Neurocognitive Sequelae in Adult Childhood Leukemia Survivors Related to Levels of Phosphorylated Tau

Iris Elens, Sabine Deprez, Marina Danckaerts, Patricia Bijttebier, Veerle Labarque, Anne Uyttebroeck, Stefaan Van Gool, Rudi D’Hooge*, Jurgen Lemiere*

Affiliations of authors: Laboratory of Biological Psychology (IE, RDH), Department of Imaging and Pathology (SD), Department of School Psychology and Child and Adolescent Development (PB), Department of Pediatrics, Pediatric Hemato-Oncology (VL, AU, JL), Department of Cardiovascular Medicine (VL), and Department of Child and Adolescent Psychiatry, University Psychiatric Centre Leuven (IE, MD, JL), KU Leuven, Leuven, Belgium; Department of Radiology, University Hospital Leuven, Leuven, Belgium (SD); Immunologisch Onkologisches Zentrum Köln, Köln, Germany (SVG)

*Authors contributed equally to this work.
Correspondence to: Rudi D’Hooge, PhD, Laboratory of Biological Psychology, Tiensestraat 102 box 3714, 3000 Leuven, Belgium (e-mail: rudi.dhooge@ppw.kuleuven.be).

Abstract

Central nervous system–directed prophylactic chemotherapy increases survival in childhood leukemia, but possible late neurocognitive sequelae remain a concern. We compared intellectual performance (WAIS IV), memory (AVLT), and executive functioning (ANT) between adult leukemia survivors (n = 31) and control individuals (n = 35). In survivors, cerebrospinal fluid (CSF) levels of phosphorylated Tau (p-Tau) during treatment and total intrathecal methotrexate dose correlated with adult intellectual performance (Pearson’s and Spearman’s coefficients, respectively). Long-term memory and attentional control, both maturing before survivors’ mean age at diagnosis, were unaffected (P > .05 on all four subtests), in contrast to cognitive flexibility and information processing (P < .05 for eight of the subtests), which mature during adolescence. CSF p-Tau and methotrexate dose negatively correlated with intellectual performance (r = −0.414, P = .04 and r = −0.484, P = .007, respectively), but not with each other (r = 0.219, P = .29). These data identify CSF p-Tau as a predictor of late neurocognitive sequelae (in addition to methotrexate dose). Early identification of children at risk could inspire interventions to prevent or remediate chemotherapy-induced cognitive sequelae.

Acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) account for 30% of childhood cancers (1). Central nervous system–directed prophylaxis prevents relapse and increases survival, but the possibility of late neurocognitive sequelae remains a major concern (2–4). Because of its devastating effects on the developing brain (3,5–9), prophylactic cranial irradiation was replaced by intrathecal and high-dosed intravenous injections of the antifolate drug methotrexate. However, intellectual and executive function (EF) deficits might still occur in a proportion of chemotherapy-treated survivors (2,4,9–12). Unfortunately, little is known about possible predictors of these late neurocognitive sequelae, but the youngest children were suggested to be most susceptible to these adverse effects (9,11–14).

The present study is the first report of adult neurocognitive performance in childhood leukemia survivors in relation to intrathecal methotrexate administered and cerebrospinal fluid (CSF) levels of Tau and phosphorylated Tau (p-Tau) during treatment as markers of axonal damage and neurofibrillary tangles, respectively. Elevated CSF levels of Tau and p-Tau have been reported after intrathecal methotrexate (15–19). Moreover, children’s neurocognitive performance, up to five years after diagnosis, was negatively correlated with total intrathecal methotrexate dose as well as CSF Tau levels (Supplementary Figure 1, available online) (16,18,20–22). Additionally, we tested the developmental chronometric hypothesis, stating that the age of occurrence of an adverse event (e.g., chemotherapy) determines its long-term cognitive impact (23). Functions with a protracted...
Table 1. Neurocognitive data of study participants

<table>
<thead>
<tr>
<th>Cognitive function</th>
<th>Test</th>
<th>Task</th>
<th>Survivors</th>
<th>Controls</th>
<th>P survivors vs controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>SD</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Intelligence</td>
<td>WAIS</td>
<td>Total IQ†</td>
<td>97</td>
<td>65–119</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verbal IQ†</td>
<td>98</td>
<td>66–122</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Performance IQ†</td>
<td>97</td>
<td>70–125</td>
<td>13</td>
</tr>
<tr>
<td>Memory</td>
<td>AVLT</td>
<td>Delayed recall (A7)‡</td>
<td>12</td>
<td>6–15</td>
<td>2.0</td>
</tr>
<tr>
<td>Executive functioning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focused attention</td>
<td>ANT</td>
<td>FA4L§</td>
<td>75</td>
<td>0.8–309</td>
<td>72</td>
</tr>
<tr>
<td>Sustained attention</td>
<td>ANT</td>
<td>SAD§</td>
<td>1100</td>
<td>590–2110</td>
<td>340</td>
</tr>
<tr>
<td>Inhibition</td>
<td>ANT</td>
<td>SSV inhib§</td>
<td>327</td>
<td>3–969</td>
<td>228</td>
</tr>
<tr>
<td>Flexibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set shifting</td>
<td>ANT</td>
<td>SSV flex§</td>
<td>552</td>
<td>335–1540</td>
<td>244</td>
</tr>
<tr>
<td>Working memory</td>
<td>AVLT</td>
<td>Sum trial A1–3‡</td>
<td>30</td>
<td>15–40</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>ANT</td>
<td>MSL§</td>
<td>334</td>
<td>70–634</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>WAIS</td>
<td>Working memory†</td>
<td>99</td>
<td>68–122</td>
<td>14</td>
</tr>
<tr>
<td>Information processing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>WAIS</td>
<td>Processing speed†</td>
<td>97</td>
<td>78–122</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>ANT</td>
<td>Baseline speed FA4L (Th1)§</td>
<td>652</td>
<td>413–1271</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline speed MSL (Th1)§</td>
<td>720</td>
<td>471–1271</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline speed SAD (Th2)§</td>
<td>706</td>
<td>530–1055</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline speed SSV (Tc1)§</td>
<td>390</td>
<td>284–585</td>
<td>391</td>
</tr>
</tbody>
</table>

*Statistical analysis was based on two-sided, one-way (repeated-measures for AVLT 1-3) analysis of covariance with group (survivors vs controls) as independent factor and an index of parental socio-economic status (33) as covariate. The results of cognitive testing of one survivor (EORTC 58951) were not included in the analysis because of a low motivational state during testing.

† Data are presented as standardized scores with 100 as mean and 15 as standard deviation. High score indicates good performance. ANT = Amsterdam Neuropsychological Tasks; AVLT = Rey Auditory Verbal Learning Test; FA4L = Focused Attention Four Letters; MSL = memory search letters; SAD = sustained attention dots; SSV inhib = shifting set visual inhibition; SSV flex = shifting set flexibility; WAIS = Wechsler Adult Intelligence Scale.

‡ Data are presented as number of items recalled. High score indicates good performance. Sum trial A1-3 was chosen as outcome measure for working memory since it takes ceiling effects of the sum trial A1-5 into account (31).

§ Data are depicted as milliseconds, but analyses were performed on log-transformed data. Performance of focused attention, inhibition, working memory, and flexibility is corrected for baseline speed. High score indicates poor performance.

Development would thus be more vulnerable than those that largely matured before the brain insult (Supplementary Figure 2, available online). In view of their differential maturation rates (24,25), we examined different memory functions and core EF components such as attention control, cognitive flexibility, and information processing.

Thirty-one adult, nonirradiated childhood ALL (n = 27) or NHL (n = 4) survivors were compared with 35 age-matched control subjects (mean age of all participants = 21.5 years, range = 16.1–29.8 years). Mean age at diagnosis was 6.4 years (range = 16.1–29.8 years; treatment between 1994 and 2004 according to the EORTC 58881 or 58951 protocol) (Supplementary Tables 1–3, available online) (26–28). Control participants were recruited via family members of the survivors and local advertising. Each participant signed a written informed consent form approved by the ethical committee of the Leuven University Hospital. We tested potential differences in depressive or anxiety symptoms using Beck Depression Inventory and State-Trait Anxiety Inventory, respectively (29). In 30 (Taul) and 26 (p-Taul) survivors, we could retrace and pool CSF biomarker levels during the entire treatment (see [19] for collection and analysis). Intelligence, memory, and EF were assessed using the Wechsler Adult Intelligence Scale (WAIS IV) (30), Rey Auditory Verbal Learning Test (AVLT) (31), and Amsterdam Neuropsychological Tasks (ANT) (32), respectively. Statistical analysis included two-sided, one-way (repeated-measures for AVLT 1-3) analysis of covariance (ANCOVA) with group (survivor vs control) as independent factor and an index of parental socio-economic status (33) as covariate. Two-sided testing of the correlation between levels of CSF biomarkers, total intrathecal methotrexate dose, and intelligence was based on Pearson’s and Spearman’s correlation coefficients, respectively. A P value of less than .05 was considered statistically significant.

Adul childhood leukemia survivors displayed statistically significantly lower total, verbal, and performance intelligence quotients (TIQ, VIQ, and PIQ, respectively) than controls (P = .001, .02, and .007, respectively), although results were still within range of normality (Table 1). There was a negative correlation between TIQ, VIQ, and PIQ and CSF p-Tau (r = −0.219, −0.242, and −0.207, respectively), which suggests involvement of intervening factors that increase CSF p-Tau levels independently from total intrathecal methotrexate as described earlier (Figure 1) (20). Total intrathecal methotrexate dose and CSF p-Tau were not statistically significantly correlated (r = −0.048, P = .007), illustrating the sensitivity of especially PIQ to intrathecal methotrexate as described earlier (Figure 1) (20). Total intrathecal methotrexate dose and CSF p-Tau levels were not statistically significantly correlated (r = −0.484, P = .007), which suggests involvement of intervening factors that increase CSF p-Tau levels independently from total intrathecal methotrexate dose, such as methylenetetrahydrofolate reductase (MTHFR) gene deficiency (34), subclinical leukemic brain infiltration (18), or intrathecal corticosteroid administration (35). The latter as part of triple (EORTC 58951), but not mono (EORTC 58881) intrathecal chemotherapy (Supplementary Figure 1, available online).

Whereas set-shifting, most measures of working memory, and processing speed were affected (P < .05 for eight of the subtests) (Table 1), neuropsychological assessments indicated intact long-term memory, focused and sustained attention, and...
inhibition (P > 0.05 on all four subtests). Notably, this differential vulnerability of cognitive functions paralleled their putative developmental trajectories (Supplementary Figure 2, available online) (24). Long-term memory and attentional control abilities (ie, sustained and focused attention, and inhibition), which did not differ from controls, have been suggested to develop largely before six years of age, the mean age of diagnosis in our survivor cohort. Conversely, cognitive flexibility (ie, set-shifting, working memory) and information processing (ie, processing speed), which were affected in various tests, predominantly mature during adolescence (23–25).

Our data suggest that increased CSF p-Tau is a better predictor than CSF Tau for long-term intellectual sequelae. This is supported by the notion that increased Tau phosphorylation—possibly resulting from methotrexate-induced changes in folate metabolism (34)—is an established marker of neurotoxic Tau aggregation (36,37). This study demonstrates that CSF p-Tau is an independent predictor of intellectual functioning in adult childhood cancer survivors and should encourage implementation of this CSF biomarker in multidimensional neurotoxicity assessment, alongside intrathecal methotrexate dose and age at therapy onset. By and large, the observed pattern of neurocognitive deficits, demonstrating mainly impairments in functions with a more protracted maturation course, supports the developmental chronometric hypothesis (6,23).

Limitations of this study include its retrospective and cross-sectional design, the relatively small sample size, and the lack of pretreatment neurocognitive data. Control participants had a higher parental socio-economic status, but this possible bias was controlled by including parental socio-economic status as a covariate. There was also no statistically significant correlation between age at start of therapy and neurocognitive measurements. This might, however, be attributed to the small sample size and the fact that the youngest children received a milder treatment in relation to their better prognosis.

Our findings might thus be particularly important for early identification of childhood leukemia survivors more at risk for long-term sequelae. These insights might allow subsequent implementation of individually tuned prevention or remediation programs, focusing on cognitive functions with more protracted development. Clearly, for patients to take maximum advantage of such interventions, vulnerable children have to be identified at the earliest stage possible.

**Funding**

This work was generously supported by the charity-based Olivia Hendrickx Research Fund (www.olivia.be).

**Notes**

The funder did not interfere with the study design, the collection, analysis, and interpretation of data, or the writing of the manuscript.

We are grateful to the participants who contributed to this study, and researchers Charlotte Sleurs, Dr. Thibo Billet, Elise Bossuyt, Charlotte van Soest, Trui Vercruysse, Linde Van den Wyngaert, Femke Pauwels, and Lien Solie, who made this work possible. The authors are also grateful to Prof. Dr. Koen Luyckx for statistical advice, Dr. Hugo Vanderstichele and Fujirebio Europe NV for practical and logistic help with collecting and analyzing the CSF samples, and the pediatric oncology team for their dedicated care for childhood cancer patients.

**References**


1. Iyer NS, Balsamo LM, Bracken MB, et al. Chemotherapy-only treatment ef-
10. Bisen-Hersh EB, Hineline PN, Walker EA. Disruption of learning processes by 
11. Askins MA, Moore BD. Preventing neurocognitive late effects in childhood


21. Steinberg S, Hartmann R, Wisniewski S, et al. [Late sequelae of CNS recur-
27. Domenesch C, Suciu S, De Moorloose B, et al. Dexamethasone (6 mg/m2/day) and prednisolone (60 mg/m2/day) were equally effective as induction ther-
31. Van Der Elst W, Van Boxtel M, Van Breukelen G, et al. Rey’s verbal learning test: Normative data for 1855 healthy participants aged 24–81 years and the test: normative data for 1855 healthy participants aged 24–81 years and the
34. Sonntag JM, Wasek B, Talerko G, et al. Altered protein phosphatase 2A methyl-
36. Butè L, Bussière T, Buèe-Scherrner V, et al. Tau protein isoforms, phosphoryla-
37. Butè L, Troquier L, Burnoul S, et al. From tau phosphorylation to tau aggrega-
38. wegenomew over de haard.