Accumulation of High Levels of Methotrexate Polyglutamates in Lymphoblasts From Children With Hyperdiploid (>50 chromosomes) B-Lineage Acute Lymphoblastic Leukemia: A Pediatric Oncology Group Study


Hyperdiploidy (>50 chromosomes, or a DNA index > 1.16) confers a favorable prognosis in B-lineage acute lymphoblastic leukemia of childhood. Children with B-lineage acute lymphoblastic leukemia whose lymphoblasts at diagnosis accumulate high levels of methotrexate (MTX) and MTX polyglutamates (MTXPGs) in vitro experience a better event-free survival than those whose lymphoblasts do not (Blood 76:44, 1990). Lymphoblasts from 13 children with hyperdiploidy (>50 chromosomes) accumulated high levels of MTXPGs (1,095 and 571 to 2,346 pmol/10^6 cells [median and 25% to 75% intraquartile range]). These levels were higher than those in B-lineage lymphoblasts from 19 children with other aneuploidy (326 and 159 to 775 pmol/10^6 cells) and 15 children with diploidy (393 and 204 to 571 pmol/10^6 cells) (P = .0015). Chromosomal trisomies in hyperdiploid cases were highly nonrandom. Chromosome 9 was not one of the chromosomes involved in trisomies, even though this chromosome contains the gene for folate polyglutamate synthetase, which is the enzyme required for MTXPG synthesis. The correlation between MTXPG level and percentage of S-phase cells was weak, suggesting that increased levels of MTXPGs could not be attributed to elevated proportions of cells in active DNA synthesis. The ability of hyperdiploid lymphoblasts to accumulate high levels of MTXPGs may increase their sensitivity to MTX cytotoxicity, accounting in part for the improved outlook for hyperdiploid patients treated with regimens that emphasize MTX as a primary component of continuation therapy.

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favorable prognosis. The hyperdiploid > 50 group accounts for a quarter of all patients with B-lineage ALL (see Seeker-Walker and Pui et al for recent reviews).

Recently, we found that children with B-lineage ALL whose lymphoblasts accumulated high levels of both MTX and MTXPGs in vitro experienced better 5-year event-free survival (EFS) than did those with lower MTX and/or MTXPG levels. We now report that lymphoblasts from children with hyperdiploid (> 50 chromosomes) B-lineage ALL accumulate high to very high levels of MTXPGs.

PATIENTS AND METHODS

Patient characteristics. Between April 1989 and January 1991, 1 to 2 mL of bone marrow was obtained with informed consent and ethical review committee approval from 80 children with B-lineage ALL at the time of diagnostic bone marrow aspiration. Patients were entered onto POG 8901, a limited-institution Pediatric Oncology Group (POG) study of in vitro MTX metabolism. B-lineage (early pre-B and pre-B cell) ALL was defined as the DNA content of leukemic compared with normal Go/G1 cells, was calculated as an approximation of chromosome ploidy. A DI of > 1.16 usually corresponds to 53 or more chromosomes.

Sample acquisition and handling. Bone marrow was collected in heparin, mixed with an equal volume of Hanks' Balanced Salt Solution (HBSS) lacking phenol red and NaHCO3, pH 7.4 (Flow Laboratory, Toronto, Ontario, Canada), and 4.0 mL layered onto 3.0 mL of Ficoll-Paque (Pharmacia Fine Chemicals, Montreal, Quebec, Canada). After centrifugation at 1,500g for 30 minutes, the mononuclear cells were separated, washed with HBSS, and counted. Five million lymphoblasts were incubated in 2.0 mL modified Eagle's minimal essential medium (MEM; Flow Laboratory), to which was added 0.67 mmol/L glycine, 0.037 mmol/L adenosine, 0.04 mmol/L thymidine, 26 mmol/L NaHCO3, 1.25 mmol/L pyruvate, 8.3 mmol/L dextrose, and 0.025 mmol/L ferric nitrate, and containing 10% newborn calf serum (GIBCO Co, Burlington, Ontario, Canada) and 1.0 µmol/L 3',5'-3H-MTX (Moravek Co, Brea, CA) for 24 hours in a P-35 culture dish (Falcon Co, Pointe Claire, Quebec, Canada) at 37°C in 5% CO2-95% O2. After incubation, cells were washed three times in 5.0 mL HBSS to remove extracellular MTX and then suspended in 2.0 mL HBSS. Trichloroacetic acid was added to cells to a final concentration of 10% and the mixture was frozen at −20°C.

Extracts were shipped to the reference laboratory at McGill University at room temperature and were stable. Samples were centrifuged and MTX and MTXPGs in the supernatant were concentrated on a SepPak cartridge (Waters Associates, Milford, MA), eluted from it in 200 µL 5.0 mmol/L tetrabutylammonium phosphate (TBAP) and 10.0 mmol/L potassium phosphate buffer, pH 5.5, and separated and quantitated using high performance liquid chromatography.

Table 1. Cytogenetic Findings, DI, Percentage of S-Phase (% S) Cells, and MTXPG Levels in Lymphoblasts From Children With Hyperdiploid (> 50 chromosomes) B-Lineage ALL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ALL (yr)</th>
<th>Sex</th>
<th>WBC (x 10^3/L)</th>
<th>DI</th>
<th>Karyotype (no. of metaphases)</th>
<th>% S Cells</th>
<th>Total MTXPGs (pmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pre-B</td>
<td>4.3</td>
<td>M</td>
<td>4.4</td>
<td>56, X; Y, + X, + 4, + 6, + 7, + 10, + 14, + 17, + 18, + 21, + 21, + dup(1) (q21q44)(3)/46,XY,Y(11)</td>
<td>—</td>
<td>2,167</td>
</tr>
<tr>
<td>2</td>
<td>Pre-B</td>
<td>5.1</td>
<td>M</td>
<td>5.0</td>
<td>1.17 53, XY; + X, + 8, + 10, + 16, + 21, + del(5)(q33),del(13)(q13q14), + der(5)(5;15)(q31q36)(5)/54,XY,Y, + X, + 6, + 10, + 11, + 18, + 21, - del(5)(q33),del(13)(q13q14), + der(5)(5;15)(q31q36)(3)/46,XY(Y9)</td>
<td>10.2</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>Early pre-B</td>
<td>8.9</td>
<td>F</td>
<td>7.0</td>
<td>1.20 56, XX; + X; + 3, + 5, + 7, + 8, + 10, + 14, + 18, + 21, + 22, (X;14)(q11;q32), + der(3)(3)(q27,q13)(5)/46,XX,Y(11)</td>
<td>12.6</td>
<td>3,287</td>
</tr>
<tr>
<td>4</td>
<td>Early pre-B</td>
<td>2.2</td>
<td>M</td>
<td>17.0</td>
<td>1.16 54, XY; + X, + 4, + 5, + 10, + 14, + 17, + 18, + 21(46),XY,Y(16)</td>
<td>7.9</td>
<td>626</td>
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<tr>
<td>5</td>
<td>Early pre-B</td>
<td>2.0</td>
<td>M</td>
<td>9.9</td>
<td>1.13 51, XY; + X, + 6, + 14, + 21(46),XY,Y(2)</td>
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<td>798</td>
</tr>
<tr>
<td>6</td>
<td>Early pre-B</td>
<td>3.0</td>
<td>F</td>
<td>4.2</td>
<td>1.20 56, XX; + X, + 4, + 6, + 10, + 14, + 17, + 18, + 21, + 21(46),XY,Y(2)</td>
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<td>7</td>
<td>Early pre-B</td>
<td>2.7</td>
<td>M</td>
<td>4.6</td>
<td>1.22 56, XY; + X, + 4, + 6, + 8, + 10, + 14, + 17, + 18, + 21(15)</td>
<td>22.9</td>
<td>2,047</td>
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<tr>
<td>8</td>
<td>Early pre-B</td>
<td>3.5</td>
<td>F</td>
<td>79.0</td>
<td>1.21 55, XX; + X, + 4, + 6, + 8, + 10, + 14, + 17, + 21(15)</td>
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<td>9</td>
<td>Pre-B</td>
<td>5.5</td>
<td>F</td>
<td>2.8</td>
<td>1.19 56, XX; + X, + 4, + 6, + 8, + 10, + 14, + 18, + 21(14), + 46,XX(6)</td>
<td>14.0</td>
<td>1,095</td>
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<td>10</td>
<td>Early pre-B</td>
<td>8.2</td>
<td>F</td>
<td>18.6</td>
<td>1.10 51,XX; + X, + 14, + 21, + del(6)(q13q21),del(9)(p13),del(11)(q14)(17)</td>
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<td>516</td>
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<tr>
<td>11</td>
<td>Early pre-B</td>
<td>3.8</td>
<td>M</td>
<td>1.5</td>
<td>1.19 56, XY; + X, + 4, + 6, + 10, + 14, + 17, + 21, + 21(46),XY,Y(10)</td>
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<td>12</td>
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<td>1.3</td>
<td>M</td>
<td>73.2</td>
<td>1.19 55, XY; + X, + 4, + 6, + 10, + 14, + 17, + 18, + 21(19),46,XY,Y(11)</td>
<td>3.9</td>
<td>422</td>
</tr>
<tr>
<td>13</td>
<td>Early pre-B</td>
<td>6.1</td>
<td>F</td>
<td>3.0</td>
<td>— 63, XX; + 2, + 2, + 5, + 6, + 7, + 8, + 10, + 11, + 12, + 14, + 17, + 18, + 21, + 22, + der(4)(1;4)(4pter—4q35;1q12—1qter), + mar(10)(10), + mar(13)(13), + mar(22)(22), + dup(11)(q31—q41), + der(4)(1;4)(4pter—4q35;1q12—1qter), + mar(3)(3)/46,XX(7)</td>
<td>—</td>
<td>851</td>
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</table>

Age and WBC values are at diagnosis.
Table 2. Cytogenetic Findings, DI, Percentage of S-Phase Cells (% S), and MTXPG Levels in Lymphoblasts From Children With Other Aneuploid B-Lineage ALL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ALL</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (×10⁹/L)</th>
<th>DI</th>
<th>Karyotype (no. of metaphases)</th>
<th>% S Cells</th>
<th>Total MTXPGs (pmol/10⁹ cells)</th>
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<tbody>
<tr>
<td>14</td>
<td>Pre-B</td>
<td>5.1</td>
<td>M</td>
<td>9.4</td>
<td>1.00</td>
<td>46,XY, dup(1)(q21q44)(7)/46,XY(13)</td>
<td>8.5</td>
<td>1,118</td>
</tr>
<tr>
<td>15</td>
<td>Early pre-B</td>
<td>3.1</td>
<td>M</td>
<td>16.0</td>
<td>1.00</td>
<td>46,XY, -12t(6;12)(p21;12), +der(12)t(6;12)(p21;12)t(17)/46,XY(3)</td>
<td>19.4</td>
<td>279</td>
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<tr>
<td>16</td>
<td>Early pre-B</td>
<td>5.0</td>
<td>M</td>
<td>116.0</td>
<td>1.00</td>
<td>46,XY, del(3)(p14), inv(14)(q11q32)(11)/46,XY, inv(14)(q11q32)(6)/46,XY(5)</td>
<td>1.4</td>
<td>775</td>
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<tr>
<td>17</td>
<td>Pre-B</td>
<td>8.6</td>
<td>F</td>
<td>206.0</td>
<td>1.00</td>
<td>46,XX, del(1)(q21;23)(4)/46,XX(6)</td>
<td>3.1</td>
<td>122</td>
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<tr>
<td>18</td>
<td>Early pre-B</td>
<td>4.1</td>
<td>F</td>
<td>3.7</td>
<td>1.00</td>
<td>47,XX, +21,t(11;19)(q11;q15), t(13;22)(q13;q13)(6)/46,XX(17)</td>
<td>5.5</td>
<td>190</td>
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<tr>
<td>19</td>
<td>Pre-B</td>
<td>5.2</td>
<td>M</td>
<td>9.3</td>
<td>46,XY, del(6)(q13q23)(2)/46,XY(6)</td>
<td>245</td>
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<td>20</td>
<td>Early pre-B</td>
<td>5.2</td>
<td>M</td>
<td>46.0</td>
<td>1.00</td>
<td>46,XY, -6, del(6)(q15), del(11)(q21q23), t(10;12)(q24;p13), +der(9)(t9;7)(q34;7)(5)/46,XY, -9, del(11)(q21q23), +der(9)(t9;7)(q34;7)(3)/46,XY(13)</td>
<td>3.5</td>
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<td>21</td>
<td>Pre-B</td>
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<td>M</td>
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<td>1.00</td>
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<tr>
<td>22</td>
<td>Non-T, non-B</td>
<td>3.5</td>
<td>F</td>
<td>5.9</td>
<td>47,XX, -1, -8, +1, +der(11)(t1;1)?; t(36;?)</td>
<td>204</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Early pre-B</td>
<td>10.0</td>
<td>F</td>
<td>172.0</td>
<td>1.00</td>
<td>46,XX, del(13)(q14); t(14)(q11;q32), +der(14)(t8;14)(q11;32)(14)/46,XX(6)</td>
<td>12.1</td>
<td>1,220</td>
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<tr>
<td>24</td>
<td>Early pre-B</td>
<td>6.0</td>
<td>F</td>
<td>12.9</td>
<td>1.00</td>
<td>47,XX, +10t(15;20)(q15;12)(8)/46,XX(12)</td>
<td>10.7</td>
<td>536</td>
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<tr>
<td>25</td>
<td>Pre-B</td>
<td>5.9</td>
<td>M</td>
<td>10.3</td>
<td>1.00</td>
<td>47,XY, -13t(13); t(13)(q13;14)(5)/46,XY(10)</td>
<td>24.6</td>
<td>658</td>
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<tr>
<td>26</td>
<td>Early pre-B</td>
<td>3.9</td>
<td>F</td>
<td>3.8</td>
<td>1.00</td>
<td>46,XX, -11, -12; dup(1)(q21q32), del(5)(q22q33), del(6)(q15), t(5;12)(q15;p13), +der(12)(t12;12)?(p13), +mar(5)/46,XX(18)</td>
<td>4.9</td>
<td>63</td>
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<tr>
<td>27</td>
<td>Early pre-B</td>
<td>6.4</td>
<td>F</td>
<td>36.0</td>
<td>1.00</td>
<td>45,XY, t(7;6)(q13q21)(4)/46,XX(5)</td>
<td>6.3</td>
<td>773</td>
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<tr>
<td>28</td>
<td>Early pre-B</td>
<td>0.1</td>
<td>F</td>
<td>122.8</td>
<td>1.00</td>
<td>46,XX, t(11)(q21;23)(16)/46,XX(4)</td>
<td>6.6</td>
<td>1,426</td>
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<tr>
<td>29</td>
<td>Early pre-B</td>
<td>9.2</td>
<td>F</td>
<td>21.9</td>
<td>1.00</td>
<td>47,XX, -12, -21, +der(12)t(12;12)?(p12;7)?; t(13;22)(q13;22); t(13;22)(q13;22); t(13;22)(q13;22)</td>
<td>2.3</td>
<td>154</td>
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<tr>
<td>30</td>
<td>Pre-B</td>
<td>1.3</td>
<td>M</td>
<td>34.6</td>
<td>46,XY, t(11)(q19;11)(q23;p13)(2)/46,XX(18)</td>
<td>326</td>
<td>326</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Early pre-B</td>
<td>6.8</td>
<td>F</td>
<td>10.4</td>
<td>1.00</td>
<td>46,XX, del(6)(q15); t(8;14)(q11;ql1)(q21)(4)/46,XX(12)</td>
<td>4.2</td>
<td>391</td>
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<tr>
<td>32</td>
<td>Pre-B</td>
<td>2.1</td>
<td>F</td>
<td>63.9</td>
<td>1.00</td>
<td>46,XX, -12, +der(12)(t12;12)?(p11;7)(3)/46,XX(21)</td>
<td>3.6</td>
<td>49</td>
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</table>

Age and WBC values are at diagnosis.

Levels of MTXPGs > 500 pmol/10⁹ cells were considered to be very high, while levels > 2,000 pmol/10⁹ cells were considered to be very high.

Because the ability of lymphoblasts to accumulate MTX and metabolize it to MTXPGs in vitro decreases if incubation is delayed more than 24 hours, incubation of fresh bone marrow samples with MTX was performed in the reference laboratory (McGill University) and in four new laboratories: the Clinical Immunology Laboratory, Montreal Children's Hospital, McGill University; Midwest Children's Cancer Center, Milwaukee, WI; Baylor College of Medicine, Houston, TX; and the University of Alberta, Edmonton, Alberta, Canada.

Establishment of this methodology in these new laboratories involved a written protocol, frequent telephone calls, and exchange and testing of culture media and buffers with the reference laboratories.
laboratory. It was validated by new laboratories demonstrating the ability to incubate L5178Y mouse leukemia cells successfully with $^3$H-MTX. Successful incubation resulted in uptake of 2,000 to 4,000 pmol MTX and MTXPGs/10^9 cells and formation of MTXPGs containing predominantly 4 glutamyl residues. For this study, patient results were judged valid if the lymphoblast MTXPG level was > 100 pmol/10^9 cells and/or the predominant MTXPG had 5 glutamyl residues.

Quality control and validation studies by the reference laboratory demonstrated that culture medium containing $^3$H-MTX was stable for 2 years when stored at -20°C. Exposure of lymphoblasts to HBSS buffer at alkaline pH during washing before cell incubation, an interval of 30 minutes, markedly decreased MTX uptake. This was due to suboptimal removal of HBSS from the cell pellet during washing. With removal of all but 50 to 100 L of buffer, elevated MTX levels were no longer noted. Nevertheless, a fourth wash was added to ensure effectiveness of washing of later samples. No difference in levels or chain-length distribution of MTXPGs was seen when samples were split and washed optimally and suboptimally, demonstrating that measurement of MTXPGs was not affected by high levels of MTX in the sample.

Five samples were shipped to the reference laboratory in less than 24 hours and were incubated successfully with $^3$H-MTX (patients no. 5, 7, 19, 25, and 39; Tables 1 through 3). Despite apparent technical success, the use of several laboratories to measure lymphoblast MTX and MTXPG levels in the present study is likely to have introduced variables not present in the earlier single institution study.

Statistical methods. Associations between MTXPG levels and quantitative covariates were conducted via the Spearman correlation analysis. Associations between MTXPG levels and qualitative factors (sex and diagnosis) were conducted by the Wilcoxon rank sum test. Regression analysis comparing percentage of cells in S-phase and MTXPG levels was conducted by standard methods. Unless otherwise stated, p values are based on two-tailed tests. Because of the skewed nature of the data regarding MTXPGs (Fig 1) and other parameters, medians and intraquartile (25% to 75%) ranges are reported, rather than means and standard deviations.

RESULTS

Karyotypic findings and results of incubation of lymphoblasts with 1.0 μmol/L $^3$H-MTX were available in 48 of the 80 patients with B-lineage ALL from whom bone marrow samples were obtained at the time of diagnosis. High MTX levels were present in 19 of these, due to incomplete removal of extracellular MTX from washed cells (see Patients and Methods). Therefore, lymphoblast MTX levels could not be analyzed in these patients. However, MTXPG levels were available in these 48 patients and were the subject of this analysis. Patients were divided on the basis of ploidy into 13 with hyperdiploid, 19 with other aneuploidy, and 15 with diploid. The 48th patient was a black male, aged 3.1 years, with early pre-B-cell ALL who had a near-haploid karyotype, comprised of two clones (27, X, +Y, +14, +18, +21 and 54, XY, +X, +Y, +14, +14, +18, +18, +21, +21 present in 5 and 8 of 13 cells analyzed, respectively), and a DI of 0.57. His initial WBC was 35.3 x 10^9/L and his lymphoblast MTX and MTXPG levels were 189 and 2,645 pmol/10^9 cells, respectively, with the predominant MTXPG having 5 glutamyl residues.

Patients with hyperdiploid ALL. There were 13 patients with a hyperdiploid clone containing > 50 chromosomes (Table 1). Four patients had translocations in addition to hyperdiploidy (patients no. 2, 3, 6, and 13) and four had structural changes other than translocations (patients no. 1, 2, 10, and 13). Eleven of the 13 patients had high lymphoblast MTXPG levels of > 500 pmol/10^9 cells, and 5 patients had very high MTXPG levels of > 2,000 pmol/10^9 cells (Fig 1). The median MTXPG level was 1,095 pmol/10^9 cells and the 25% to 75% intraquartile range was 571 to 2,346 pmol/10^9 cells.

Chromosome trisomies were nonrandom, with preferential involvement of several specific chromosomes (X, 4, 6, 7, 10, 14, 17, 18, and 21; Table 1). Although none of the patients had Down's syndrome, two or more extra copies of chromosome 21 were present in the leukemic cells of 11 patients. Trisomies were not observed of chromosome 9, on which the gene for FPGS is located. Eight patients had trisomies of both chromosomes 4 and 10.

The DI was > 1.16 in 8 of the 11 patients in whom it was measured (Table 1). Of these eight patients, six had MTXPG levels > 500 and four had levels > 2,000 pmol/10^9 cells.

Patients with other aneuploid ALL. Nineteen patients had other aneuploidy by cytogenetic analysis (Table 2). Seven patients showed multiple abnormalities of chromosomal structure and number. Six patients had 47 chromosomes and one had 45 chromosomes. Fifteen patients had translocations. Other abnormalities included inversions, deletions, duplications, derivatives, and markers (Table 2). The median and intraquartile range of MTXPG levels for these 19 patients were 326 and 159 to 775 pmol/10^9 cells.

![Fig 1. Levels of MTXPGs in lymphoblasts in B-lineage childhood ALL by ploidy. Groups are hyperdiploid (51 to 65 chromosomes with and without structural changes); other aneuploid (46 to 47 chromosomes with structural changes); and diploid (46 normal chromosomes). Bars are median levels.](www.bloodjournal.org)
Eight patients had MTXPG levels > 500 pmol/10^9 cells. None had levels > 2,000 pmol/10^9 cells (Fig 1).

Patients with diploid ALL. Fifteen patients had a normal diploid karyotype. Their clinical and diagnostic features are shown in Table 3. The median and intraquartile range of MTXPG levels were 393 and 204 to 571 pmol/10^9 cells. Five patients had MTXPG levels > 500 pmol/10^9 cells. None had levels > 2,000 pmol/10^9 cells (Fig 1).

MTXPG levels and ploidy. Three-way comparison of MTXPG levels in hyperdiploid (A) versus other aneuploid (B) versus diploid (C) ALL showed a significant relationship (P = .0015). This was true as well for two-way comparisons: (A) versus (B), P = .002; (A) versus (C), P < .001; and (A) versus (BC), P < .001. A similar relationship was found between high MTXPG levels and DI > 1.16 (P = .009, three-way comparison). Despite the strong correlation between hyperdiploidy and increased accumulation of MTXPGs in lymphoblasts in vitro, there were no significant correlations between MTXPG levels and age (P = .18), sex (P = .85), WBC (P = .80) and diagnosis (early pre-B vs pre-B ALL) (P = .62), or with hemoglobin level (P = .35) and platelet count (P = .97) (not shown) at diagnosis.

Relation of MTXPG chain-length to ploidy. The distribution of MTXPGs by chain-length in lymphoblasts from patients with high hyperdiploid, other aneuploid, and diploid ALL is shown in Table 4. The predominant form was MTXGlul in all ploidy groups. Long-chain MTXPGs accounted for more than 60% of total MTXPGs. There was a trend to shorter chain-length MTXPGs in hyperdiploid, based on percent MTXGlul (P = .025, three-way comparison; P = .063, hyperdiploid vs not). No significant difference was found comparing percent MTXGlul+4+5+6 by ploidy.

MTXPG level and percentage of S-phase cells. The accumulation of high levels of MTXPGs in hyperdiploid lymphoblasts might be explained by their increased proliferative activity. The relationship of proliferative activity of the leukemic cells to the extent of accumulation of MTXPGs in lymphoblasts was assessed by comparing these measurements in the 40 patients in whom both determinations were available. There was a positive ordinal relationship between these two measures (P = .0012 by Spearman association). However, there was too much scatter to consider either variable as a useful predictor of the other. For example, on the basis of ordinary linear regression, only 7% of the variation of one measure could be explained by the other (R^2 = 7%). Of several transformations of the data attempted, the highest R^2 value was 16%, which occurred when the log of MTXPG levels were plotted against percentage of cells in S-phase (Fig 2). These findings indicated that the elevated MTXPG levels could not be explained by the proliferative function of the leukemic cell population.

DISCUSSION

Recent studies have highlighted the prognostic significance of cytogenetic abnormalities in childhood ALL.23-32 A striking finding has been the good prognosis associated with hyperdiploidy.23-28,30-32 Pui et al reported on 138 children with hyperdiploid with 4 years of follow-up. They suggested an 80% EFS for those with numeric changes only and 60% EFS for those with both numeric and structural changes. Rivera et al reported 86% 4-year EFS in 74 children with hyperdiploid ALL. MTX was a major component of continuation and central nervous system therapy in many of these studies.23,25,26,30,32,45 Interestingly, Fletcher et al found no favorable prognostic role for hyperdiploidy. They reported an overall 5-year EFS of 78% for 165 patients. For hyperdiploid, pseudodiploid, and diploid subgroups, 5-year EFS were 80%, 73%, and 81%, respectively. They attributed their excellent results in these and other cytogenetic groups to intensive chemotherapy, which included MTX during induction, intensification, and sanctuary therapy.47 Hyperdiploidy, measured by flow cytometry and expressed as a DI > 1.16, also carries an excellent prognosis relative to other ploidy groups.33-36 Look et al compared results in children who received intrathecal MTX plus high-dose MTX infusions with those who received cranial radiation therapy and sequentially administered pairs of drugs as intensification therapy. The beneficial effect of hyperdiploidy was much greater in those who received

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>No. of Samples</th>
<th>MTXGlu1 (%)</th>
<th>MTXGlu2 (%)</th>
<th>MTXGlu4 (%)</th>
<th>MTXGlu5 (%)</th>
<th>MTXGlu6+15+16 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid</td>
<td>13</td>
<td>12 (7-14)</td>
<td>27 (18-31)</td>
<td>27 (24-30)</td>
<td>34 (26-40)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td>Other aneuploid</td>
<td>19</td>
<td>9 (4-13)</td>
<td>19 (14-24)</td>
<td>25 (21-27)</td>
<td>41 (35-48)</td>
<td>4 (2-7)</td>
</tr>
<tr>
<td>Diploid</td>
<td>15</td>
<td>12 (10-19)</td>
<td>22 (14-25)</td>
<td>26 (23-30)</td>
<td>31 (26-45)</td>
<td>2 (0-4)</td>
</tr>
</tbody>
</table>

Values are median and 25% to 75% intraquartile ranges. Sums of median percents do not equal 100.

*MTXGlu1+4 are MTXPGs with 2 to 6 glutamyl residues.
parenteral intensification therapy with MTX, with a 5-year EFS of 82% (95% confidence limits, 65% to 98%). This analysis suggested that MTX may be important to the cure of hyperdiploid ALL. Recently, Trueworthy et al showed that a DI > 1.16 was the most important single prognostic feature defining excellent outcome in children with ALL treated with a regimen containing intensive MTX treatment during continuation therapy.²⁶ Further, Pui et al showed that a DI > 1.16 was the single persisting prognostic factor that defined success of treatment after discontinuation of chemotherapy.⁴⁹ Again, MTX was an important component of therapy of these patients.³⁴-³⁶,⁴⁹

To confirm and extend our previous findings linking combined lymphoblast MTX and MTXPG levels with EFS,³⁹ we compared MTXPG levels in hyperdiploid with those in other aneuploid and diploid lymphoblasts. Unfortunately, MTX levels could not be analyzed (see Patients and Methods), resulting in an incomplete picture of MTX metabolism. However, hyperdiploid patients had significantly higher MTXPG levels (P = .0015) than patients with other aneuploid and with diploid lymphoblasts (Fig 1). Furthermore, nearly half of the patients with hyperdiploidy had very high MTXPG levels, ie, > 2,000 pmol/10⁸ cells, a feature unique to these patients (Fig 1). These findings provide additional evidence linking MTX metabolism in lymphoblasts at diagnosis with response to multidrug antileukemic therapy in which MTX is a prominent component. Patient follow-up is too short in the present study to permit comparison of findings with therapeutic outcome.

There was a trend to shorter MTXPG chain-lengths in hyperdiploidy (Table 4). If confirmed in a larger series of patients, this finding might reflect altered substrate concentrations or FPGS activity.⁸

The mechanism for these high and very high MTXPG levels is unknown. Trisomies of chromosomes in our patients with hyperdiploidy were nonrandom. Frequent trisomies were similar to those reported by others.³⁷ MTXPGs are synthesized by FPGS, which is encoded by a gene located on chromosome 9.⁴⁴ Whereas trisomy of chromosome 9 was present in other patients with hyperdiploidy,³⁴ it was not present in any of our 13 patients (Table 1). Therefore, there is no evidence that an increased FPGS gene dosage explains the increased accumulation of MTXPGs in hyperdiploid ALL. Harris et al have shown that children with B-progenitor cell ALL with trisomies of both chromosomes 4 and 10 have a very low risk of treatment failure.⁵⁰ Eight of our patients had these two trisomies (Table 1). Of these, seven had high MTXPG levels and four had very high MTXPG levels. It is tempting to speculate that genes whose products promote FPGS gene expression may be located on one or both of these chromosomes.

Proliferating cells accumulate higher levels of MTXPGs than do resting cells.³¹-³³ As previously described,³³ we found an increase in percent S-phase cells in hyperdiploid compared with other aneuploid and diploid blasts (Fig 2). If this increase in percent S-phase cells reflects increased proliferative activity, rather than slower passage of blasts through S-phase,³³ then increased proliferative rate may contribute to increased accumulation of MTXPGs. However, the association was weak, suggesting that other factors exist and play a more prominent role. Some of these factors may be identified by measuring the rate of blast cell uptake of MTX, the intracellular level of MTX, and the activities of FPGS and gammaglutamyl hydrodases in lymphoblasts. Gammaglutamyl hydrodases are enzymes that hydrolyze folate and MTX polyglutamates to monoglutamate.³⁴,³⁵

Several mechanisms have been advanced to explain why hyperdiploidy confers a better prognosis than pseudodiploidy or diploidy. An increase in the number of cells in S-phase due to either increased proliferative rate or increased residence of cells in S-phase may make these blast cells more sensitive to cell-phase-specific drugs, such as vincristine, 6-mercaptopurine, and MTX. Hyperdiploid cells have a tendency toward terminal differentiation and therefore are more sensitive to corticosteroid treatment.³⁵ Tsuchiya et al have suggested that extra chromosomes may moderate the tumorigenicity of malignant cells, either through the action of putative antioncogenes or by altering the balance of the number of chromosomes with and without oncogenes.

The present findings, together with the clinical findings of Look et al and the observation by Whitehead et al linking high lymphoblast MTX and MTXPG levels and improved EFS, suggest an additional possibility. It is likely that the ability of hyperdiploid lymphoblasts to accumulate high and very high levels of MTXPGs makes them particularly sensitive to MTX cytotoxicity. This may explain in part the apparent improved outlook for hyperdiploid patients treated with regimens emphasizing MTX as a primary component of continuation therapy.

REFERENCES

Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): A Pediatric Oncology Group study. Blood 76:117, 1990


43. Ott RL: An Introduction to Statistical Methods and Data Analysis. Boston, MA, Duxbury, 1984, p 190


Accumulation of high levels of methotrexate polyglutamates in lymphoblasts from children with hyperdiploid (greater than 50 chromosomes) B-lineage acute lymphoblastic leukemia: a Pediatric Oncology Group study

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