed therapy to prevent relapse. In modern treatment protocols, prophylactic cranial irradiation has been replaced with intrathecal chemotherapy to reduce severity of neurocognitive side effects (1). Although this shift has reduced toxicity, patients treated with chemotherapy alone remain at elevated risk for

neurocognitive problems (5–9). An essential drug for ALL therapy is methotrexate (MTX), which blocks DNA synthesis by depleting folate via inhibition of dihydrofolate reductase, resulting in increase in homocysteine, which is toxic to cellular membranes (4).

Roughly 20% to 40% of long-term survivors of childhood ALL experience neurocognitive impairment (10); high treatment intensity, younger diagnosis age, and female sex increase risk for impairment (11,12). Survivors manifest decline in functions over time (13,14), with deficits in intelligence, academic skills

(8,15), and specific cognitive domains, including processing speed, executive function, and attention (9,16).

Attention involves prioritized processing of information in the face of competing information. A contemporary network theory defines three functional components of attention: alerting, orienting, and conflict (17). Alerting subsumes increasing and maintaining an alert state. Orienting involves selection of target information among numerous sensory inputs. Conflict involves engagement of complex mental operations during detection and resolution of conflict by shifting attention.

Functional magnetic resonance imaging (fMRI) plays an important role in understanding neurocognitive late effects in cancer survivors (18,19). ALL survivors treated with chemotherapy display smaller cortical volumes of the left hippocampus, amygdala, thalamus and nucleus accumbens (20), lower white matter volume (12,21,22), and altered fractional anisotropy compared with healthy controls (22,23). Poor neurocognitive performance is associated with smaller white and grey matter volume (12,21,22). Recently, we reported higher plasma concentration of MTX to be associated with executive dysfunction, thicker cortex, and higher brain activity in frontal regions (9). However, the causes of neurocognitive deficits in ALL survivors remain unclear, and elucidation of the neural basis is needed to design and evaluate remedial cognitive interventions (24,25). We conducted an fMRI study in a large cohort of long-term survivors of childhood ALL treated with chemotherapy alone to investigate neural systems for attention and executive function that may be disrupted by methotrexate exposure. Given the role of frontal brain regions in the modulation of attention and executive function, we expected activity in these regions to be associated with treatment intensity.

Methods

Study Population

This study was approved by the Institutional Review Board at St. Jude Children's Research Hospital, and written informed consent was obtained from survivors and/or their parents. Between 2000 and 2010, 408 children were treated on the Total Therapy XV chemotherapy protocol (ClinicalTrials.gov, NCT00137111) (1). To be eligible for the current long-term follow-up, survivors had to be five or more years postdiagnosis and ≥ 8 years of age. Survivors were excluded if English was not their primary language; if they relapsed, received cranial radiation, had a history of head injury or neurological condition unrelated to ALL treatment; or if they had been diagnosed with a genetic disorder associated with neurocognitive impairment (eg, Down syndrome). No survivors were prescribed psychotropic medication such as methylphenidate or atomoxetine at the time of testing. Of the 302 eligible survivors, 213 (71%) completed neurocognitive testing, and evaluable imaging data were obtained for 165 (78%).

Treatment

Children were treated with either low-risk or standard-risk therapy approaches, based on established risk parameters. The low-risk arm received 13–18 triple intrathecal (TIT) treatments with MTX, hydrocortisone, and cytarabine; consolidation treatment with intravenous high-dose methotrexate (HDMTX) at 2.5 gm/m² per dose for four courses; and dexamethasone pulses at 8 mg/m² per day for five days per course, in addition to other

chemotherapeutic agents. The standard-/high-risk arm received 16–25 TIT injections, consolidation treatment with intravenous HDMTX at 5.0 gm/m² per dose for four courses, and dexamethasone pulses at 12 mg/m² per day for five days per course. Prophylactic cranial irradiation was not administered to any patient. Serum MTX concentrations were measured at six, 23, and 42 hours following intravenous administration and quantified as area under the curve (AUC) averaged across all courses (26). Leucovorin rescue began 42 hours after HDMTX and was examined through sensitivity analyses.

Neurocognitive Evaluation

Neurocognitive tests were administered by certified examiners under the supervision of a board-certified clinical neuropsychologist. Testing procedures followed standardized clinical guidelines, with fixed test order and schedules controlled to reduce interference and fatigue. The test battery included measures of attention (27), executive function (28), intelligence (29), processing speed (30), memory (31), and fine motor dexterity (32). Measures of interest for executive function included Number Letter Switch and Verbal Fluency from the Delis-Kaplan Executive Function System, and the Digit Backward test from the age-appropriate Wechsler scale (Supplementary Table 1, available online). Attention measures included continuous performance test (CPT) omissions, variability, and detectability indices (27) and the attention network task (ANT), with measures of alerting, orienting, and conflict resolution. Impairment was defined as having a score below the 10th percentile of age-adjusted population normative data (33). Overall impairment was defined as being impaired on either executive function or attention.

Functional MRI

Brain imaging was performed on 3T scanners (Trio or Skyra, Siemens, Malvern, PA) using a standard head coil. Functional images were obtained with single-shot T2*-weighted echoplanar imaging (TR = 2.06 seconds, TE = 30 ms, FOV = 192 mm, matrix = 64×64, slice thickness = 5 mm). Images were acquired in axial planes parallel to the anterior commissure–posterior commissure axis. While undergoing fMRI, survivors again completed CPT and AN (34) tests. During fMRI-CPT (35), survivors were instructed to push a button for every letter except X. The fMRI-CPT was presented in a block design, with periods of task performance interleaved with control periods, during which the survivors simply maintained fixation on a small cross at the center of the screen. The ANT was recreated from Fan et al. (34), in which stimuli consisted of a row of five arrows (1 central and 4 flankers) pointing either left or right, with counter-balanced directional frequency. Three cue conditions were used for orientation before the stimulus: a center cue, a spatial cue, and no cue. The survivors' task was to respond to the direction of the center arrow (target condition), which was either congruent or incongruent with the flankers. Incongruent flankers require cognitive flexibility.

Statistical Analysis

Neurocognitive performance was transformed into age-adjusted standard scores using normative data. Performances between survivors and population norms were compared using one-sample *t* tests and *P* values adjusted for false discovery

rate, and only measures that differed from normative data were examined for associations with treatment exposure. Descriptive statistics were calculated using R software (<http://cran.r-project.org/>) for demographics and task performance during neurocognitive (raw scores and standardized scores) and fMRI testing. Correlations were tested using Pearson's test. Repeated-measures analysis of variance was used to examine change in behavioral performance across multiple task conditions.

Image data analysis was performed using Statistical Parametric Mapping software (SPM8, Wellcome Institute of Neurology, London, UK). Functional images from each survivor were realigned to correct for interscan head motion, normalized to the Montreal Neurological Institute brain template and smoothed with a Gaussian kernel of 6 mm full width at half-maximum (FWHM). The smoothed and normalized images were resliced to 2 mm isotropic resolution. Preprocessed images were analyzed using fixed-effects general linear models, with task-induced activity modeled as a boxcar function and treating low-frequency signal components as nuisance covariates. Statistical contrasts were calculated to identify attention networks. Contrast images from each survivor were used as variables in second-level random-effect analyses to identify group patterns of brain activation.

Specific clinical and neurocognitive variables, selected a priori based on our previous publications (5,10,16), were tested for correlations and entered in multivariable models to identify brain areas where activity was associated with behavioral performance. Voxel-level analysis was conducted with family-wise error correction for multiple comparisons, with a minimum cluster size of 5 voxels (T threshold = 3.5). Additional cluster-level analysis was conducted with a voxel uncorrected threshold P value of less than .001 and minimum cluster size of 5 voxels. Effects were considered statistically significant for a corrected P value of less than .05. The anatomical name reported for each supratentorial cluster of activation was determined with the Talairach demon, and the location of clusters was crosschecked by visual comparison with the Talairach atlas.

All statistical tests were two-sided, and a P value of less than .05 was considered statistically significant (available online).

Results

Behavioral Performance

Table 1 presents demographic and clinical characteristics of the sample. Survivors were mostly male (51.5%), Caucasian (74.5%), and adolescents (mean [SD] age at evaluation = 14.4 [4.7] years; age at diagnosis = 6.7 [4.4] years; time since diagnosis = 7.7 [1.7] years). Maternal and paternal educations were 13.8 (2.5) years and 13.6 (3.0) years, respectively. Performance on the clinical neurocognitive battery is presented in Supplementary Table 1 (available online). Compared with population norms, survivors demonstrated higher rates of impairment on number-letter switching ($P < .001$), verbal fluency ($P < .001$), digit span backward ($P = .004$) and forward ($P < .001$), Rey copy ($P < .001$), block design ($P = .001$), grooved pegboard ($P < .001$), coding ($P < .001$), and letter sequencing ($P = .002$). Results from multivariable general linear models for prediction of executive dysfunction are reported in Supplementary Table 2 (available online). Methotrexate exposure was higher in survivors with impaired executive function (methotrexate AUC $P = .048$) and processing

Table 1. Demographic and clinical characteristics of ALL survivors

Category	Mean (SD)	Median (IQR)
Demographics		
Sex, No. (%)		
Male	85 (51.5)	—
Female	80 (48.5)	—
Race/ethnicity, No. (%)		
Caucasian	123 (74.5)	—
African American	21 (12.7)	—
Hispanic	14 (8.5)	—
Other	7 (4.3)	—
Current age, y	14.4 (4.7)	13.2 (8.2–26.5)
Education, y		
Maternal	13.8 (2.5)	—
Paternal	13.6 (3.0)	—
Treatment		
Age at diagnosis, y	6.7 (4.4)	5.3 (3.3–8.6)
Years since diagnosis	7.7 (1.7)	7.5 (6.3–9.1)
Treatment risk stratum, No. (%)		
Low	93 (56.4)	—
Standard	72 (43.6)	—
Dexamethasone oral, mg/m ²	1100.3 (295.3)	1099.6 (991.5–1252.7)
Methotrexate, IV standard dose, g/m ²	4.0 (2.4)	3.7 (3.1–4.4)
IV high dose, g/m ² *		
Low risk	13.0 (7.4)	12.1 (10.5–14.2)
Standard risk	20.0 (4.5)	19.7 (18.0–21.7)
Intrathecal, mL†	168.1 (56.3)	144.0 (134.0–192.0)
TIT chemotherapy, No. of counts	14.4 (4.1)	12.0 (12.0–16.0)

*HD IV methotrexate was defined as daily dose of more than 1 g/m² of IV methotrexate. Cumulative doses are presented separately for the low-risk and standard/high-risk groups. ALL = acute lymphoblastic leukemia; IQR = interquartile range; IT = intrathecal; IV = intravenous; TIT = intrathecal injection of MTX plus hydrocortisone plus cytarabine.

†Intrathecal methotrexate is administered, where 1 mL contains methotrexate 1 mg, hydrocortisone 2 mg, and cytarabine 3 mg.

speed (total TIT counts $P = .02$) compared with survivors without impairment.

Reaction time for incongruent stimuli was longer than for congruent stimuli ($P < .001$); the fastest responses were obtained with spatial cues, whereas no cues led to the slowest responses (Table 2). Behavioral performance on the CPT and ANT when administered in the clinic was similar to performance when administered during fMRI scanning (CPT reaction time correlation: $r = .61$, $P < .001$; ANT conflict: $r = .48$, $P < .001$) (Supplementary Table 3, available online). The fMRI CPT commission rate was modestly correlated with fMRI ANT alerting and conflict scores ($r = .27$, $P = .001$, and $r = .28$, $P < .001$, respectively). CPT performance during fMRI was associated with cognitive flexibility, working memory, omissions, variability, and detectability during clinical testing (Table 3). The ANT conflict score was associated with verbal fluency, variability, and omissions (Table 3). Age at diagnosis was associated with fMRI CPT and ANT task performances. Omissions were higher for those diagnosed at a younger age ($r = -.35$, $P < .001$). Survivors who were younger at diagnosis showed higher alerting ($r = -.23$, $P = .005$) and conflict scores ($r = -.28$, $P < .001$). Neither methotrexate dose or exposure nor sex was associated with task performance during fMRI ($P > .05$).

Table 2. Mean ANT reaction time and accuracy for each condition during fMRI*

Cue condition	Congruent		Incongruent		Mean	
	Mean reaction time (SD), ms	Accuracy, % (SD)	Mean reaction time (SD), ms	Accuracy (SD), %	Mean reaction time (SD), ms	Accuracy (SD), %
No cue	861.6 (221.7)	91.0 (25.0)	1020.0 (314.2)	86.0 (25.0)	941.5 (283.2)	88.0 (25.0)
Center cue	834.4 (218.9)	91.0 (24.0)	952.6 (260.3)	87.0 (26.0)	893.5 (247.3)	89.0 (25.0)
Spatial cue	771.9 (188.7)	91.0 (24.0)	938.3 (293.8)	87.0 (24.0)	855.9 (260.7)	89.0 (24.0)
Mean	822.7 (213.2)	91.0 (24.0)	970.3 (291.9)	87.0 (25.0)	897.0 (266.2)	89.0 (25.0)

*ANT = attention network task; fMRI = functional magnetic resonance imaging; ms = milliseconds.

Table 3. Associations between task performance during fMRI and attention, and executive measures in survivors of childhood ALL treated with chemotherapy only

Measure	Omission rate		Commission rate		Reaction time		Accuracy rate		Alerting		Orienting		Conflict	
	CPT	P*	CPT	P*	CPT	P*	CPT	P*	ANT	P*	ANT	P*	ANT	P*
Cognitive flexibility	−0.17	.04	−0.19*	.02	0.12	.16	0.19*	.02	0.11	.21	−0.02	.82	−0.11	.20
Verbal fluency	−0.03	.77	−0.09	.29	−0.01	.94	0.09	.29	0.09	.30	−0.01	.94	−0.22	.008
Working memory	−0.15	.07	−0.17	.04	0.11	.17	0.17	.04	0.05	.57	0.05	.53	0.06	.51
CPT variability	0.26	.002	0.42	<.001	−0.04	.59	−0.42	<.001	0.06	.47	−0.07	.40	0.41	<.001
CPT omission	−0.24	.003	−0.36	<.001	0.15	.06	0.36	<.001	−0.15	.07	0.06	.47	−0.21	.01
CPT detectability	−0.22	.007	−0.54	<.001	0.30	<.001	0.54	<.001	−0.09	.29	0.04	.66	−0.05	.57

*Pearson's correlation test (two-sided). ALL = acute lymphoblastic leukemia; ANT = attention network task; CPT = continuous performance task; fMRI = functional magnetic resonance imaging.

fMRI Results

Results of random-effects analyses for fMRI CPT and ANT tasks are presented in Table 4. Higher brain activation during CPT (task main effect, CPT > control fixation) was identified in the right cerebellum, bilateral insula, left thalamus, right midfrontal, left fronto-parietal, and right anterior cingulate cortices (Figure 1, red). During the CPT task, younger age at diagnosis was associated with lower brain activation in the bilateral superior temporal and parietal cortices (Figure 2, red). Higher methotrexate exposure was associated with lower brain activation (CPT > fixation) in the bilateral midfrontal, parietal, and right temporal areas (Figure 2, green).

The ANT alerting effect (index of arousal) center cue compared with no cue condition showed higher brain activation in the right midtemporal and bilateral fronto-parietal cortices (Figure 1, pink). ANT orienting activity (index of spatial orientation), defined as spatial cue trials compared with center cue trials, was found in the right lingual gyrus and bilateral occipital cortex (Figure 1, blue). ANT conflict (index of flexibility), defined as incongruent trials vs congruent trials, activated the bilateral insula, bilateral temporo-occipital, and right frontal cortices (Figure 1, green). Higher methotrexate exposure was associated with higher ANT alerting activity in the bilateral frontal cortex, anterior cingulate cortex (ACC), and basal ganglia (Figure 2, pink). Younger age at diagnosis was associated with lower conflict resolution in the left parietal lobe and right hippocampus/parahippocampal gyrus (Figure 2, blue). A schematic representation showing associations between brain activity during the CPT and the ANT and clinical risk factors (age at diagnosis and methotrexate exposure measured as area under the curve) in long-term survivors of ALL is illustrated in Figure 3. No statistically significant association between brain activation and sex was found.

Discussion

Our study represents the largest cohort of long-term survivors of ALL treated on a single, contemporary chemotherapy-only protocol for which fMRI was used to assess brain networks related to attention and executive function. Patterns of activation show heterogeneous regional brain responses, with reduced activation in distinct areas, suggesting compromised task involvement, and increased activation in other areas, suggesting compensatory task involvement. Brain activation during attention tasks was associated with age at diagnosis and exposures to intravenous HDMTX, important risk factors for cognitive deficits in children treated for leukemia. Children diagnosed at a younger age showed decreased activity in areas involved in conflict resolution and sustained attention. Increased and decreased activation in regions involved in alerting and sustained attention, respectively, were associated with higher HDMTX exposure. Increased activity in prefrontal brain regions during the ANT and decreased activity in parietal and temporal regions during the CPT were associated with higher methotrexate exposure and may suggest increased effortful processing to compensate for inefficiency in posterior brain regions (Figure 3).

Younger age at diagnosis and higher methotrexate exposure were associated with lower CPT brain activation in the right temporal and bilateral fronto-parietal regions. These regions are part of the ventral and dorsal attention networks and are known to be involved in response inhibition, shifting of attention, language, executive functions, and decision-related processes (17,36,37). CPT performance during fMRI was similar to performance on the CPT during clinical testing, which did not differ from the normative population. However, lower activation, years after diagnosis, suggests diminished engagement of these brain regions during sustained and selective attention related to treatment. Thus, although survivors' performance on

Table 4. Results from the random-effects analyses for the CPT and ANT alerting, orienting, and conflict conditions*

Contrast	Brain region	Side	Talairach coordinates	Z score
CPT > fixation (task main effect; sustained attention)	Cerebellum	R	12 -48 -22	>8
	Insula	L	-32 18 10	>8
	Insula	R	32 24 6	>8
	Thalamus	L	-13 -9 9	>8
	Midfrontal cortex	R	40 48 26	>8
	Precentral	L	-42 -20 62	>8
	Postcentral	L	-44 -33 59	>8
	ACC	R	9 19 39	>8
ANT alerting (center cue > no cue; index of arousal)	Middle frontal gyrus	R	42 4 56	6.01
	Inferior frontal cortex	R	50 22 34	4.78
	Superior parietal cortex	R	36 -54 56	4.99
	Superior parietal cortex	L	-20 -68 58	3.83
	Inferior parietal cortex	R	52 -48 48	4.41
ANT orienting (spatial cue > center cue; index of spatial orientation)	Superior occipital cortex	L	-26 -92 24	4.08
	Middle occipital cortex	R	28 -94 12	4.04
	Lingual gyrus	R	14 -92 -7	3.80
ANT conflict (incongruent > congruent; index of flexibility)	Insula	R	36 24 -2	5.21
	Insula	L	-32 22 -2	4.11
	Superior occipital cortex	R	26 -68 40	4.95
	Inferior occipital cortex	L	-44 -74 -10	4.84
	Inferior frontal gyrus	R	48 10 28	4.41
	Inferior temporal gyrus	R	48 -60 -16	4.37
	Superior temporal cortex	R	56 -24 18	4.50
	Superior temporal cortex	L	-50 -44 24	4.19
Decreased CPT with younger age at diagnosis	Superior parietal cortex	L	-28 -56 64	4.44
	Supramarginal gyrus	R	54 -30 44	3.85
	Middle temporal gyrus	R	58 -28 -6	3.89
Decreased CPT with increased MTX AUC	Parietal cortex	L	-30 -4 50	4.00
	Middle frontal gyrus	R	40 0 54	3.56
	Middle frontal gyrus	L	-28 0 54	3.76
	Middle superior frontal cortex	R	14 56 32	4.22
Increased ANT alerting with increased MTX AUC	Middle superior frontal cortex	L	-10 50 34	4.09
	Caudate nucleus	R	7 11 6	3.73
	Caudate nucleus	L	-10 8 6	4.01
	Putamen	L	-24 13 6	3.65
	Anterior cingulate cortex	L	-10 26 28	3.83
Decreased ANT conflict with younger age at diagnosis	Parietal cortex	L	-58 -14 28	4.50
	Hippocampus	R	26 -14 -20	4.33

*ACC = anterior cingulate cortex; ANT = attention network task; CPT = continuous performance task; L = left; MTX AUC = methotrexate area under the curve; R = right.

this simple attention task was sufficient, decreased brain activation with increased methotrexate exposure suggests that these temporal and fronto-parietal regions may have been affected by therapy (38). Lower activation related to conflict resolution was found in the right hippocampus and left parietal areas in survivors diagnosed at younger ages. The hippocampus is a site of postnatal neurogenesis, and therapy-induced hippocampal damage has been proposed to play a role in neurocognitive impairment among cancer survivors (39). Previous studies have reported smaller cortical surface area and volume in the hippocampus among long-term ALL survivors compared with healthy controls (20,40).

In survivors of ALL one year off chemotherapy, sustained attention problems were identified more often in children exposed to intravenous HDMTX compared with standard treatment (7). Consistent with previous results (9), our study highlights increased brain activation (ANT alerting) related to higher methotrexate exposure in frontal areas, the basal ganglia (caudate nuclei and putamen), and the anterior cingulate

cortex. Increased activity in these frontal and subcortical areas seems to be compensating for damaged or inefficient brain regions in order to achieve a comparable level of behavioral performance in survivors exposed to higher doses of methotrexate. Previous studies have reported smaller volumes of basal ganglia and ACC in long-term survivors of ALL compared with sex- and age-matched controls (40,41). The midfrontal gyrus and the basal ganglia have been implicated in efficient processing of warning signals involved in generating anticipatory responses (42). The basal ganglia play a role in a wide range of functions, from movement control and sensory feedback to set-shifting, attention, visual perception, memory, learning, and executive functions (43,44). The basal ganglia have high metabolic demands during childhood, and thus might be especially vulnerable to treatment-induced injury (45). The caudate nucleus of the basal ganglia is part of the frontostriatal circuit supporting executive functions (46), and caudate damage can lead to impaired planning and problem solving, attention, learning, memory, and verbal fluency (47–49). The central role of the ACC

Figure 1. Functional magnetic resonance imaging (fMRI) brain activation for the continuous performance task (CPT) and the three anterior cingulate cortex (ANT) attentional networks. Images are displayed in neurological convention. All the activation maps had thresholds that were set at $P < .001$ (uncorrected) with an extent of 5 voxels for visualization. CPT > fixation (red): right cerebellum, bilateral insula, left thalamus, right midfrontal, left fronto-parietal, and right anterior cingulate cortices. Alerting network (center cue > no cue; pink): right midtemporal and bilateral fronto-parietal cortices. Orienting network (spatial cue > center cue; blue): the right lingual gyrus and bilateral occipital cortex. Conflict network (incongruent > congruent; green): bilateral insula, bilateral temporo-occipital, and right frontal cortices. ANT = attention network task; CPT = continuous performance task.

has been repeatedly reported to involve executive control and conflict resolution (34,50,51). Elevated task-related activity in these areas suggests compensatory engagement for behavioral performance in alerting.

Findings of altered activation in prefrontal and anterior cingulate cortices during sustained and shifting attention are consistent with studies of phenotypes of attention deficit hyperactivity disorder (ADHD) and executive dysfunction in a variety of developmental and acquired brain injury populations. ADHD and executive dysfunction have been associated with reduced thickness in dorsolateral and anterior cingulate frontal cortices (52), as well as reduced size of frontostriatal white matter tracts (53). Functional activation in these areas during performance on attention and working memory tasks is reduced in individuals with ADHD (54). Unlike previous studies in developmental ADHD, we found compensatory increased activity in the anterior cingulate cortex for ANT alerting to be related to higher exposure to methotrexate. These differences in anterior cingulate cortex activation might be related to differences in genetic variation vs acquired injury on brain function (ie, the result of methotrexate exposure in an otherwise healthy brain).

Performance on the Rey Copy and Grooved Pegboard tests was below the normal range but did not correlate with fMRI task performance. Pathophysiological correlates of impairment on these tests remain unknown. Further research is needed to investigate why compensatory mechanisms failed for organization/planning and motor speed in ALL survivors and to clarify how changes in brain activation sustain normal performance

on other tasks. Moreover, ANT alerting and orienting performance during fMRI did not correlate with other clinical measures, and it remains unclear whether this performance is at a normal level or impaired. Future studies are necessary to investigate the clinical relevance of the ANT alerting- and orienting-related brain activation changes in survivors of childhood ALL.

Our results must be interpreted in light of several limitations. The study did not include a typically developing control group. However, our sample size and clinical data permitted examination within relatively wide age ranges and treatment with varying amounts of methotrexate. We did not track which of the two MRI scanners was used for each participant and could not adjust for this variable. However, both scanners are 3T from the same manufacturer, and slight differences in scanner might add to error variance, weakening power to detect differences, though this would not account for statistically significant differences. The number of fMRI tasks was limited because the study was focused on understanding brain networks associated with attention and executive functions. Other cognitive domains, including working memory, will need to be studied in the future. We did not have fMRI data before diagnosis or during therapy with which to compare long-term survivorship. The peak age at diagnosis for ALL is between two to four years, and therapy typically begins the day after diagnosis. Thus, engagement of such young children systematically in fMRI activation tasks is not feasible.

Despite limitations, this study shows that attention and executive function-related brain activation is associated with

Figure 2. Brain areas showing statistically significant correlations with clinical risk factors. Images are displayed in neurological convention. All the activation maps are shown with thresholds set at $P < .001$ (uncorrected) and extent of 5 voxels for visualization. Activation maps revealed neural correlates of altered brain function. Continuous performance task activation decreased in survivors diagnosed at a younger age in bilateral superior temporal and bilateral parietal cortices (red) and decreased with methotrexate area under the curve (MTX AUC) in the bilateral midfrontal, parietal, and right middle temporal areas (green). Conflict-related activity decreased in survivors diagnosed at younger ages in the left parietal lobe and right hippocampus (blue). Alerting-related activity increased with MTX AUC in the bilateral frontal, caudate nuclei, left putamen, and anterior cingulate cortex (pink). CPT = continuous performance task; MTX AUC = methotrexate area under the curve.

Figure 3. Schematic representation of associations between brain activity and clinical risk factors in long-term survivors of acute lymphoblastic leukemia (ALL). Decreased activation in younger survivors could imply a compromised task involvement. Increased activity in prefrontal and subcortical brain regions during the attention network task (ANT; pink) and decreased activity in parietal and temporal regions during the continuous performance task (CPT; green) were both associated with higher methotrexate exposure and may suggest increased effortful processing as a method to compensate for inefficiency in posterior brain regions in patients at highest risk for attention deficits. Red: CPT-age at diagnosis; blue: conflict-age at diagnosis; green: CPT-methotrexate AUC; pink: alerting-methotrexate AUC. CPT = continuous performance task; MTX AUC = methotrexate area under the curve.

important clinical risk factors for neurocognitive deficits in long-term survivors of ALL treated with chemotherapy only. The pattern of behavioral and imaging responses provides

evidence for altered engagement of neural systems to maintain task performance despite therapy-induced brain injury. Thus, functional imaging may help to clarify the pathophysiology of

neurocognitive deficits often seen in ALL survivors and to select patients for remedial intervention based on neural phenotypes.

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