Evidence for Cerebellar-Frontal Subsystem Changes in Children Treated With Intrathecal Chemotherapy for Leukemia

Enhanced Data Analysis Using an Effect Size Model

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Background: Following brain insult in early childhood, the later maturing neocerebellum and frontal lobes frequently show abnormalities.

Objective: To investigate the morphologic characteristics and function of a proposed cerebellar-frontal subsystem in children treated for acute lymphoblastic leukemia (ALL) with intrathecal methotrexate using quantitative magnetic resonance imaging, neuropsychological measures, nonlinear multiple regression analysis, and a statistical effect size model that augments interpretive validity of nonsignificant statistical findings, particularly from small sample size studies.

Design: Comparison and relationship of magnetic resonance imaging morphometry of cerebellar lobuli I-V and VI-VII and prefrontal cortices, and performance on 5 neuropsychological tests assessing visual-spatial attention, short-term memory, and visuomotor organization and coordination between childhood survivors of ALL and a matched control group.

Participants: Ten childhood survivors of ALL treated between 1982 and 1989 with standard 3-year intrathecal chemotherapy, and matched control subjects.

Main Outcome Measures: Morphometric results of cerebellar lobuli I-V and VI-VII and prefrontal cortices, and results of Trail-Making Tests, Rey-Osterreith Complex Figure Test, WISC-III Coding.

Results: Significant effect size model values for outcome measures in the ALL group support deficits in lobuli VI-VII and prefrontal cortices, and neuropsychological performance. Multiple regression analysis results were consistent with hypothesized involvement of a cerebellar-frontal brain subsystem.

Conclusion: Treatment of children with ALL with intrathecal methotrexate before 5 years of age has structural and functional effects on the developing neocerebellar-frontal subsystem.

Arch Neurol. 1998;55:1561-1568

NITRATHCAL methotrexate impairs proliferation of leptomeningeal leukemic cells, and is a major component of preventive central nervous system (CNS) treatment for childhood acute lymphoblastic leukemia (ALL).\(^1\) Reported neuroanatomical changes following methotrexate treatment include cerebral calcifications and atrophy,\(^2,3\) and children treated with both cranial irradiation (240 Gy) and methotrexate chemotherapy before the age of 5 years have shown decreases in measures of cerebellar morphology.\(^4,5\)

CEREBELLAR-FRONTAL SUBSYSTEM VULNERABILITY TO EARLY DAMAGE

Consistently, results from neuropsychological studies on clinical populations have shown the cerebellum and prefrontal lobes related to deficits in attention, spatial working memory, and planning.\(^6,8\) Attention and short-term memory deficits, particularly in the visual-spatial-motor domain, have previously been reported in survivors of ALL.\(^3,5,6,11\)

The present study investigated the morphometry of the cerebellar vermis in children who were treated before the age of 5 years with intrathecal chemotherapy, but with no cranial irradiation. We hypothesized that if cerebellar changes as a consequence of exposure to intrathecal methotrexate were found, they would follow our developmental chronometry hypothesis,\(^3\) which predicts that the later developing neocortical posterior vermis (lobuli VI-VII) would be more affected than the earlier maturing anterior part (lobuli 1-V). Further, it was hypothesized that the relatively later developing cerebellar lobuli VI-VII and prefrontal cortices are components of the brain.
PARTICIPANTS AND METHODS

PARTICIPANTS

Written informed consent was obtained from all children and parents before testing, and all children and parents participated in a clinical interview related to prenatal, perinatal, and early postnatal periods of development. Ten survivors of childhood ALL (4 boys and 6 girls; age range, 6 years 9 months to 13 years 5 months) were recruited from The University of New Mexico Pediatric Oncology Program, Albuquerque. The present study reflects all available children who underwent a homogeneous 3-year protocol for CNS prophylaxis between 1982 and 1989 and were classified as low-risk, non-T, early B cases. Treatment for ALL, over a 3-year period included: CNS prophylaxis, induction, consolidation, and maintenance. All children received cytosine arabinoside, hydrocortisone, and methotrexate intrathecally for CNS prophylaxis (with no irradiation). Selection criteria for the present study included the following: 1 to 5 years of age at diagnosis, 3 years or more in continuous remission after discontinuation of ALL treatment, no premorbid sensory or motor disabilities, no developmental psychiatric or metabolic disorders or focal brain lesions, and no family history of alcoholism or other drug abuse.

Ten healthy controls (4 boys and 6 girls; age range, 6.0 years to 13.0 years) were selected to match the ALL group in age, sex, and socioeconomic status. In several cases, the relatives of ALL subjects were available as control subjects. The selection criteria described above, except for details about the ALL treatment, also applied to the control group. The present report used data from those children who successfully completed neuropsychological testing and magnetic resonance imaging (MRI) of the brain, with images clearly readable for quantitative morphometry.

MAGNETIC RESONANCE IMAGING

Scanning

Magnetic resonance imaging scans of the head were performed with a Siemens 1.5-T imaging system in the New Mexico Veterans Affairs Imaging Center, Albuquerque. Acquired were axial spin echo images using repetition time (TR) of 2500 milliseconds, echo time (TE) of 22 and 90 milliseconds, and 5-mm slice thickness with 0.4-mm interslice gap and sagittal T1-weighed sequence (TR, 40 milliseconds; TE, 6 milliseconds; TI, inversion time; 300 milliseconds; FL, flip angle 40°, 1.5-mm slice thickness and no interslice gap). Coronal images were reconstructed from the 3-dimensional sagittal data set at 1-mm slice thickness with no interslice gap.

No sedation was used, but prior to MRI acquisition each child met with one of us (K.T.C.) for a short relaxation session. To help each child in minimizing head movement while in the magnet, a red dot 1 cm in diameter was placed on the ceiling of the MRI tube. Children were instructed to focus on the dot during scanning, and prepare a short story to tell after the imaging ended. Scanning took about 28 minutes per subject.

Morphometry

Planimetric measurements were performed on commercially available computer hardware (model 7100/66, Apple Computer Inc, Cupertino, Calif; http://www.apple.com/) using the public domain National Institutes of Health program, version 1.32 (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). For all neuroanatomical variables, 3 separate measurements of each region of interest were performed, and a mean area (squared millimeters) from these values was then calculated. Two independent raters performed all neuroanatomical measurements, with an average correlation of 90% interrater reliability and 93% intrarater reliability.

Measurements of anterior vermal lobuli I-V (lingula, central lobule, and culmen) and superior-posterior VI-VII lobuli (declive and folium, including tuber) were performed on the midsagittal image that most clearly showed the cerebral aqueduct and the longest primary and prepyramidal fissures. Vermal measurements were obtained by tracing the boundaries of vermal lobuli I-V, bordered inferiorly by the primary fissure, and lobuli VI-VII, bordered inferiorly by the prepyramidal fissure. Measurement of the pons followed the boundaries of the pontomesencephalic junction, the dorsal tegmentum, the pontomedullary junction, and the ventral basal pons. The pons, located in the same topographical brain area as the cerebellum, served as a regional anatomical control measure to further investigate our hypothesis that the structures of the developing brain are affected selectively by toxic effects of methotrexate, determined by their individual properties and course of maturation.

To obtain an estimate of left and right prefrontal cortex volume, a consistent method of demarcation was used on the midsagittal slice, and corresponding coordinate values (x, y) were extrapolated to coronal slices. On the midsagittal slice, the posterior boundary (x) was defined as 5 mm anterior to the genu of corpus callosum, and a line through the posterior and anterior comissures was drawn across the medial frontal lobe; this line provided a y-coordinate value for the coronal slices, and served as an inferior boundary. Starting at the coronal slice indicated by the x-coordinate of the midsagittal posterior boundary, and moving anteriorly at 5-mm slice intervals until cortex was no longer visible (8-9 slices), the lateral boundary of cortex was traced downward from the longitudinal fissure until the inferior boundary line was met, then, moving back upward, the remaining cortex was traced. Each coronal mean area measurement was multiplied by 5 mm, and the resulting values were summed to obtain an estimate of volume. Extrapolation from the midsagittal demarcation boundaries to coronal slices indicates that measures of prefrontal association cortex largely comprised Brodmann areas 9, 10, and 46. Figure 1 shows cerebellar regions of interest, and Figure 2 shows prefrontal regions of interest.

Whole brain volumes (WBVs) were estimated from 10 coronal slices. The first anterior slice where brain tissue initially appeared was determined, and the fourth slice posterior to this point was used as the first WBV slice. The last that, influenced by their corresponding protracted course of development and neural connections, may organize into a subsystem; ie, interrelated and interacting components of the CNS involved in a particular process or function. Accordingly, it was hypothesized that if the later developing neocerebellum was found to be abnormal, there would be a corresponding abnormality in later developing prefrontal cortices, and poorer performance on visual-spatial attention, short-term memory, and visuomotor organization and coordination tests.
posterior slice where brain tissue clearly could be identified was determined, and the fourth slice anterior to this point was used as the 10th WBV slice. The remaining slices were spaced at equal intervals between the anterior and posterior slices. The hemispheres, and when apparent the brainstem (bordered inferiorly by the pontomedullary junction), and cerebellum were outlined. Ventricular planimetric areas were subtracted out. From the first slice to the next to last slice, the mean area was multiplied by the distance between it and the next consecutive slice. The resulting values were summed to yield an estimate of total volume.

NEUROPSYCHOLOGICAL TESTS

Central to the purposes of this study were tests that provide information on visual-spatial attention, short-term memory, and visuomotor organization and coordination skills. The tests included Trail-Making Test, parts A and B (TMT-A, TMT-B);20 Rey-Osterreith Complex Figure Test, Copy (CFT-C) and Immediate Recall (CFT-IR);20 and Wechsler Intelligence Scale for Children, Third Edition (WISC-III) Coding.22 (Detailed information on these tests can be found in texts by Lezak23 and Spreen and Strauss.24)

The TMT assesses visual-spatial attention, search, and sequencing; visuomotor coordination; and cognitive flexibility.21,24 The test has been shown to load on “rapid visual search” and “visual-spatial sequencing” constructs in factor analysis,21 and to correlate highly with other visual search tests, but not with verbal tests.26 Part A requires the individual to connect consecutively numbered circles and measures the ability to track a single train of numbers. Part B requires individuals to connect consecutively connect numbered and lettered circles (1-A-2-B, etc), and involves the ability to flexibly switch between sets of stimuli by inhibiting the irrelevant set and attending to the alternative set. Speed is an important indicator of performance, and although errors are recorded, the main measure is the total time taken for task completion.

The CFT-C and CFT-IR provide information on visual-spatial attention, visuomotor and perceptual organization and integration, visual-spatial memory, and planning skills.23,24 A detailed, multi-element geometric design is presented to copy, and immediately afterward the individual is required to reproduce it from short-term memory. The number of correctly reproduced details of the figure is used as a measure of performance.

Finally, coding is a symbol substitution task intended to provide information on attention, visual scanning and tracking, short-term memory, and cognitive flexibility.27 Within WISC-III, coding correlates high with symbol search (a task that involves attention and concentration, perceptual discrimination, short-term memory, cognitive flexibility, and visuomotor coordination) and low with the verbal scale.27 For younger children (6-7 years of age; coding A), the test requires individuals to match and place simple visual symbols (eg, lines, circle) within geometrical figures (eg, triangle, square), and for older children (8-16 years of age; coding B), to match a particular symbol with a particular number. The number of correct pair matches completed within 120 seconds is used as the measure of performance.

STATISTICAL ANALYSIS

Data analyses were performed with SPSS version 6.1 for the computer software program UNIX (SPSS Inc, Chicago, Ill). For each family of analyses, Student 2-tailed t tests for independent samples were computed, and compared with a Bonferroni-adjusted α level. For all analyses, the Levene test for equality of variance was not significant; therefore, t values were based on pooled variance estimates. Reported P values can be considered as providing an index as to whether replication of the present study using a different sample would produce similar significant results in the same direction, ie, one group higher and one group lower on some measure.28,29 Effect size model values also were computed. The d statistic was used as a measure of ES. Interpretation of ES is based on a convention suggested by Cohen13—0.20 is considered a "small" effect; 0.30, "medium"; and 0.80 or greater, "large." Post hoc power analyses were performed using the d statistic to estimate power from Cohen’s tables by linear interpolation.13 With very large ESs, d was used to calculate Cohen’s f and used with more extensive tables or to calculate f, and used with Pearson-Hartley charts to estimate power.13,16 In computing and interpreting power, sample ES values are assumed to generalize to the population. Finally, 95% confidence intervals were computed for ES13 and mean differences.

SUBSYSTEM ANALYSIS

Hierarchical regression analysis30 and partial correlation31 techniques were used to explore the hypothesis that a cerebellar-frontal subsystem may be involved in the processes of visual-spatial attention, and visuomotor organization and coordination. A representative measure, Frontal-Spatial (F-S), was formed by combining mean performance on the present neuropsychological tests, which assess functions in the visual-spatial-motor domain. Our composite label F-S was chosen to distinguish it from other functions that may be associated with frontal subsystems, eg, frontal-verbal. The subsystem model assumed that the lobuli VI-VII development would relatively precede extended prefrontal cortical development; therefore, the lobuli VI-VII variable was entered first in all analyses. Proportion of variance accounted for (R2), expressed as a percentage, was used as a measure of association between the neuroanatomical variables and F-S. To obtain a measure of the proportion of variance in F-S accounted for by the interaction between the neuroanatomical variables, partial correlation analyses were performed between F-S and the product of lobuli VI-VII and either the left or right prefrontal measure, controlling simultaneously for both separate lobuli VI-VII and the respective prefrontal variable.31 The result can be interpreted as the amount of variance in F-S accounted for solely by the interaction of lobuli VI-VII and prefrontal variables.31

EFFECT SIZE MODEL OF DATA ANALYSIS

We used a statistical effect size (ES) model of enhanced data description and analysis by reporting ES and post hoc power analyses,13,14 and confidence intervals. “Effect size” is a standardized quantitative index that can represent the magnitude of change that one variable produces in another variable as reflected in the difference between 2 means.13,15 Power is the probability that a statistical test will yield a statistically significant result and
The null hypothesis will be rejected. The most feasible way for a researcher to increase the power of a statistical test is to increase sample size. Because often only small samples are available in clinical neuroscience research, the knowledge of ES model values is particularly valuable for interpreting results. For example, a nonsignificant $P$ value may be interpreted as evidence for no treatment effect and/or no difference between means. However, ES model measures may reveal moderate to large ES and confidence interval values, but the study may have relatively low power. In a replication study with more subjects, power would increase, and thus the likelihood would increase of finding statistically significant results. Effect size model measures, then, provide additional quantitative information that can be used to augment statistical inferences drawn from the outcome of a single study, and, compared with the passive use of “significant” vs “nonsignificant” $P$ values to make decisions concerning treatment effects and/or differences between means, allow for a more active process of reasoning about and interpretation of one’s results.

### RESULTS

#### DEMOGRAPHICS

Table 1 presents demographic data for the 2 groups. To determine whether the ALL and control groups were adequately matched, a multivariate analysis of variance was conducted on the differences between the 2 groups with respect to age, handedness, sex, and socioeconomic status. The main effect of group was not significant ($P = .94$).
Table 2. Control and Acute Lymphoblastic Leukemia (ALL) Group Values for Lobuli I-V and VI-VII, Left and Right Prefrontal Cortices, Pons, and Whole Brain Volume

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Mean Difference (95% CI)</th>
<th>Effect Size (95% CI)</th>
<th>P</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobuli I-V, mm²</td>
<td>425.53 (33.37)</td>
<td>396.46 (50.19)</td>
<td>29.07 (−10.97 to 69.11)</td>
<td>0.68 (0.28 to 1.08)</td>
<td>.14</td>
</tr>
<tr>
<td>Lobuli VI-VII, mm²</td>
<td>322.70 (56.32)</td>
<td>268.73 (36.47)</td>
<td>53.97 (9.40 to 98.54)</td>
<td>1.14 (0.64 to 1.64)</td>
<td>.02</td>
</tr>
<tr>
<td>L front, cm³</td>
<td>29.25 (2.60)</td>
<td>24.89 (3.42)</td>
<td>4.36 (1.51 to 7.21)</td>
<td>1.44 (0.87 to 2.01)</td>
<td>.005</td>
</tr>
<tr>
<td>R front, cm³</td>
<td>30.79 (3.79)</td>
<td>26.93 (2.15)</td>
<td>3.86 (0.63 to 7.18)</td>
<td>1.12 (0.63 to 1.61)</td>
<td>.02</td>
</tr>
<tr>
<td>Pons, mm²</td>
<td>510.33 (48.48)</td>
<td>484.92 (54.23)</td>
<td>25.41 (−22.92 to 73.74)</td>
<td>0.49 (0.13 to .85)</td>
<td>.28</td>
</tr>
<tr>
<td>WBV, cm³</td>
<td>119.67 (5.87)</td>
<td>120.89 (8.57)</td>
<td>1.22 (−8.76 to 6.34)</td>
<td>0.16 (−0.19 to .51)</td>
<td>.74</td>
</tr>
</tbody>
</table>

* CI indicates confidence interval; L front, left prefrontal cortex; R front, right prefrontal cortex; and WBV, whole brain volume.

Table 3. Control and Acute Lymphoblastic Leukemia (ALL) Group Values for 5 Neuropsychological Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (SD)</th>
<th>Mean Difference (95% CI)</th>
<th>Effect Size (95% CI)</th>
<th>P</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-A</td>
<td>0.22 (1.16)</td>
<td>0.22 (0.65)</td>
<td>0.00 (−0.93 to 0.92)</td>
<td>.005 (−0.33 to 0.34)</td>
<td>.99</td>
</tr>
<tr>
<td>TMT-B</td>
<td>0.53 (0.70)</td>
<td>−0.58 (0.88)</td>
<td>1.11 (0.31 to 1.91)</td>
<td>1.40 (0.80 to 2.00)</td>
<td>.009</td>
</tr>
<tr>
<td>CFT-C†</td>
<td>0.52 (0.77)</td>
<td>−0.25 (0.82)</td>
<td>0.77 (−0.006 to 1.56)</td>
<td>.96 (0.49 to 1.43)</td>
<td>.05</td>
</tr>
<tr>
<td>CFT-IR†</td>
<td>0.20 (0.83)</td>
<td>−0.79 (1.01)</td>
<td>0.99 (0.12 to 1.86)</td>
<td>1.07 (0.59 to 1.55)</td>
<td>.03</td>
</tr>
<tr>
<td>WISC-III Coding†</td>
<td>0.003 (0.96)</td>
<td>−1.42 (1.52)</td>
<td>1.42 (0.23 to 2.62)</td>
<td>1.12 (0.63 to 1.61)</td>
<td>.02</td>
</tr>
</tbody>
</table>

* CI indicates confidence interval; TMT-A, Trail-Making Test part A; TMT-B, Trail-Making Test part B; CFT-C, Rey-Osterreith Complex Figure Test, Copy; CFT-IR, Rey-Osterreith Complex Figure Test, Immediate Recall; and WISC-III Coding, Wechsler Intelligence Scale for Children, Third Edition, Coding.
† Bonferroni-adjusted α = .05/5 = .01; CFT-C, CFT-IR, and coding P values are not significant, but their large effect size and CIs along with low power indicate that the treatment clearly had an effect in this sample, and that an effect likely exists in the population, but owing to low power (most likely because of small sample size), a significant P value was not obtained.

Table 4. Correlation Coefficients Between Neuropsychological Tests and Frontal-Spatial (F-S) Composite

<table>
<thead>
<tr>
<th>Test</th>
<th>TMT-B</th>
<th>CFT-C</th>
<th>CFT-IR</th>
<th>WISC-III Coding</th>
<th>F-S</th>
<th>r (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-B</td>
<td>0.60 (.01)</td>
<td>0.64 (.004)</td>
<td>0.54 (.06)</td>
<td>0.82 (&lt;.001)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>CFT-C</td>
<td>0.58 (.008)</td>
<td>0.63 (.004)</td>
<td>0.84 (&lt;.001)</td>
<td>0.76 (&lt;.001)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>CFT-IR</td>
<td>0.33 (.15)</td>
<td>0.76 (&lt;.001)</td>
<td>0.81 (&lt;.001)</td>
<td>0.81 (&lt;.001)</td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>

* See first footnote to Table 3 for an explanation of abbreviations.

BRAIN MORPHOMETRY

Table 2 presents morphometric measures. Results for each family of variables were compared with a Bonferroni-adjusted α level (B-α) of .05/2 = .03. First, for cerebellar vermis, the lower ALL group mean for cerebellar lobuli VI-VII was significant (P = .02, d = 1.14, power approximately 0.67). In contrast, the lower ALL group mean for lobuli I-V was not significant (P = .14, d = 0.68, power approximately 0.30). Second, for left and right prefrontal lobe volume, the lower ALL group mean for left prefrontal cortex was significant (P = .005, d = 1.41, power approximately 0.84). Similarly, the lower ALL group mean for right prefrontal cortex was significant (P = .02, d = 1.12, power approximately 0.65). Pons and WBV data were analyzed. Complete WBV data were not available for 2 control subjects, and were not included in the analysis. Group differences for the pons were not significant (P = .28, d = 0.49, power approximately 0.18). Likewise, WBV differences were not significant (P = .74, d = 0.16, power approximately 0.06).

NEUROPSYCHOLOGICAL MEASURES

Table 3 shows the neuropsychological results. Test raw scores were converted to standard z scores using published test norms. Data not available were TMT-A for 1 subject with ALL, TMT-B for 1 control subject and 1 ALL subject, and CFT-C for 1 control subject, and therefore were not included in their respective analyses. Results were compared with B-α = .01. For TMT-B, lower mean performance by the ALL group was significant (P = .009, d = 1.40, power approximately 0.80). Lower mean performance by the ALL group approached significance for CFT-IR (P = .03, d = 1.07, power approximately 0.63) and WISC-III Coding (P = .02, d = 1.12, power approximately 0.67). Lower ALL group performance was not significant for CFT-C.
components (its compound variable) accounted for the lobuli VI-VII combined linear, quadratic, and cubic portions of these data. Polynomial regression analysis indicated that a cubic polynomial provided a better description of the scatterplots and curve-fitting analysis suggested that F-S measures and F-S were nearly zero. However, examination of scatterplots and curve-fitting analysis suggested that a cubic polynomial provided a better description of these data. Polynomial regression analysis indicated that the lobuli VI-VII combined linear, quadratic, and cubic components (its compound variable) accounted for about 17% of F-S variance, the compound left prefrontal measure accounted for about 14%, and the compound right prefrontal measure accounted for about 26%. The combination of lobuli VI-VII and left prefrontal compound variables accounted for about 39% of F-S variance, and lobuli VI-VII and right prefrontal accounted for about 56%. The percentages of F-S variance accounted for by combination and interaction of compound lobuli VI-VII and left (LF) and right (RF) prefrontal cortex measures for both groups of subjects are shown in the tabulation below.

**Table 5. Linear Shared Proportion of Variance Between Lobuli VI-VII, Left and Right Prefrontal Cortices, and Frontal-Spatial (F-S) Measures for Acute Lymphoblastic Leukemia (ALL) and Control Groups**

<table>
<thead>
<tr>
<th></th>
<th>ALL Group (n = 10)</th>
<th>Control Group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobuli VI-VII</td>
<td>&lt;0.01 (.82)</td>
<td>0.10 (.39)</td>
</tr>
<tr>
<td>L front</td>
<td>0.06 (.94)</td>
<td>0.42 (.04)</td>
</tr>
<tr>
<td>R front</td>
<td>0.01 (.83)</td>
<td>0.44 (.04)</td>
</tr>
</tbody>
</table>

* L front indicates left prefrontal cortex; R, right prefrontal cortex.

For the ALL group, it was found that a cubic polynomial also provided a better description of the data. The lobuli VI-VII compound variable accounted for about 44% of F-S variance, the compound left prefrontal measure accounted for about 93%, and the compound right prefrontal measure accounted for about 48%. The combination of lobuli VI-VII and left prefrontal compound variables accounted for about 99% of F-S variance, and lobuli VI-VII and right prefrontal accounted for about 79%.

### Interaction

For the ALL group, the lobuli VI-VII and left prefrontal interaction accounted for about 20% of F-S variance; the lobuli VI-VII and right prefrontal interaction accounted for less than 1%. For the control group, the lobuli VI-VII and left prefrontal interaction accounted for about 41% of F-S variance; the lobuli VI-VII and right prefrontal interaction accounted for about 21%.

### ALL SURVIVORS’ BRAIN SEQUELAE INVOLVE CEREBELLAR-FRONTAL DEFICITS

The present study found that in children treated for ALL with chemotherapy only before the age of 5 years, the posterior cerebellar vermis (lobuli VI-VII) and left and right prefrontal association cortices were significantly reduced, concurrent with neuropsychological deficits in visual-spatial attention, short-term memory, and visuomotor organization and coordination. It should be emphasized that one aim of the present study was to explore whether these data are consistent with the hypothesis that the later developing cerebellar lobuli VI-VII and prefrontal cortices may be particularly vulnerable to the effects of intrathecal methotrexate, and may comprise an interrelated and interacting subsystem, which may produce an effect different from that of either structure alone.12 The present subsystem analyses using multiple regression and partial correlation techniques yielded results that are compatible with this hypothesis; for both groups, the combination of both lobuli VI-VII and prefrontal cortex measures accounted for substantially greater amounts of F-S function variance than either variable alone. Except for the ALL group lobuli VI-VII and right prefrontal cortex, the interaction of lobuli VI-VII and prefrontal cortex measures accounted for notable amounts of F-S variance. These results suggest that a cerebellar-frontal deficit is involved in brain sequelae of ALL survivors, and provide further evidence indicating a cerebellar-frontal relationship to cognitive deficits, including deficits in visual-spatial attention and memory tasks.8,33-35

### TOXIC EFFECTS ON NEOCEREBELLUM AND FRONTAL CORTEX

The neurogenesis and migration of granule cells in the human cerebellum continue through the first several years of life, causing an increase in the size of this structure. It is essential for the normal development of the cerebellum that during the early postnatal period there is normal structural and functional interactions of granule cells with Purkinje cells and radial glial fibers.36,37 Early disruption of this interaction by toxic insult, such as irradiation and/or chemotherapy, may inhibit the development of interactions between granule and Purkinje cells, and result in cerebellar hypoplasia and atrophy.38
A study in monkeys documented the transport of herpes simplex virus from prefrontal cortex to the lateral cerebellum, demonstrating a prefrontal-cerebellar connection. In humans, results of a functional MRI study have shown that neural systems including the cerebellum and dorsolateral prefrontal cortex are involved in cognitive reasoning tasks. An abnormally developing cerebellum, then, may contribute to deficits in higher cognitive functions, due to an anatomical and functional cerebellar-frontal association.

Our developmental chronometry hypothesis posits that as the neocerebellum and frontolateral areas are relatively later-developing structures of the brain, a neocerebellar-frontal subsystem with a slow rate of maturation may have prolonged vulnerability to damage, and as such may be a common nonspecific site of abnormality in many disorders of childhood. Toxic insult to this subsystem in young children, when the processes of development are prolific, may manifest itself later in ontogeny as structural, functional, and interconnection abnormalities. Although our present observations suggest that cerebellar-frontal subsystem abnormality may be common among survivors of ALL, this may not necessarily be a specific diagnostic marker for this population; cerebellar-frontal brain subsystem deficits may occur in the course of the developing brain of any child following substantial genetic, viral, toxic, or traumatic insults.

**CEREBELLAR-FRONTAL DEFICITS MAY CONTRIBUTE TO NONVERBAL LEARNING DISABILITIES**

Although ALL survivors show some deficits in verbal tasks, more severe deficits are noted in visual-spatial orientation, visuomotor coordination, spatial memory, and arithmetic, particularly in children treated before 5 years of age. Such deficits have been interpreted as reflecting abnormalities in the right hemisphere and in white matter integrity. 

These suggestions are derived from neurobehavioral data, and do not yet have direct empirical support from neuroanatomical data. For the ALL group in the present study, the neuropsychological profile of deficits shows some similarities to findings that others have related to right brain hemispheres were abnormal, the left hemisphere appears to be more affected (d = 1.41, mean difference = 43.59) than the right (d = 1.12, mean difference = 38.61). The left anterior brain has been related to processing sequentially presented stimuli and to programming rapid motor sequence responses. The present tests, particularly TMT and Coding, strongly require sequencing ability. Given that sequencing abnormalities in the ALL population have been reported, and that most children surviving ALL are considerably more deficient in the visual-spatial rather than the verbal domain, the observed left brain deficit may be more strongly related to poor sequencing in the visual-spatial domain, rather than to poor verbal abilities.

**EARLY NEUROPSYCHOLOGICAL COGNITIVE INTERVENTION SUGGESTED**

Pragmatically, the question of interest of the present study is whether the experimental results make it reasonable to conclude that if children are treated with chemotherapy before the age of 5 years, it is likely that they will subsequently demonstrate deficits in areas vital to normal growth and development, thus requiring special remediation in those areas. Our findings include some nonsignificant P values regarding the answer to this question; however, it does not necessarily follow that the conditions have been met to confidently conclude that deleterious treatment effects are not actually occurring in the population. Effect sizes and confidence intervals for group differences in neuropsychological performance (Table 3) indicate that potentially large and clinically important differences may actually exist in the population, but may not have been detected at a level of significance in this particular study due to low sample size and subsequent low to moderate statistical power. Based, then, on additional information from ES model values, the present data can reasonably be interpreted as providing persuasive evidence that intrathecal methotrexate treatment of children before the age of 5 years has both structural and functional effects on the developing brain. Because frontal and other cortical areas of the developing brain reflect a dynamic and changing system, with the potential for functional reorganization at least into late childhood (for review, see Joseph), neuropathological-cognitive rehabilitation should be provided as early as possible after completion, or near completion, of the treatment protocol to optimize the developmental potential of frontal functions in survivors of childhood ALL.

Accepted for publication April 20, 1998.

We thank William Orrison, MD, and the staff of the New Mexico Veterans Affairs Imaging Center for their help in completing the brain imaging part of this study; staff from the Department of Pediatrics, University of New Mexico Hospital for referring children treated for ALL; Richard J. Harris, PhD, for comments on the final version of the manuscript; and Barbara Brooks and Dina Hill for help in psychometric testing.

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