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Kellen Gandy

*St. Jude's Children's Research Hospital*

Matthew A Scoggins

*St. Jude's Children's Research Hospital*

Nicholas Phillips

*St. Jude's Children's Research Hospital*

Ellen van der Plas

*University of Iowa*

Slim Fellah

*Washington University School of Medicine in St. Louis*

*See next page for additional authors*

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### Recommended Citation

Gandy, Kellen; Scoggins, Matthew A; Phillips, Nicholas; van der Plas, Ellen; Fellah, Slim; Jacola, Lisa M; Pui, Ching-Hon; Hudson, Melissa M; Reddick, Wilburn E; Sitaram, Ranganatha; and Krull, Kevin R, "Sex-based differences in functional brain activity during working memory in survivors of pediatric acute lymphoblastic leukemia." *JNCI Cancer Spectrum*. 6, 2. pkac026 (2022).  
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**Authors**

Kellen Gandy, Matthew A Scoggins, Nicholas Phillips, Ellen van der Plas, Slim Fella, Lisa M Jacola, Ching-Hon Pui, Melissa M Hudson, Wilburn E Reddick, Ranganatha Sitaram, and Kevin R Krull

## Sex-Based Differences in Functional Brain Activity During Working Memory in Survivors of Pediatric Acute Lymphoblastic Leukemia

Kellen Gandy, PhD <sup>1</sup>, Matthew A. Scoggins, PhD,<sup>2</sup> Nicholas Phillips, MD, PhD <sup>1</sup>, Ellen van der Plas, PhD <sup>3</sup>, Slim Fellah, PhD,<sup>4</sup> Lisa M. Jacola, PhD <sup>5</sup>, Ching-Hon Pui, MD,<sup>6,7</sup> Melissa M. Hudson, MD <sup>1,6</sup>, Wilburn E. Reddick, PhD <sup>2</sup>, Ranganatha Sitaram, PhD <sup>2</sup>, Kevin R. Krull, PhD <sup>1,5,\*</sup>

<sup>1</sup>Department of Epidemiology and Cancer Control, St. Jude's Children's Research Hospital, Memphis, TN, USA, <sup>2</sup>Department of Diagnostic Imaging, St. Jude's Children's Research Hospital, Memphis, TN, USA, <sup>3</sup>Department of Psychiatry, University of Iowa Hospital and Clinics, Iowa City, IA, USA, <sup>4</sup>Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA, <sup>5</sup>Department of Psychology, St. Jude Children's Research Hospital, Memphis, TN, USA, <sup>6</sup>Department of Oncology, St. Jude's Children's Research Hospital, Memphis, TN, USA, and <sup>7</sup>Department of Pathology, St. Jude's Children's Research Hospital, Memphis, TN, USA

\*Correspondence to: Kevin R. Krull, PhD, Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, 262 Danny Thomas Pl, Mail Stop 735, Memphis, TN 38105, USA (e-mail: kevin.krull@stjude.org).

### Abstract

**Background:** Long-term survivors of pediatric acute lymphoblastic leukemia are at elevated risk for neurocognitive deficits and corresponding brain dysfunction. This study examined sex-based differences in functional neuroimaging outcomes in acute lymphoblastic leukemia survivors treated with chemotherapy alone. **Methods:** Functional magnetic resonance imaging (fMRI) and neurocognitive testing were obtained in 123 survivors (46% male; median [min-max] age = 14.2 years [8.3-26.5 years]; time since diagnosis = 7.7 years [5.1-12.5 years]) treated on the St. Jude Total XV treatment protocol. Participants performed the n-back working memory task in a 3 T scanner. Functional neuroimaging data were processed (realigned, slice time corrected, normalized, smoothed) and analyzed using statistical parametric mapping with contrasts for 1-back and 2-back conditions, which reflect varying degrees of working memory and task load. Group-level fMRI contrasts were stratified by sex and adjusted for age and methotrexate exposure. Statistical tests were 2-sided ( $P < .05$  statistical significance threshold). **Results:** Relative to males, female survivors exhibited less activation (ie, reduced blood oxygen dependent-level signals) in the right parietal operculum, supramarginal gyrus and inferior occipital gyrus, and bilateral superior frontal medial gyrus during increased working memory load (family-wise error-corrected  $P = .004$  to  $.008$ , adjusting for age and methotrexate dose). Female survivors were slower to correctly respond to the 2-back condition than males ( $P < .05$ ), though there were no differences in overall accuracy. Performance accuracy was negatively correlated with fMRI activity in female survivors (Pearson's  $r = -0.39$  to  $-0.29$ ,  $P = .001$  to  $.02$ ), but not in males. **Conclusions:** These results suggest the working memory network is more impaired in female survivors than male survivors, which may contribute to ongoing functional deficits.

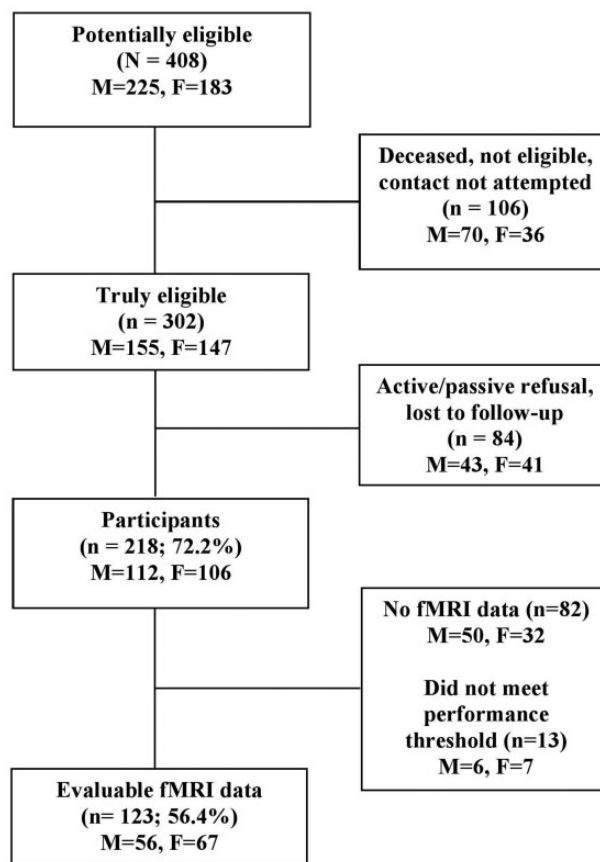
Leukemia is the most common pediatric cancer, with the majority of children being diagnosed between the ages of 2 and 5 years (1). Survival rates of childhood acute lymphoblastic leukemia (ALL) exceed 90% on contemporary treatment protocols that have replaced prophylactic cranial irradiation with risk-stratified intrathecal therapy, high-dose intravenous methotrexate, and dexamethasone (2,3). Although survival rates have improved, leukemia patients treated on contemporary chemotherapy protocols remain at elevated risk for long-term neurocognitive impairment and altered patterns of brain function (4,5). Given the young age at diagnosis and high rate

of survival, it is critical to examine the long-term impact of cancer and its treatment on brain function and neurocognitive abilities to mitigate the risk for potential neurocognitive late effects (ie, cognitive deficits that emerge in the years following therapy). Long-term survivors of childhood ALL ( $\geq 5$  years from diagnosis) most commonly exhibit deficits in attention, executive function, and processing speed (5,6). These neurocognitive impairments can contribute to poor quality of life, reduced academic achievement, and increased unemployment among survivors, demonstrating a strong burden to the individual (7,8).

Received: October 29, 2021; Revised: January 31, 2022; Accepted: February 24, 2022

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**Figure 1.** Flow chart of recruitment procedures. fMRI = functional magnetic resonance imaging; M = male; F = female.

The degree of neurocognitive impairment in long-term ALL survivors is known to be associated with certain treatment exposures (ie, intrathecal therapy, high-dose methotrexate, dexamethasone, etc) and age at diagnosis, but recent evidence suggests that these interactions may be sex specific (9-11). A higher risk of neurocognitive impairment has been observed in female survivors compared with males, with research studies noting statistically significant deficits in attention and executive function (12-15). Neuroimaging investigations have also reported sex-specific differences in brain connectivity patterns in long-term ALL survivors, with females displaying greater brain alterations compared to males (10,16). For example, females exhibited widespread cortical thinning that corresponded with younger age at diagnosis and higher dexamethasone exposure, a pattern not observed among males. Additionally, female survivors displayed disrupted brain connectivity within the cerebello-thalamo-cortical network, which is involved in higher-ordered cognitive processes (ie, executive function, working memory) and rich in glucocorticoid receptors. These findings suggest that females may be particularly vulnerable to the neurotoxic effects of leukemia and its treatment, especially regarding corticosteroid exposure. To advance understanding about sex-based differences among childhood ALL survivors treated with chemotherapy, we undertook a large-scale neuroimaging investigation using task-based functional magnetic resonance imaging (fMRI) to examine sex-specific differences in brain activity during a working memory task.

## Methods

### Study Population

A total of 408 children with newly diagnosed ALL were treated on the Total XV protocol at St. Jude Children's Research Hospital between 2000 and 2010 (ClinicalTrials.gov, NCT0013711) (2). Survivors were eligible for the separate prospective neuroimaging study if they were 5 years and more from diagnosis of ALL, English speaking, and 8 years and older at the time of study enrollment. Survivors were ineligible for the study if they had received cranial radiation therapy for central nervous system (CNS) relapse, had relapse or secondary neoplasms, were diagnosed with a genetic disorder associated with cognitive impairments (ie, Trisomy 21), or had a history of head trauma or neurological conditions unrelated to cancer therapy. Of the 302 eligible survivors, 213 (71%) completed neurocognitive testing, and 123 (58%) had evaluable functional neuroimaging data. Figure 1 summarizes the flow of study recruitment procedures. Demographic data (ie, sex) and clinical characteristics were obtained from the survivors' medical records. Sex refers to the biological characteristics of males and females. This study was approved by the institutional review board, and written informed consent was obtained by the participants and/or their legal guardians.

### Total Therapy XV Treatment

Children diagnosed with ALL were stratified and treated in low-risk or standard/high-risk treatment arms (17). Compared with

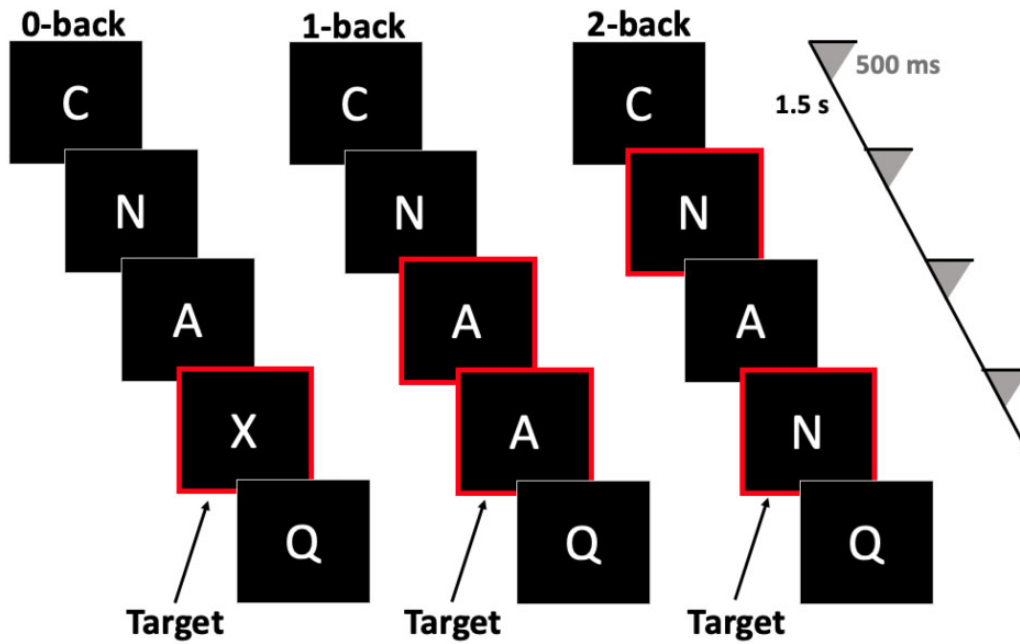


Figure 2. Illustration of the n-back task paradigm used in the fMRI. fMRI = functional magnetic resonance imaging; ms = milliseconds; s = seconds.

patients treated in the standard/high-risk arm, those treated in the low-risk arm received fewer doses of triple intrathecal treatment with methotrexate, hydrocortisone and cytarabine (13-18 vs 16-25), lower dosage of intravenous high-dose methotrexate (2.5 vs 5.0 gm/m<sup>2</sup>) for every other week for 4 courses as consolidation treatment, and lower dosage of dexamethasone pulses at 8 vs 12 mg/m<sup>2</sup> per day for 4 days per course for 25 courses. All long-term survivors in this study were treated using these protocols. Serum concentrations of methotrexate were obtained at 3 different time periods following intravenous administration (ie, 6, 23, and 42 hours post administration), and an average area under the curve (AUC) was computed. Dexamethasone AUC was also calculated based on serum samples, which were collected during therapy (ie, before, 1, 2, 4, 8 hours post oral administration).

### Neurocognitive Evaluation

Neurocognitive evaluations were administered by certified examiners under the supervision of a board-certified clinical neuropsychologist on ALL survivors who were 5 to 10 years from their initial cancer diagnosis. Standardized clinical guidelines for cognitive evaluations were implemented to reduce interference, test order effects, and fatigue. The neurocognitive battery included standardized measures of intelligence (18), executive function (19), attention (20), processing speed (21), memory (22), and fine motor dexterity (23). The neurocognitive tests relevant to the aim of this study included metrics of working memory (ie, Weschler Digit Span Backwards), verbal fluency (ie, Delis-Kaplan Executive Function System Verbal Fluency), and cognitive flexibility (ie, Delis-Kaplan Executive Function System Trail Making), because all of these neurocognitive functions are related to the n-back task (24). Neurocognitive impairment was defined as age-adjusted normative scores below the 10th percentile.

### N-Back Task

The n-back task is a measure of working memory load and capacity (25,26). The task involved 3 separate conditions: the 0-back, 1-back, and 2-back conditions, which reflect varying degrees of working memory and task load. Participants completed the n-back task while undergoing a fMRI exam. An illustration of the task paradigm and parameters is presented in Figure 2. A 2-second instruction screen was presented between each block with 5 seconds of silence after the instructions. Performance accuracy was calculated separately for each condition using the following formula: accuracy = (hits + correct rejections)/total stimuli; correct rejections = number of distractors – commission errors; hits = number of targets – omission errors. We applied performance cutoffs for the n-back task, based on previous literature, to ensure that participants were actively engaged in the task and had an appropriate understanding of the task instructions (24). For the 1-back and 2-back conditions, participants were excluded if they did not achieve at least 30% target accuracy (ie, with 25% target accuracy representing random selection). Given that the 0-back task is easier than the other 2 conditions, a more stringent performance threshold was applied (ie, cut off <75% target accuracy) (25). Participants who did not meet the performance threshold (ie, validity screening) were not included in the behavioral or fMRI analysis.

### Functional MRI

fMRI was performed on 3T scanners (Trio or Skyra, Siemens, Malvern, PA, USA) using a 14-channel standard head coil. The functional images were acquired using a single-shot T2\*-weighted echo-planar imaging sequence (Repetition Time [TR] = 2.06 seconds, Time to Echo [TE] = 30 ms, Field-of-view [FOV] = 192 mm, matrix = 64 × 64, slice thickness = 5 mm) during the n-back task. Functional neuroimaging data were preprocessed (realigned, slice time corrected, normalized, and

smoothed) and analyzed using statistical parametric mapping (SPM) (27) with contrasts developed for the 1-back vs 0-back, 2-back vs 0-back, and 2-back vs 1-back conditions. Group-level fMRI contrasts were stratified by sex and adjusted for age at evaluation and methotrexate dosage (ie, methotrexate AUC).

### Statistical Analysis

Normative age-standardized scores were calculated among survivors to determine neurocognitive impairment in domains of working memory, verbal fluency, and cognitive flexibility (ie, scores <10th percentile) and compared by sex while adjusting for methotrexate AUC. The behavioral performance on the n-back task was analyzed using general linear models that adjusted for age at evaluation and methotrexate AUC. Functional neuroimages were preprocessed and analyzed using SPM (27). The SPM preprocessing steps included correction for interscan head motion using realignment techniques, normalization of images to the Montreal Neurological Institute brain template, and image smoothing with a 6-mm full width at half-maximum Gaussian kernel. The preprocessed functional images were then resliced to 2 mm isotropic resolution for analysis using general linear models. Statistical calculations were computed using SPM based on an initial voxel-level analysis, which included a family-wise error correction for multiple comparisons, with a minimum cluster size of 5 voxels ( $T$  threshold = 3.5). Differences in brain activity were considered statistically significant for corrected  $P$  values of less than .05. Cluster-level analyses was also conducted with a voxel uncorrected threshold  $P$  value of less than .001 and minimum cluster size of 5 voxels. Anatomical labels for the clusters were determined with SPM, and the location of clusters was crosschecked by visual comparison with the Talairach atlas. Associations between fMRI activity, neurocognitive performance, treatment exposures, patient demographics, and n-back task performance were examined using multiple linear regressions and Pearson correlations in XLSTAT 22.5.1. All statistical tests were 2-sided with a  $P < .05$  threshold for statistical significance.

### Results

fMRI and neurocognitive testing were obtained in 123 survivors (46% male, 54% female; median [min-max] age = 14.2 years [8.3-26.5 years]; time since diagnosis = 7.7 years [5.1-12.5 years]). The demographic information and clinical characteristics of this cohort are presented in Table 1. Neurocognitive and n-back task performance are presented in Table 2. No behavioral differences in performance accuracy during the n-back task were observed between male and female survivors. However, female survivors had statistically significant slower reaction times during the 2-back condition ( $P = .048$ ). Standardized neurocognitive performance (ie, working memory, verbal fluency, and cognitive flexibility tasks) was not associated with fMRI brain activity during the n-back task. However, male survivors demonstrated greater deficits in cognitive flexibility than females ( $P = .003$ ). There were no other differences between females and males for the other neurocognitive outcomes relevant to this study (ie, working memory or verbal fluency;  $P > .05$ ).

For fMRI, females had reduced brain activity (ie, reduced blood oxygen level-dependent [BOLD] signal) in the right parietal operculum, supramarginal gyrus and inferior occipital gyrus, and bilateral superior medial frontal gyrus during increased working memory task load (ie, 2-back vs 1-back contrast

**Table 1.** Demographic and clinical characteristics of acute lymphoblastic leukemia survivors by sex<sup>a</sup>

Category	Overall	Females	Males	P
		No. (%)		
Sex				
Male	56 (46)	0	56	—
Female	67 (54)	67	0	—
Treatment risk stratum				
Low risk	52 (42.3)	46 (68.7)	25 (32.6)	.007
Standard/high risk	71 (57.7)	21 (31.3)	31 (67.4)	.007
Mean (SD)				
Age at evaluation, y	15.1 (4.7)	15.9 (4.9)	14.3 (4.5)	.07
Age at diagnosis, y	7.2 (4.5)	6.6 (4.3)	7.8 (4.7)	.15
Methotrexate AUC	32.8 (11.6)	30.2 (10.1)	35.9 (12.5)	.003
Dexamethasone AUC	542.7 (285.5)	531.3 (255.9)	556.2 (316.6)	.84
Total IT injections, No.	14.4 (3.9)	13.5 (3.0)	15.5 (4.6)	.005

<sup>a</sup>AUC = area under the curve; IT = intrathecal injection of methotrexate. Student  $t$  test comparisons with  $P$  values less than .05 represent statistical significance.

compared with males (family-wise error-corrected  $P = .004$  to .008, adjusting for age at evaluation and methotrexate dose) (see Figure 3). Brain activation patterns were not associated with treatment risk group, age at diagnosis, executive dysfunction status, or total number of intrathecal injections. Multiple linear regression models revealed that performance accuracy on the n-back task accounted for 11.8% to 17.5% of the variation in BOLD signaling in the bilateral superior medial frontal gyrus during increased working memory load in female survivors, but not in male survivors ( $P < .05$ ). Performance accuracy on the n-back task was negatively correlated with BOLD activation in this brain region during the 1-back (left medial frontal gyrus: Pearson  $r = -0.394$ ,  $P = .001$ ; right medial frontal gyrus: Pearson  $r = -0.322$ ,  $P = .008$ ) and 2-back conditions (left medial frontal gyrus: Pearson  $r = -0.358$ ,  $P = .003$ , right medial frontal gyrus: Pearson  $r = -0.290$ ,  $P = .02$ ) in female survivors. Performance accuracy was not associated with fMRI activity in male survivors. Additionally, dexamethasone AUC accounted for 8.2% to 18.7% of the variation in BOLD signaling in the right parietal operculum, supramarginal gyrus, and inferior occipital gyrus among females during increased working memory load ( $P < .05$ ). Dexamethasone AUC was negatively correlated with the amplitude of BOLD signals in these brain regions (parietal operculum: Pearson  $r = -0.433$ ,  $P = .001$ ; supramarginal gyrus: Pearson  $r = -0.286$ ,  $P = .03$ ; inferior occipital gyrus: Pearson  $r = -0.347$ ,  $P = .008$ ).

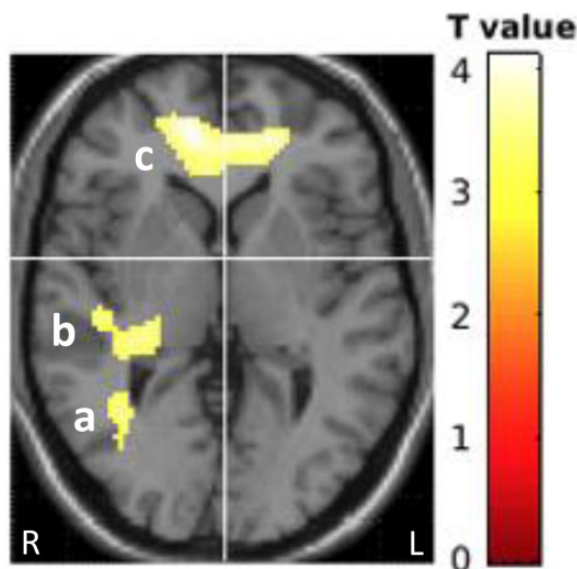
### Discussion

The findings from this study suggest that female sex is associated with brain dysfunction in the working memory networks of ALL survivors. Despite the lack of difference in behavioral performance accuracy between female and male survivors on the n-back task, there were sex-specific variations in reaction time (ie, processing speed) and brain activation patterns. Compared with males, female survivors were statistically significantly slower ( $P < .05$ ) at correctly identifying target stimuli during the n-back task condition that required the greatest level of working memory load (ie, 2-back). Although reaction times typically become slower as a cognitive task becomes more difficult, these effects were exacerbated in female survivors. These

**Table 2.** Neurocognitive evaluations, n-back task performance, and fMRI outcomes among acute lymphoblastic leukemia survivors by sex<sup>a</sup>

Cognitive and fMRI Outcomes	Males (n = 56) M (SD)	Females (n = 67) M (SD)	P
Wechsler digit span backwards, Z score	-0.31 (1.11)	-0.20 (0.89)	.64
D-KEFS trail-making task, Z score	-0.70 (1.19)	-0.15 (0.98)	.003
D-KEFS verbal fluency, Z score	-0.51 (0.96)	-0.26 (0.85)	.37
0-back task accuracy, %	95.7 (0.06)	95.1 (0.06)	.88
1-back task accuracy, %	91.0 (0.11)	90.1 (0.13)	.83
2-back task accuracy, %	78.7 (0.16)	79.6 (0.12)	.49
0-back hits reaction time, ms	512.9 (74.2)	540.0 (88.2)	.21
1-back hits reaction time, ms	591.2 (136.3)	637.9 (141.2)	.31
2-back hits reaction time, ms	652.3 (140.7)	723.3 (179.3)	.048
R. Inferior occipital gyrus BOLD signal	0.133 (0.36)	-0.124 (0.38)	<.001
R. Supramarginal gyrus BOLD signal	0.289 (0.41)	-0.061 (0.45)	<.001
R. Parietal operculum BOLD signal	0.091 (0.22)	-0.104 (0.27)	<.001
R. Superior medial frontal gyrus BOLD signal	0.268 (0.52)	-0.046 (0.46)	<.001
L. Superior medial frontal gyrus BOLD signal	0.254 (0.57)	-0.072 (0.53)	<.001

<sup>a</sup>BOLD = blood oxygen level-dependent; D-KEFS = Delis-Kaplan Executive Function System; fMRI = functional magnetic resonance imaging; L = left; R = right. Statistical analyses were conducted using general linear models adjusting for age at evaluation and methotrexate area under the curve, with P values less than .05 representing statistical significance. fMRI outcomes were corrected for multiple comparisons using family-wise error. BOLD signal values represent peak voxel activation within the region of interest. Z scores were age standardized and thus were only adjusted for methotrexate area under the curve in the general linear models.



**Figure 3.** Sex-specific variations in blood oxygen level-dependent signaling during increased working memory task load (ie, 2-back vs 1-back condition) presented in radiological convention. Female survivors display reduced activity in the (a) right inferior occipital gyrus, (b) supramarginal gyrus and parietal operculum, and (c) bilateral superior medial frontal gyrus, compared to males. L = left; R = right. T value threshold statistically significant if greater than 3.5.

findings suggest that the working memory network may become overloaded during complex tasks, with consequent impairment in processing speed and regional changes in functional brain activation. The current literature in noncancer populations does not support the notion of sex-specific differences in behavioral performance (ie, reaction time, accuracy) (28-30) or fMRI brain activity (31) during the verbal n-back task, suggesting that the differences observed in this study are unique to female ALL survivors who have undergone chemotherapy. These findings demonstrate that female survivors may

be particularly sensitive to treatment exposures, which likely contribute to long-term neurocognitive late effects.

Previous neuroimaging investigations in noncancer populations have established that working memory is supported by activation of the fronto-parietal brain regions, including the cingulate, parietal, and prefrontal cortices (32). Interestingly, reduced BOLD activation was exclusively observed in these same brain regions among female survivors, suggesting a sex-specific vulnerability for impaired brain function in the years following chemotherapy. The reductions in brain activation among females may reflect emerging brain deficits that have yet to manifest into overt working memory impairments. Given that neurocognitive late effects are often subtle, functional neuroimaging techniques may be particularly useful for detecting discrete and domain-specific neurocognitive functions (6).

Performance accuracy on the 1-back and 2-back conditions was associated with BOLD activity during increased working memory load among female survivors. Considering that the fMRI contrast of interest was derived from the 2-back vs 1-back conditions, it is not surprising that accuracy on these trials accounted for some of the variation in BOLD signaling. In addition, dexamethasone exposure had a sex-specific impact on BOLD activation, with female survivors displaying a statistically significant inverse relationship between these 2 factors.

These findings are consistent with previous literature that observed sex-dependent variations on the impact of dexamethasone exposure on neuroanatomical brain structure in long-term ALL survivors (10). The increased density of glucocorticoid receptors in female survivors within these brain regions may increase the neurotoxicity of chemotherapy agents and steroids administered during ALL therapy. The neurotoxic agents used in chemotherapy are capable of penetrating and damaging the blood-brain barrier, which in turn can cause increased cytokine-induced inflammation (33), neural apoptosis (34), and decreased hippocampal neurogenesis (35). Additional longitudinal studies investigating neuroimaging and neurocognitive outcomes in relation to these treatment factors are warranted to help inform the validity of this proposed mechanism of neurocognitive impairment in long-term ALL survivors.

Research has indicated that the frontal lobes are involved in higher-ordered cognitive processes, including working memory (36), and are among the last brain regions to develop, with full maturation not met until the third decade of life (37-39). While caudal regions of the brain are more developed in children, rostral brain regions (ie, the prefronto-parietal cortex) are less developed and may be particularly susceptible to the neurotoxic effects associated with chemotherapy and steroid exposure. As previously mentioned, the peak incidence of ALL occurs between 2 and 5 years of age. Although rapid brain development occurs during this period, it is important to note that the child's brain size is only 80%-90% of that achieved during adulthood and biologically significant changes in synaptic pruning, myelination, and reorganization have yet to occur (40). Given that children have yet to develop the myelination and dendritic arborization, their neurons need to be protected from chemotherapy-induced toxic insult to avoid cognitive deficits in the years following treatment. Given this trajectory of brain development in the frontal lobe, neurocognitive processes supported by the fronto-parietal regions (ie, working memory and attention) may be particularly vulnerable to the neurotoxicity associated with CNS-directed therapy in children, especially among females.

The findings from this study have limitations to consider, including the lack of a healthy control group. However, the large sample size permitted within group comparisons and clinically relevant outcomes was still observed. Different types of MRI scanners (trio and skyra) were used, though both MRIs were developed by the same manufacturer and had the same magnet strength of 3 T to ensure homogeneity in the data collection process. Variations in the type of scanner may introduce unwanted variance and reduce power to detect differences in BOLD signaling, but these factors would not account for the statistically significant differences observed. Other treatment variables that differed between male and female survivors, such as total number of intrathecal methotrexate injections or treatment risk stratification, were not adjusted for in the statistical models. Instead, methotrexate AUC dosage was adjusted for in the models because it highly correlates with the number of injections and treatment risk stratification but provides more information regarding methotrexate clearance and toxicity. Despite these limitations, this study demonstrated sex-based differences in brain activation patterns associated with working memory in a large cohort of long-term ALL survivors. These findings suggest that neural systems involved in working memory are detrimentally affected by contemporary chemotherapy protocols for pediatric ALL, and these effects may be exacerbated by female sex. Considering evidence indicating that sex hormones play varying roles in protecting the central nervous system from chemotherapy-related insult (41-44), future research investigations may benefit from including longitudinal assessments of sex hormones before, during, and following chemotherapy to determine the extent of their involvement in facilitating or reducing treatment-induced neurotoxicity.

Inclusion of structural and functional neuroimaging investigations (ie, task-based and resting-state fMRI, diffusion tensor imaging, etc) in long-term cancer survivors is essential to advance our understanding of how chemotherapy and other treatment factors can affect brain connectivity, network efficiency, and white matter integrity. Identification of risk factors associated with altered brain function and neurocognitive deficits is important to inform the development of interventions for current childhood cancer survivors and to improve treatment strategies for those who are newly diagnosed.

## Funding

This work was supported by the National Cancer Institute at the National Institutes of Health T32 Institutional Research Training Grant (T32 CA225590 to KRK), National Institute of Mental Health (MH085849 to KRK), National Cancer Institute (CA195547 to MMH and LLR; CA021765), and by the American Lebanese Syrian Associated Charities (ALSAC).

## Notes

**Role of the funder:** The funding source did not have a role in the study design, the collection, analysis, and interpretation of the data, the preparation and writing of the manuscript, or the decision to submit the manuscript for publication.

**Disclosures:** The authors report no conflict of interest or disclosures.

**Author contributions:** Conceptualization, data curation, formal analysis, visualization, and writing original draft: KG, KRK; Methodology: KG, MAS, SF, KRK; Writing, review, and editing: KG, MAS, NP, EvdP, SF, LMJ, CP, MMH, WER, RS, KRK; Supervision and funding acquisition: KRK.

**Acknowledgements:** We thank the research participants for their involvement in the study.

## Data Availability

The data supporting this article will be shared upon reasonable request to the corresponding author.

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