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ORIGINAL REPORT

Reduced Neuroanatomic Volumes in Long-Term Survivors of Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose
To compare regional brain volumes in adult long-term survivors of childhood acute lymphoblastic leukemia (ALL) and healthy controls.

Patients and Methods
We investigated 130 survivors of childhood ALL diagnosed between 1970 and 2002 with magnetic resonance imaging (MRI) and neuropsychological testing at a median of 22.5 years after diagnosis. Morphometric analyses including whole-brain segmentation were performed using a validated automated procedure; 130 healthy adults served as controls.

Results
Compared with healthy controls, ALL survivors showed significantly smaller volumes of cortical gray matter, cerebral white matter, amygdala, caudate, hippocampus, thalamus, and estimated intracranial volume. Effect sizes ranged from small to medium. The strongest effect was found for the caudate, which on average was 5.2% smaller in ALL survivors. Caudate volumes were also smaller when controlling for intracranial volume, suggesting a specific effect. Neither age at diagnosis nor treatment variables such as radiation therapy or drug dose had a major impact on neuroanatomic volumes. Neuropsychological assessment revealed reduced processing speed, executive function, and verbal learning/memory in survivors compared with controls but no difference in estimated general intellectual ability. In ALL survivors, but not in controls, neuropsychological test results correlated with volumes of cortical gray matter, caudate, and thalamus as well as intracranial volume.

Conclusion
Structural MRI of long-term survivors of childhood ALL demonstrated smaller volumes of multiple brain structures compared with healthy controls. Because of possible selection biases, these results must be interpreted with caution. Future studies are required to clarify the significance of these findings and the neurobiologic mechanisms involved.

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INTRODUCTION

Survival rates of childhood acute lymphoblastic leukemia (ALL) have improved from close to zero before 1970 to approximately 90% today.[1,2] Consequently, there is a growing population of long-term survivors. Adequate CNS-directed therapy is imperative for continuous remission after ALL treatment. CNS irradiation, although effective and widely used previously, has largely been abandoned because of the risk of severe late effects.[3-5] However, even chemotherapy alone has been shown to have negative long-term effects on neurocognitive functions,[6-8] with subtle effects on attention, executive functioning, visual processing, and visual-motor functioning.[9] Possible mechanisms include direct neurotoxic effects leading to atrophy of gray matter (GM) and/or demyelination of white matter (WM) fibers, secondary immunologic responses causing inflammatory reactions, and microvascular injury.[10-13] Individual differences in vulnerability seem to exist, leading to investigation of genetic and other risk factors.[14]

The typical age peak of childhood ALL is between 1 and 6 years. These children are thus exposed to potentially toxic regimens for a prolonged time in a period of rapid brain maturation and cognitive development.[15-17] Neuroimaging may enhance our understanding of the anatomic substrates of potential toxic treatment effects. Previous studies using

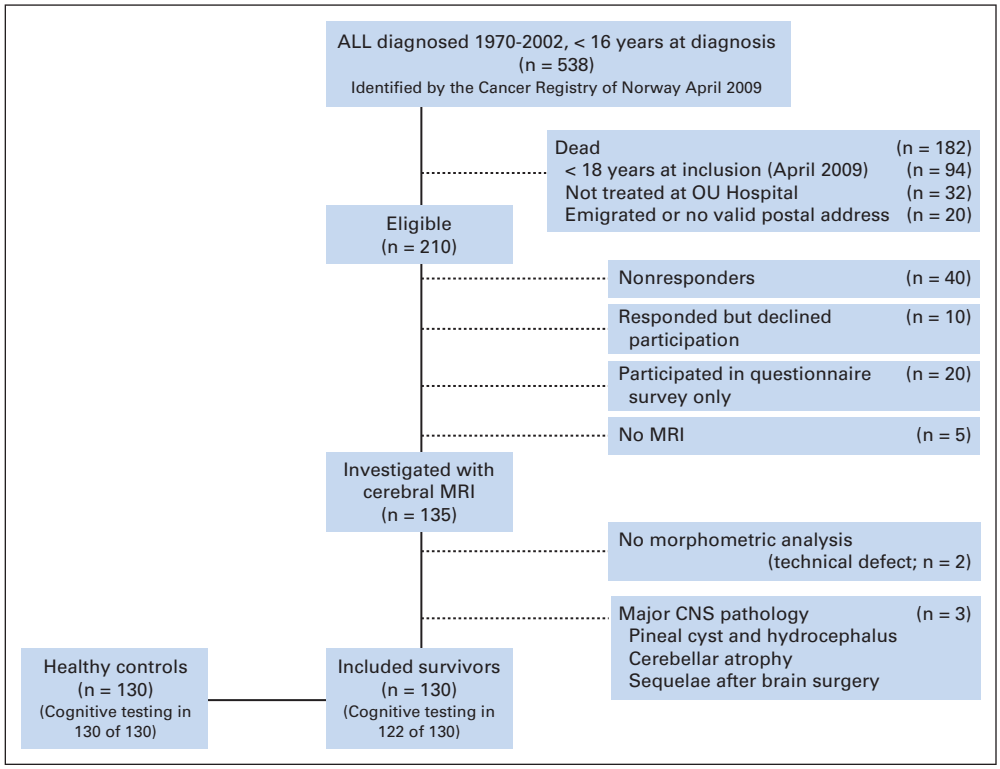

Fig 1. Flow diagram of survivors and controls. Survivor eligibility criteria: treated at University of Oslo (OU) Hospital, alive ≥ 5 after therapy completion, and age at investigation ≥ 18 years. Control eligibility criteria: normal or corrected to normal vision and hearing, no history of injury or disease known to affect CNS function, not receiving psychiatric treatment, not using drugs known to affect CNS function, free of significant CNS pathology on magnetic resonance imaging (MRI); majority of controls were recruited from newspapers advertisements; some were students or employees at OU, and some were from local schools. ALL, acute lymphoblastic leukemia.

computed tomography (CT) or conventional magnetic resonance imaging (MRI) have shown inconsistent results.[5,18-20] More-recent research using single photon emission CT,[21] functional MRI,[22] and diffusion tensor imaging[23-25] has provided new insights into possible underlying pathophysiologic mechanisms. Morphometric studies, measuring neuroanatomic volumes, have found smaller WM volumes in ALL survivors compared with controls, possibly related to altered myelination.[26-29]

Using automated whole-brain segmentation, it is possible to explore morphometric characteristics of a large number of neuroanatomic structures. The primary aim of our study was to gain insight into the neurodevelopmental consequences of ALL treatment by comparing a range of cortical and subcortical volumes between a large cohort of survivors and healthy individuals without history of cancer. A secondary aim was to explore the impact of disease and treatment on brain volumes. On the basis of the assumed interfering role of ALL treatment on neurodevelopmental processes as well as previous studies, we hypothesized that ALL survivors would have significantly smaller WM volume, and possibly also cortical and subcortical volumes, compared with healthy controls.

PATIENTS AND METHODS

Sample

The study was approved by the regional committee for medical and health research ethics. Of 538 patients diagnosed with ALL at Oslo University Hospital (covering half of the Norwegian population) from 1970 to 2002 and age < 16 years, 210 were eligible for the study (Fig 1). Additional eligibility criteria are listed in Figure 1. We used cerebral MRI to investigate 135 survivors. Five were excluded from morphometric analyses because of major CNS pathology or lost data. Thus, the final sample included 130 patients.

We drew 130 healthy controls matched as closely as possible for sex and age from two ongoing projects at the Center for the Study of Human Cognition, University of Oslo.[30-32] A majority of controls were recruited through newspaper advertisements, some were students or employees at the University of Oslo, and some were from local schools. Written informed consent was obtained from all participants. Standardized health screening interviews were conducted, and controls were required to be right handed and fluent Norwegian speakers. Additional eligibility criteria are listed in Figure 1.

Neuropsychological Testing

All healthy controls and 122 of the ALL survivors underwent neuropsychological testing. In addition to an estimate of intelligence quotient (IQ) from the Wechsler Abbreviated Scale of Intelligence,[33] three domains of neurocognitive function were assessed: processing speed, which included the color-naming and word-reading conditions of the Color-Word Interference Test[34]; executive function, including the inhibition and inhibition/shifting conditions of the Color-Word Interference Test; and verbal learning/memory, including the total learning and delayed recall scores of the California Verbal Learning Test.[35] Raw test scores were standardized and averaged within each domain, and the processing speed and executive function measures were reversed so that higher scores reflected better performance. Additionally, an executive function measure residualized for processing speed was calculated. Sample characteristics and test results for survivors and controls are listed in Table 1.

Disease and Treatment Characteristics in ALL Survivors

Information on cancer disease and treatment was obtained by review of patients' records. ALL treatment intensity developed substantially during the period from 1970 to 2002.[36] Throughout all treatment periods, prevention of CNS leukemia included repeated intrathecal injections of methotrexate. Only a few patients in this sample received cranial irradiation, because prophylactic radiation therapy in the Nordic countries was replaced with intravenous methotrexate combined with intrathecal therapy early in the inclusion period.[36] Because no uniform protocols were used before 1992, it is difficult to grade treatment intensity according to protocols. Instead, we chose to estimate patients' therapy load by calculating cumulative doses of

Table 1. Demographics and Neuropsychological Assessments in ALL Survivors and Healthy Controls

Characteristic	Survivors (n = 130)	Controls (n = 130)	*P*
Demographic*			
Age at MRI scan, years			.366
Median	28.4	26.0	
Range	18.6-46.5	16.9-48.5	
Female sex			.902
No.	65	66	
%	50	51	
Height, cm			.374
Mean	173.8	174.9	
SD	10.5	8.7	
Education, years			.077
Median	12.0	15.0	
Range	10-18	10-18	
Daily cigarette smoking			**.035**
No.	11	27	
%	10.9	21.4	
Alcohol, units per week			.142
Median	2.5	3	
Range	0-25	0-20	
Neuropsychological testing†			
Estimated IQ			.083
Mean	114.0	111.9	
SD	9.41	9.56	
Processing speed, Z score			**< .001**
Median	−0.05	0.36	
Range	−5.39-1.65	−1.63-1.95	
Executive function, Z score			**< .001**
Median	−0.29	0.56	
Range	−4.64-1.33	−2.74-2.02	
Executive function/processing speed, Z score‡			**< .001**
Median	−0.23	0.41	
Range	−4.03-2.37	−3.82-1.73	
Verbal learning/memory, Z score			**.006**
Median	0.11	0.35	
Range	−2.89-1.27	−2.97-1.57	

NOTE. Bold font indicates significance ($P < .05$).
Abbreviations: ALL, acute lymphoblastic leukemia; IQ, intelligence quotient; MRI, magnetic resonance imaging; SD, standard deviation.
*Information missing for height (15 controls), education (one control), smoking (29 controls; four survivors), and alcohol (27 controls; 11 survivors).
†Not available for estimated IQ (eight survivors), speed/executive function (nine survivors; seven controls), and verbal learning/memory (eight survivors; 25 controls).
‡Executive function residualized for processing speed.

the most-important drugs and assessing the number of intrathecal methotrexate injections (Table 2).

MRI Acquisition and Analysis

Imaging data were collected using a 12-channel head coil on a 1.5 T Siemens Avanto scanner (Siemens Medical Solutions, Erlangen, Germany). The same scanner and sequences were used for patients and controls. The pulse sequences used for morphometry were two repeated 160-slice sagittal T1-weighted magnetization-prepared rapid gradient echo sequences (repetition time [TR], echo time [TE], time to inversion, and flip angel were 2400 ms, 3.61 ms, 1,000ms, and 8°, respectively). The two runs were averaged to increase the signal-to-noise ratio. The protocol also included a 176-slice sagittal three-dimensional T2-weighted turbo-spin echo sequence (TR and TE were 3,390 and 388 ms, respectively) and a 25-slice coronal fluid-attenuated inversion recovery sequence (TR and TE were 7,000 to 9,000 and 109 ms, respectively) to aid the neuroradiologic examination.

Image processing and analyses were performed with FreeSurfer 5.1 (http://surfer.nmr.mgh.harvard.edu/). The procedure for automated brain segmentation has been described in detail elsewhere[37,38] and yields volumetric estimates for total cerebral and cerebellar GM and WM, subcortical structures, corpus callosum, and ventricular regions. Additionally, the cortical GM[39] and gyral cerebral WM[31] were automatically segmented, and lobar volumes were calculated. In most cases, left- and right-hemisphere regions are measured separately; in our study, mean values across the hemispheres were used in all analyses to reduce the number of comparisons. All volumes were inspected for accuracy, and minor manual edits were performed for most participants. Intracranial volume (ICV) was estimated using an atlas normalization procedure,[40] and ICV-corrected residuals for the other volumes were calculated and used in follow-up statistical analyses. Coronal slices illustrating segmentation in a representative patient are shown in Figure 2.

Statistical Analyses

Analyses were performed using PASW Statistics version 18.0 (SPSS, Chicago, IL). Descriptive statistics of groups (survivors *v* controls) included *t* tests for continuous data and χ^2 tests for categorical data. Nonparametric tests were used where appropriate. To test the effect of group (survivors *v* controls) on neuroanatomic volumes, one-way between-group analyses of covariance (ANCOVAs) were performed for the whole sample, with raw volumes as dependent variables, group as a fixed factor, and age and sex as covariates. Second, the same analyses were repeated with height and length of education as additional covariates to control for the effect of body size and education, respectively. Third, to correct for individual differences in brain size, we performed ANCOVAs on residual volumes after correcting for ICV, with age and sex included as covariates. For some of the measured volumes, ANCOVAs were performed after log transformation because of non-normal distributions. In ALL survivors, Spearman's rank order correlations controlling for age and sex were performed to explore the relationships between age at diagnosis and treatment characteristics and neuroanatomic volumes and the relationships between neuropsychological test results and brain volumes. Uncorrected *P* values are reported throughout the manuscript, and Bonferroni corrections for multiple comparisons are also included where appropriate.

RESULTS

Demographics and Clinical Characteristics

No significant group differences between ALL survivors and healthy controls were found for age, sex, height, alcohol use, or estimated IQ (Table 1). The control group had a higher fraction of smokers ($P = .035$) and a trend toward longer education ($P = .077$). Because of different inclusion criteria, all controls were right handed, whereas the survivor group included 9% left handers. This was considered acceptable in the current study because mean values for the two hemispheres were used in all analyses.

Disease and treatment characteristics of the ALL survivors are summarized in Table 2. Median follow-up time (time from diagnosis to MRI) was 22.5 years. Despite this long observation time, survivors were relatively young at investigation (median age, 28.4 years). This is explained by the young age at diagnosis (median, 5.3 years), which is typical for childhood ALL. Eighteen patients had received cranial radiation therapy.

Neuroanatomic Volumes in ALL Survivors Compared With Healthy Controls

ALL survivors showed significantly smaller cortical GM, cerebral WM, amygdala, caudate, hippocampus, and thalamus volumes compared with healthy controls (Table 3; Fig 3). The effect sizes ranged

Table 2. Clinical Characteristics and Treatment During Three Time Periods in ALL Survivors

Characteristic	Total (N = 130)	1970 to 1981 (n = 33)	1982 to 1991 (n = 55)	1992 to 2002 (n = 42)
Clinical				
Age at diagnosis, years				
Median	5.3	4.1	4.2	7.4
Range	0.3-15.9	0.3-12.2	0.6-14.5	0.8-15.9
Follow-up, years				
Median	22.5	32.1	23.6	14.7
Range	7.4-40.0	28.9-40.0	18.6-29.1	7.4-19.1
WBC at diagnosis*				
Median	10.3	6.6	12.0	10.3
Range	0.5-409.0	2.0-229.0	0.8-409.0	0.5-255.0
Relapsed disease				
No.	19	6	10	3
%	14.6	18.2	18.2	7.1
Treatment burden				
Cerebral irradiation				
No.	18	7	7	4
%	13.8	21.2	12.7	9.5
Dose, Gy				
Median	20.0	20.0	19.8	18.0
Range	12.0-25.0	20.0-24.0	18.0-25.0	12.0-18.0
Stem-cell transplantation				
No.	3	0	2	1
%	2.3	0	3.6	2.4
Chemotherapy, mg†				
Vincristine				
Median	22.0	34.0	22.0	28.0
Range	8.0-102.0	10.0-72.0	12.0-58.0	8.0-102.0
Methotrexate IV				
Median	21,000	1,500	21,000	40,000
Range	0-75,000	0-39,000	1,500-64,000	0-75,000
No. of intrathecal injections				
Median	13.0	8.0	13.0	14.0
Range	0.0-36.0	0.0-26.0	8.0-33.0	4.0-36.0
Antracyclines				
Median	120.0	0.0	120.0	240.0
Range	0.0-510.0	0.0-240.0	0.0-510.0	40.0-510.0
Oral steroids‡				
Median	4,380	6,102	4,380	5,520
Range	1,860-14,550	1,860-17,250	2,280-9,780	2,280-14,890

Abbreviations: ALL, acute lymphoblastic leukemia; IV, intravenous.
*Not available in 15 patients.
†Cumulative dose per m^2 body surface; not available in two patients.
‡Prednisolone plus dexamethasone; prednisolone equivalents: 1 mg prednisolone = dexamethasone dose (mg) × 6.5.

from small to medium, as indicated by the partial η^2 values (.019 to .053). The most pronounced effect was seen in caudate, which on average was 5.2% smaller in ALL survivors. Note also that ICV was significantly smaller in ALL survivors. Using Bonferroni correction for multiple testing, only total cortical GM and caudate volumes remained significantly smaller in ALL survivors. No significant volume difference was found for cerebellar GM, cerebellar WM, brainstem, accumbens, corpus callosum, pallidum, putamen, or ventricles. Separate analyses for lobar GM and WM volumes revealed significantly smaller volumes in survivors in all regions except occipital WM (Appendix Table A1, online only). Excluding the 18 ALL survivors with a history of previous CNS radiation therapy did not change these results, with the same structures significantly different between survivors and controls. Also, including body height or length of education as a covariate did not alter the results. We also found similar volume differences, with the same structures significantly different when comparing selected subgroups of the ALL survivors (n = 108) and controls (n = 108) with identical lengths of education (median, 15.0 years; range, 10 to 18 years). Finally, to correct for individual differences in total brain size, we performed ANCOVAs on residual volumes after correcting for ICV, with age and sex included as covariates. ALL survivors still showed significantly smaller caudate volume (Z-score difference, 0.0334; $P = .007$; $F = 7.342$; $p\eta^2 = .028$).

Impact of Age at Diagnosis and Therapy on Neuroanatomic Volumes in ALL Survivors

Spearman's correlations, controlling for age and sex, showed no significant associations between age at diagnosis of ALL and neuroanatomic volume. Furthermore, we found no significant correlations between treatment variables and those structures shown to

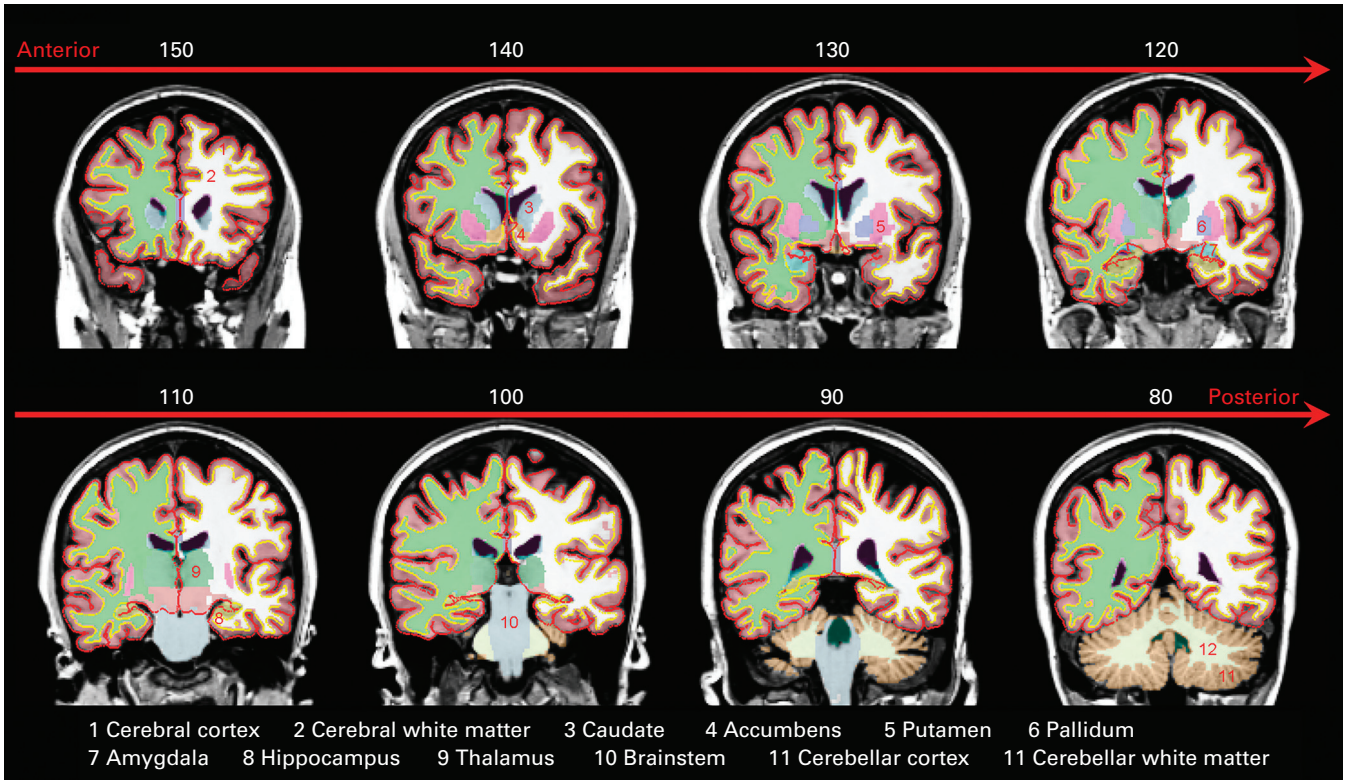

Fig 2. Automated whole-brain segmentation. Different brain volumes are shown in different colors for representative childhood acute lymphoblastic leukemia survivor in coronal views in radiologic convention. Corpus callosum and ventricles are not labeled in these slices.

significantly differ between survivors and controls (ie, ICV, cortical GM, cerebral WM, amygdala, caudate, hippocampus, and thalamus). A few other structures showed negative correlations with treatment variables (accumbens area [$r_s = -.19$; $P = .028$] and corpus callosum [$r_s = -.18$; $P = .040$] with radiation therapy; pallidum [$r_s = -.21$; $P = .016$] with total antracycline dose), whereas ventricle volumes were positively correlated with radiation therapy (lateral ventricles [$r_s = .20$; $P = .023$]; third and fourth ventricles [$r_s = .21$; $P = .017$]).

Table 3. Neuroanatomic Volume in ALL Survivors and Controls

Structure	Controls (n = 130)		ALL Survivors (n = 130)		ANCOVA			Volume Difference (%)
	Mean (μL)	SD	Mean (μL)	SD	F	*P*	Partial η^2	
Intracranial volume	1,642,919	165,256	1,599,426	148,801	8.084	**.005**	0.031	−2.6
Cortical GM	249,099	24,515	241,529	23,428	11.511	**.001**	0.043	−3.0
Cerebral WM	268,655	31,847	260,952	27,904	7.134	**.008**	0.027	−2.9
Cerebellar GM	56,793	5,901	57,077	6,181	0.288	.592	0.001	0.5
Cerebellar WM	14,420	1,544	14,495	1,705	0.106	.745	0.000	0.3
Accumbens	698	116	679	99	2.185	.141	0.008	−2.3
Amygdala	1,680	219	1,614	204	8.617	**.004**	0.033	−3.9
Brainstem	21,796	2,185	21,862	2,322	0.028	.866	0.000	0.3
Caudate*	3,758	444	3,563	446	14.325	**< .001**	0.053	−5.2
Corpus callosum	3,287	477	3,225	441	1.432	.233	0.006	−1.9
Hippocampus	4,380	396	4,255	418	7.362	**.007**	0.028	−2.9
Pallidum	1,726	182	1,705	181	1.066	.303	0.004	−1.2
Putamen	5,736	629	5,651	646	1.248	.265	0.005	−1.5
Thalamus	7,278	591	7,124	666	4.900	**.028**	0.019	−2.1
Lateral ventricle*	7,530	3,642	7,603	4,115	0.002	.966	0.000	1.0
Third and fourth ventricles*	2,646	608	2,710	705	0.469	.494	0.002	2.4

NOTE. Group differences were tested with ANCOVAs, with age and sex included as covariates. Bold font indicates significance ($P < .05$).
Abbreviations: ALL, acute lymphoblastic leukemia; ANCOVA, analysis of covariance; GM, gray matter; SD, standard deviation; WM, white matter.
*After log transformation because of non-normal distribution.

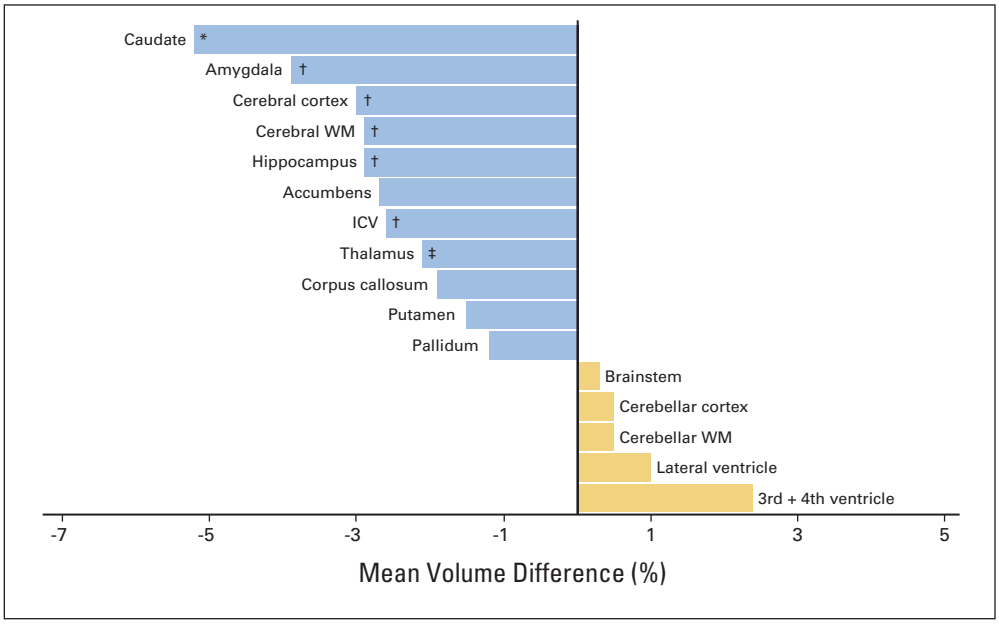

Fig 3. Mean differences in neuroanatomic volumes between acute lymphoblastic leukemia (ALL) survivors (n = 130) and controls (n = 130). Negative differences indicate smaller volumes in ALL survivors compared with controls. ICV, intracranial volume; WM, white matter. (*) $P < .001$. (†) $P < .01$. (‡) $P < .05$.

When corrected for multiple comparisons (Bonferroni), none of these correlations remained significant (Appendix Table A2, online only).

Neuroanatomic Volumes and Results of Neuropsychological Testing

Spearman's correlations, controlling for age and sex, were performed between neurocognitive scores and those neuroanatomic volumes shown to differ between ALL survivors and controls. In ALL survivors, processing speed was correlated with cortical GM ($r_s = .18$; $P = .048$), caudate ($r_s = .18$; $P = .047$), and thalamus ($r_s = .23$; $P = .012$). Executive function was correlated with ICV ($r_s = .20$; $P = .033$), cortical GM ($r_s = .22$; $P = .016$), caudate ($r_s = .21$; $P = .020$), and thalamus ($r_s = .18$; $P = .047$), but no correlations where seen for the executive function measure residualized for processing speed. In all cases, better performance was associated with larger volumes. None of these correlations remained significant when corrected for multiple comparisons, and no correlations were demonstrated among controls.

DISCUSSION

The results of this cross-sectional study with the largest number of participants to date indicate that a number of neuroanatomic structures, including total cortical GM, total cerebral WM, amygdala, caudate, hippocampus, and thalamus, are smaller in adult survivors of childhood ALL compared with healthy controls. Our findings of reduced ICV in ALL survivors indicate general rather than regionally specific neurodevelopmental effects of ALL treatment. Importantly, these volume differences, despite being relatively small, were observable decades after therapy and in mostly well-functioning individuals with estimated IQs not significantly different from the controls.

Our study confirmed the results from several previous studies reporting smaller WM volumes in ALL survivors[26-29] but also found smaller cortical GM volumes in survivors. The largest study by Reddick et al,[29] involving 112 child and adolescent survivors and 33 healthy siblings, documented WM volume loss, which was exacerbated in irradiated patients and associated with impaired neurocognitive performance. Kesler et al[28] found no difference in total brain volume but significantly reduced global and regional WM volumes in ALL survivors compared with controls. Another study reported smaller frontal WM volumes in irradiated survivors, and diffusion tensor imaging indicated reduced WM integrity in survivors, the effect being most pronounced after combined treatment with irradiation and chemotherapy.[27] Finally, Carey et al[26] described two specific regions of reduced WM in the right frontal lobe in ALL survivors compared with healthy controls.

In previous studies of survivors, there was little focus on subcortical structures. It is known that the basal ganglia during childhood have high metabolic demands and thus may be particularly vulnerable.[41] Porto et al[27] reported reduced GM concentration within the caudate and thalamus in irradiated survivors of childhood ALL. In accordance with these results, we found smaller volumes of caudate and thalamus in survivors compared with controls. The largest group difference was found in the caudate, which was a specific effect, persisting when correcting for ICV. The significance of this finding is uncertain, because studies supporting a particular vulnerability of the caudate to cancer therapy are lacking. The caudate, as part of the striatum, is a part of the frontostriatal circuit supporting executive functions,[42] and caudate lesions have been shown to lead to impaired planning and problem solving, attention, learning, memory, and verbal fluency.[43-45] Remarkably, several of these functions have also been described as common neurocognitive late effects in survivors of childhood cancer.[9] Additionally, we also found reduced volumes of hippocampus and amygdala, which, however, did not persist when corrected for ICV. The hippocampus is thought to play a crucial role in postnatal neurogenesis, and hippocampal damage induced by irradiation or chemotherapy has been proposed to play a role in neurocognitive impairment among cancer survivors.[46]

Young age at diagnosis is a risk factor for neurocognitive sequelae in patients undergoing CNS irradiation.[6,47] An MRI study of children

before, during, and after therapy for ALL showed that those with WM changes were significantly younger than those without changes.[19] In the present study, we found no association between age at diagnosis and neuroanatomic volume. One reason may be that the effects of age at diagnosis and therapy intensity counteract each other; the youngest children carry the best prognosis and thus typically require less-intensive therapy.

It has been shown that survivors of childhood ALL are at risk of decreased adult height.[48] Height is known to be associated with brain volume.[49] However, in our study, the height of survivors and controls did not differ significantly, and the group differences in brain volumes were not explained by height.

Our study failed to identify unique etiologic factors with a high impact on neuroanatomic volume in ALL survivors. Reasons for this could be the significant heterogeneity of the survivor group, the long time span covered, and the long follow-up interval, which may have given rise to unknown confounders unrelated to disease or treatment. CNS irradiation is a major risk factor for neurocognitive late effects in survivors of childhood ALL.[3,47] Our results showed no major impact of radiation therapy. However, the fraction of irradiated survivors was small, and the radiation doses applied were relatively low. If we are unable to identify a single drug or treatment variable as the main causative agent, which alternative explanations may exist? More-general factors such as generally reduced health during a prolonged period resulting from malnutrition and recurrent infections, a state of long-lasting stress,[50] repeated general anesthesias,[51] and effects resulting from deprivation[52] might be considered. According to the accelerated-aging hypothesis, cancer and cancer therapy may accelerate the trajectory of cognitive dysfunction and GM decrease associated with the process of aging.[13] This may be of particular significance in the long-term survivors included in our study.

ALL survivors in this sample had education levels similar to those of the general population and had estimated IQs not significantly different from those of controls—in fact, in the high average range. Nevertheless, their processing speed, executive function, and verbal learning/memory were inferior compared with controls, in accordance with previous literature.[6,53] Previous studies have reported an association between WM integrity and cognition in childhood cancer survivors.[24,25,29] In our study, we found associations between reduced volumes of cortical GM, caudate, thalamus, and ICV and impaired neuropsychological performance in survivors. Similar findings were reported recently in long-term survivors of childhood Hodgkin lymphoma, in whom attention deficits were related to reduced cortical thicknesses.[54] Thus, it is likely that the smaller neuroanatomic volumes of the patients account for some of the specific cognitive reductions compared with the controls, even though they still function within the normal range.

This study is limited by its cross-sectional design and possible bias resulting from the procedure used to recruit healthy controls. Survivors and controls showed no significant differences with regard to age, sex, height, or alcohol use, but controls had a lower fraction of cigarette smokers and a trend toward longer education. Furthermore, both groups showed relatively high general cognitive function and may not be seen as representative of the full range of individual differences. Even if survivors and controls did not differ with regard to estimated IQ, and controlling for length of education did not change the results, it cannot with certainty be ruled out that the differences in brain volume and neurocognitive performance at least in part may have been the result of a high-functioning control group rather than an impaired ALL group. Patients were diagnosed during a period of more than 30 years, and therefore, management was far from uniform. The long follow-up time is regarded as a strength of the study, but it also allows for accumulated effects of confounders not related to the previous leukemia.

In conclusion, this study shows that long-term ALL survivors had significantly smaller volumes of a number of brain structures compared with healthy controls, and neuropsychological performance was correlated with volumes of cortical GM, caudate, thalamus, and ICV. Given the possible limitations we have mentioned, future studies are needed to confirm and clarify the significance of these findings and the neurobiologic mechanisms involved.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Bernward Zeller, Paulina Due-Tønnessen, Ellen Ruud
Administrative support: Ellen Ruud
Provision of study materials or patients: Adriani Kanellopoulos, Kristine B. Walhovd
Collection and assembly of data: All authors
Data analysis and interpretation: Bernward Zeller, Christian K. Tamnes, Adriani Kanellopoulos, Inge K. Amlien, Stein Andersson, Anders M. Fjell, Kristine B. Walhovd, Ellen Ruud
Manuscript writing: All authors
Final approval of manuscript: All authors

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Appendix

Table A1. Lobar Volumes in ALL Survivors and Controls Group

Structure	Controls (n = 130)		ALL Survivors (n = 130)		ANCOVA			Volume Difference (%)
	Mean (μL)	SD	Mean (μL)	SD	F	*P*	Partial η^2	
Frontal GM	95,991	10,220	93,230	9,947	8.147	**.005**	0.031	−2.9
Parietal GM	66,241	6,615	64,262	6,671	8.597	**.004**	0.032	−3.0
Temporal GM	59,474	6,515	57,799	5,745	8.168	**.005**	0.031	−2.8
Occipital GM	24,222	2,723	23,383	2,651	8.592	**.004**	0.032	−3.5
Insular GM	7,351	905	7,118	775	7.678	**.006**	0.029	−3.2
Frontal WM	85,821	9,834	83,517	8,661	6.264	**.013**	0.024	−2.7
Parietal WM	62,987	7,425	61,050	6,354	8.296	**.004**	0.031	−3.1
Temporal WM	36,528	4,585	35,376	3,689	7.918	**.005**	0.030	−3.2
Occipital WM	22,839	2,653	22,398	2,883	2.600	.108	0.010	−1.9
Insular WM	9,686	1,173	9,375	843	7.973	**.005**	0.030	−3.2

NOTE. Differences were tested with ANCOVAs, with age and sex included as covariates. Bold font indicates significance ($P < .05$).
Abbreviations: ALL, acute lymphoblastic leukemia; ANCOVA, analysis of covariance; GM, gray matter; SD, standard deviation; WM, white matter.

Table A2. Associations Between Treatment Variables and Neuroanatomic Volume in ALL Survivors (n = 130)

Structure	Spearman Correlation*					
	MTX					
	IV†	Intrathecal§	Steroids†	Antracyclines†	Vincristine†	Irradiation‡
Intracranial volume	*0.010*	*0.052*	*0.007*	*−0.082*	*0.018*	*−0.056*
Cortical GM	*−0.017*	*0.041*	*0.029*	*−0.094*	*0.058*	*−0.007*
Cerebral WM	*0.024*	*0.001*	*−0.077*	*−0.124*	*0.017*	*−0.123*
Cerebellar GM	0.126	0.115	−0.030	0.022	−0.018	−0.169
Cerebellar WM	0.020	0.080	0.029	−0.022	0.129	0.026
Accumbens	−0.078	−0.065	−0.078	−0.082	0.059	**−0.194**
Amygdala	*0.041*	*0.068*	*−0.028*	*−0.020*	*−0.084*	*−0.141*
Brainstem	0.001	0.106	0.051	−0.021	0.169	−0.033
Caudate	*−0.087*	*−0.045*	*0.093*	*−0.105*	*0.105*	*0.045*
Corpus callosum	−0.043	−0.070	−0.136	−0.100	−0.072	**−0.180**
Hippocampus	*0.062*	*0.146*	*0.021*	*−0.042*	*0.031*	*−0.019*
Pallidum	−0.112	−0.114	−0.045	**−0.214**	0.043	−0.059
Putamen	−0.076	−0.049	0.096	−0.096	0.092	−0.033
Thalamus	*0.009*	*0.025*	*0.038*	*−0.078*	*0.084*	*−0.152*
Lateral ventricle	0.047	0.041	0.071	−0.022	−0.021	**0.202**
Third and fourth ventricles	−0.037	0.071	0.111	0.019	0.074	**0.211**

NOTE. Italics indicate structures significantly different between survivors and controls. Bold font indicates significance ($P < .05$). None of the correlations remained significant after Bonferroni correction for multiple testing ($P < .003$).
Abbreviations: ALL, acute lymphoblastic leukemia; GM, gray matter; IV, intravenous; MTX, methotrexate; WM, white matter.
*Controlling for age and sex.
†Cumulative doses per m^2.
‡Received radiation therapy: yes or no.
§No. of intrathecal injections.