Serum Lactic Dehydrogenase Level Has Prognostic Value in Childhood Acute Lymphoblastic Leukemia

By Ching-Hon Pui, Richard K. Dodge, Gary V. Dahl, Gaston Rivera, A. Thomas Look, David Kalwinsky, W. Paul Bowman, Judith Ochs, Minnie Abromowitch, Joseph Mirro, and Sharon B. Murphy

Serum lactic dehydrogenase (LDH) levels were measured at diagnosis in 293 children with "standard-risk" acute lymphoblastic leukemia (ALL) to determine the prognostic value of this biologic feature. Standard risk assignment was based on an initial leukocyte count of <100 x 10^9/L, the absence of a mediastinal mass, the absence of meningeal involvement, and the presence of lymphoblasts lacking sheep erythrocyte receptors or surface immunoglobulin. Serum LDH levels ranged from 97 to 6,595 U/L, with a mean of 547 U/L. Higher LDH levels were associated with higher leukocyte counts, lower blast cell DNA indices, lower platelet counts, a larger spleen size, and nonwhite race. LDH levels were not related to the percentage of marrow S-phase cells, liver size, French-American-British (FAB) classification, hemoglobin levels, age, sex, or the presence of the common ALL antigen on marrow blasts. PatiCients with the highest LDH levels (>1,000 U/L) were most likely to fail treatment, whereas those with the lowest levels (<300 U/L) had the lowest risk of failure (P < .0001). The prognostic significance of serum LDH level was retained in a subset of patients that included only those with leukocyte counts <25 x 10^9/L (P = .0018). When 11 presenting characteristics were subjected to multivariate analysis, serum LDH level was found to have independent prognostic strength, contributing clinically important information to that gained from leukocyte count. Early measurement of serum LDH could be useful in identifying a group of standard-risk ALL patients with a high relapse hazard.

MATERIALS AND METHODS

Patients and treatment. Serum LDH levels were measured in 293 (89%) of 330 children with newly diagnosed ALL who were admitted to Total Therapy Study X for standard-risk ALL. They had an initial leukemia count <100 x 10^9/L, no mediastinal mass, no initial CNS disease, and lymphoblasts lacking surface immunoglobulins or sheep erythrocyte receptors. Informed consent was obtained for all patients, and the investigation was approved by the institution's clinical trials committee.

All patients received remission induction therapy with prednisone, vincristine, and asparaginase, and then were randomized into two groups. One received cranial irradiation (1,800 rad) plus intrathecal methotrexate, followed by three different drug pairs administered in a rotational manner for 18 months: 6-mercaptopurine and methotrexate (week 1 through 36), cyclophosphamide and doxorubicin (week 36 through 54), and teniposide and cytarabine (week 54 through 72). The other group received intermediate-dose methotrexate (1 g/m^2) plus intrathecal methotrexate weekly x 3, followed by a standard backbone of oral daily 6-mercaptopurine and weekly methotrexate, interrupted every six weeks for an intermediate-dose methotrexate pulse in the first 18 months. Both regimens concluded with 12 months of oral 6-mercaptopurine and methotrexate.

Serum LDH determination. Total serum LDH activity was measured with the Monitor Kinetic AMB-610 assay on the KDA analyzer (American Monitor Corp, Indianapolis). Samples with enzyme activities greater than 700 U/L were diluted and reassayed; the values obtained were then multiplied by the dilution factor. The normal values for our laboratory range from 30 to 300 U/L.

Lymphoblast phenotype. Leukemic blast cells from bone marrow aspirates were separated on a Ficoll-Hypaque gradient and studied for spontaneous rosette formation with sheep erythrocytes incubated at 37 °C. Cell surface antigens were detected by a standard indirect immunofluorescence assay with monoclonal antibodies. Cell samples were classified as common, T cell, or undifferentiated, based on the pattern of lymphoblast surface markers. DNA content determinations. Leukemic marrow samples were stained with a DNA-specific dye, propidium iodide, and were analyzed by flow cytometry as previously described. Histograms of cellular DNA content were analyzed for percentages of S-phase cells. The DNA index (ratio of DNA content in leukemic G0/G1 cells vs normal diploid G0/G1 cells) was determined for the same cell.

The ability to identify, at diagnosis, subsets of patients with acute lymphoblastic leukemia (ALL) who have a better or worse prognosis than average has contributed greatly to the success of modern treatment programs and to the definition of the risk–benefit ratio. Nonetheless, there remains a need to account for the disparate treatment responses of patients who, by established risk criteria, should have similar clinical courses.

Serum lactic dehydrogenase (LDH) activity is increased in most patients with ALL,3,13 or non-Hodgkin's lymphoma.4–11 Although levels of the enzymes are recognized to have prognostic importance in both children and adults with lymphoma,4–11 little is known about their significance in patients with ALL. In one recent study of 66 children with ALL,13 increased serum LDH levels correlated with higher initial leukocyte counts but not with treatment outcome, possibly because the patients were treated over a ten-year period and may not have received uniform therapy. Here we report serum LDH findings for 293 children with standard-risk ALL who were entered in a single clinical trial comparing methods of continuation chemotherapy. The results of multivariate analysis indicate that serum LDH has independent predictive value, adding information to that provided by leukocyte count, age, race, French-American-British (FAB) classification, liver size, and spleen size—the standard measures of prognosis in childhood ALL.

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samples. This measure correlates closely with chromosome number (ploidy); hence, leukemic cells with a normal chromosome complement have a DNA index of 1.0.

**Statistical analysis.** The distributions of serum LDH levels in the two treatment groups were similar, so that data for all patients were pooled for analysis. Mean serum LDH levels for different subsets of patients were compared statistically by standard analysis-of-variance (ANOVA) procedures. Time-to-failure curves were constructed by the Kaplan-Meier procedure and the results were compared by the Cox-Mantel test. Time to failure is defined as the interval between achievement of remission and relapse or death due to any cause. Patients who failed to enter remission were considered to be failures and were assigned a failure time of zero.

The influence of potentially significant prognostic factors on time to failure was estimated with the Cox proportional-hazards model, which permits comparison of treatment outcome for two or more subsets of patients while simultaneously adjusting for the effect of other factors (covariates) in the model. Covariates whose values show extreme variability (eg, leukocyte count and LDH levels) can be accommodated in the Cox model by using the natural logarithm. Because cell phenotype data and percentage of S-phase cells were not available for a substantial proportion of patients and individually were not important prognostic factors (data not shown), they were excluded from analysis in the Cox model. A second analysis with the Cox model was performed to accommodate DNA index, a feature of known independent significance that was available for a subset of patients having flow cytometric measurements of blast cell DNA content.

**RESULTS**

Serum LDH levels for the 293 patients ranged from 97 to 6,595 U/L with a mean of 547 U/L. In general, patients with higher LDH levels had higher leukocyte counts, lower DNA index, lower platelet counts, and a larger spleen size (Table 1), but there was no obvious relationship between LDH findings and the percentage of S-phase cells, liver size, FAB classification, hemoglobin level, age, sex, or the presence or absence of common acute lymphoblastic leukemia antigen (CALLA) on lymphoblasts. Nonwhite patients appeared to have higher LDH levels than did white patients, but this impression could not be substantiated statistically.

To assess the influence of LDH level on treatment outcome, we arbitrarily divided the patients into three groups defined by LDH levels: <300, 300 to 1,000, and >1,000 U/L. Two patients refused treatment shortly after the diagnosis; hence, 291 patients were evaluable for the treatment response. As depicted in Fig 1, children with higher LDH levels were more likely to fail. Those with the highest values, >1,000 U/L, had poorer outcomes than those with intermediate values, 300 to 1,000 U/L (P = .01), who in turn fared worse than patients having the lowest values, <300 U/L (P = .0038). Similar relationships were found when the analysis was limited to patients with initial leukocyte counts <25 × 10^9/L (Fig 2).

Because of the recognized interrelationships among various clinical and biologic risk factors in childhood ALL, we determined the impact of serum LDH level on time to failure while simultaneously adjusting for the effects of other covariates. When tested as single regressor variables in the Cox model (without inclusion of DNA index), serum LDH level,
leukocyte count, age, race, liver size, platelet count, and spleen size each showed a significant ($P < .10$) relationship to treatment outcome (Table 2). In the multivariate model, serum LDH level retained its original predictive status, whereas the remaining covariates showed decreased significance. The prognostic information provided by leukocyte count, platelet count, and spleen size was not important in the model. When the same analysis was performed for a subset of patients ($n = 194$) with cellular DNA content determinations, serum LDH levels still showed independent prognostic value (Table 3).

Table 2. Relationship of Serum LDH Level and Other Presenting Features With Time to Failure in 283 Patients

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LDH, serum lactic dehydrogenase.

*From the likelihood ratio test.

†Comparison of time to failure, using the Cox proportional hazards model, for patients with the better-worse category of the indicated feature, without adjustment for the effects of other variables.

‡As above, except that stepwise regression analysis was used to identify the best predictors of treatment outcome, taking into account the competing effects of all ten covariates entered in the Cox model. A feature had to attain a significance level of .10 to be retained in the model.

DISCUSSION

Lactic dehydrogenase, a pyridine-linked enzyme found in virtually all animal tissues, functions primarily in the metabolism of glucose, catalyzing the reduction of free pyruvate to lactate during the last step of glycolysis, as well as the conversion of lactate to pyruvate during gluconeogenesis. Its concentration is highest in liver followed in descending order by skeletal muscle, heart, and kidney. Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly integrated; hence, the cells tend to utilize from five to ten times as much glucose as do normal tissues, converting most of it into lactate. The increased serum levels of LDH generally found in cancer patients reflect greater production and release of the enzyme by malignant cells or alterations in the metabolic interplay among organs in which LDH exists in high concentrations, or perhaps a combination of the two, is not clear.

Our results (Table 1) show that serum LDH levels in children with ALL are roughly correlated with leukocyte counts but not with the percentages of blast cells in S-phase. This suggests that the total body burden of leukemic cells is a more important determinant of serum LDH level than the cell proliferative rate. Certain pathologic conditions other than cancer (eg, hepatitis and hemolytic anemia) are known to cause an increase in serum levels of LDH, but none of them appears to have influenced the findings reported here. Two of our patients had hepatitis at diagnosis, but their serum LDH levels (251 and 409 U/L) were not remarkably different from normal. By contrast, bone marrow necrosis may have contributed to the high LDH levels (1,189 and 1,290 U/L) noted in two other patients.

It should be stressed that this study dealt with a relatively homogeneous group of patients—those with a standard risk of
treatment indicative of poor-prognosis ALL (e rosette formation, leukocyte count >100 x 10^9/L, central nervous system (CNS) involvement or a mediastinal mass), the predictive strength of serum LDH level may have been altered. In fact, in preliminary studies (C.-H. Pui, unpublished [four-year] observations), such patients had significantly higher serum LDH levels than did those without high-risk features, most likely because of the greater bulk of their disease.

Clinical investigators have recognized for many years that patients with the same form of ALL can have entirely different responses to treatment. In multivariate analyses, leukocyte count and other presenting characteristics that are linked to the extent of disease at diagnosis have consistently emerged as the best predictors of treatment outcome. Nonetheless, a substantial proportion of patients with low leukocyte counts fail in modern treatment programs. Of the 17 children in this study with counts <25 x 10^9/L, but serum LDH levels >1,000 U/L, nine have already had an adverse event, and only 20% are projected to be in continuous complete remission at 3.5 years (Fig 2). We cannot explain with certainty the prognostic value of the serum marker in the absence of a high leukocyte count, but think it noteworthy that five of the nine patients who failed had markedly enlarged livers and spleens or massive nephromegaly at diagnosis. Serum LDH level seems to provide a more reliable estimate of the total body burden of leukemic cells than does the leukocyte count alone. Results of the multivariate Cox analysis (Table 2)—in which serum LDH level and liver size, but not leukocyte count, retained prognostic significance after adjustment for other covariates—support this idea.

Recent reports have indicated that the chromosomal abnormalities of leukemic blast cells underlie patient characteristics such as leukocyte count, age, and sex, and may be responsible for different treatment outcomes in certain subsets of patients. The results of the present analysis confirm the prognostic strength of DNA index or cellular DNA content, a measure of chromosome number. Nonetheless, serum LDH level emerged with independent predictive value in a mathematical model that included DNA index. From a practical standpoint, this serum measurement is routinely available for all newly diagnosed leukemia patients and is quite inexpensive to perform, in contrast to cytogenetic or flow cytometric studies, which may not be available in all treatment centers.

Finally, we recognize that intensification of therapy can abolish the effects of many conventional prognostic features. Whether this applies to serum LDH level will require additional study. If our findings are substantiated, serum LDH level could be a useful addition to existing systems of risk assignment in childhood ALL.

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