Profiles and prognostic values of serum LDH isoenzymes in patients with haematopoietic malignancies

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Abstract

Serum lacticodehydrogenase (LDH) is commonly increased in patients with haematopoietic malignancies and has been shown to be a prognostic factor in patients with non-Hodgkin's lymphoma (NHL) and myeloma. We have examined the LDH isoenzyme content in serum of 326 patients, including 252 patients with NHL (202 at diagnosis and 50 at relapse), 28 patients with Hodgkin's disease, 17 patients with CLL, 16 patients with myeloproliferative syndromes and 13 patients with multiple myeloma. Among these, 160 pts (49%) had increased serum LDH. The analysis of LDH isoenzyme profiles in all patients showed increased percentages of isoenzyme 2 in patients with NHL, CLL and myeloproliferative syndromes, but not in samples from patients with myeloma or Hodgkin's disease. Isoenzyme alterations were then analyzed for their prognostic value in patients with NHL. In univariate analyses, increased isoenzyme 2 percentages, increased isoenzyme 3 values, total serum LDH, performance status, stage and tumour aggressiveness were prognostic variables for survival. In a multivariate analysis increased LDH isoenzyme 3 values, high isoenzyme 2 percentages and the performance status, but not total serum LDH, were independent prognostic factor for survival. High isoenzyme 3 values were predictive of early death in NHL patients. In patients with relapsing NHL, the overall survival was 12 months in patients with normal isoenzyme 3 but only 2 months in patients with increased isoenzyme 3 values. We conclude that there are characteristic alterations in serum LDH profiles in patients with haematopoietic malignancies and that some of these may be more interesting in terms of prognostic value than total serum LDH.
Introduction

Serum LDH is a useful prognostic marker in hematological malignancies, including NHL and myeloma (1, 2). It is also frequently increased in patients with myeloproliferative syndromes, and seldom increased in patients with CLL or Hodgkin's disease. In patients with NHL, total serum LDH is one of the components of the International Prognostic Index, which is a strong predictor of survival (3, 4). However little is known concerning the serum content in LDH isoenzymes, or the significance of altered isoenzymes in these situations. Increased serum LDH can be encountered in a great many situations unrelated to neoplasia. Our aim was to determine whether characteristic alterations in LDH isoenzymes were observed in patients with haematopoietic malignancies, and whether these may have prognostic value.

Lactic dehydrogenase catalyses a critical step in the glycolysis pathway, the reversible transformation between pyruvate and lactate. LDH is a tetramer composed of two types of monomers, H (for "heart", also designated as "B"), and M (for "muscle", also designated as "A") (6). The five isoenzymes of LDH can be distinguished by gel electrophoresis, and correspond to the various combinations of these monomers: isoenzyme 1 (H4), isoenzyme 2 (MH3), isoenzyme 3 (M2H2), isoenzyme 4 (M3H), isoenzyme 5 (M4). The forms with a high H content are found in tissues with a steady oxygen supply (heart, brain), whereas tissues which produce large amounts of lactic acid, such as muscle, are rich in M monomer. The ratio of H and M monomers has been reported to be regulated by the state of hypoxia in tissues (5). Various cytokines have been reported to alter the LDH isoenzyme profile by enhancing synthesis of one of the monomers (6, 7).

LDH isoenzyme alterations have been studied in a number of solid tumours (8-11). Serum LDH isoenzymes have also been analyzed in hematological malignancies (12-14). Increased isoenzyme 3 content has been reported in patients with multiple myeloma, and chronic granulocytic leukaemia. Patel et al. have reported that an increase in LDH isoenzyme 2 values was observed in 84% of patients with untreated leukaemia (15). We have previously reported that isoenzyme 2 is frequently increased in the serum of patients with NHL, but not in the CSF, even in case of meningeal involvement (16).

In this report, we have analyzed the serum LDH profiles in patients with various haematopoietic malignancies. We have also analyzed whether some of these alterations may be useful prognostic value by confronting these characteristics with other well known prognostic factors such as tumour stage or aggressiveness, and patient performance status and performing univariate and multivariate analysis for overall survival in patients with NHL.
Material and Methods

Patients and samples
Serum samples were drawn from 326 patients treated at the Department of Hematology in the Centre Hospitalier Lyon Sud between January 1996 and December 1998. Characteristics of patients at diagnosis are shown in Table 1. Diagnoses included 252 patients with NHL (202 at diagnosis and 50 relapsing patients), 28 patients with Hodgkin's disease, 17 patients with CLL, 16 patients with myeloproliferative syndromes and 13 patients with multiple myeloma.

One hundred and sixty patients (49%) had increased serum LDH. The median age was 60 years (range 15-89). None of these patients had an obvious reason to have increased LDH when serum was drawn (hemolysis, pulmonary embolism, myocardial infarction, post-surgical period). Among the patients with NHL, 139 were considered to have aggressive histology (diffuse large cell, Burkitt or lymphoblastic lymphoma), 187 had disseminated disease (stage 3 or 4), and 166 had a good ECOG performance status (PS 0 or 1).

Assay of enzyme activity and isoenzyme profiles
Total LDH activity was determined by a routinely used spectrophotometric method (Boehringer Kit) in a discrete analyzer (Hitachi 917 Boehringer). The extinction was automatically recorded at 340 nm. LDH isoenzymes were separated by agarose gel electrophoresis (Titan gel LD-kit isoenzyme procedure, Helena Laboratories, Beaumont, TX, USA). Lactate was used as the substrate to detect enzyme activity. LDH isoenzymes were visualized by nitroblue tetrazolium reduction to formazan. The gels were scanned with an integrated densitometer (Cellosystem 2, Sebia, Issy-l’És-Moulineaux, France) for determination of the different LDH isoenzyme activities. Normal LDH isoenzyme percentages for our laboratory were the following: isoenzyme 1: 13-28%; isoenzyme 2: 27-38%; isoenzyme 3: 21-31%; isoenzyme 4: 6-16%; isoenzyme 5: 4-17%.

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>326</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Non Hodgkin's lymphoma</td>
<td>252</td>
</tr>
<tr>
<td>- At diagnosis</td>
<td>- 202</td>
</tr>
<tr>
<td>- At relapse</td>
<td>- 50</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukaemia</td>
<td>17</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>13</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
<td>16</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>28</td>
</tr>
<tr>
<td>Increased total serum LDH</td>
<td>160 (49%)</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics


**Statistical analysis**

Comparison of isoenzyme 2 contents according to performance status, stage distribution, indolent vs. aggressive histology, and increased vs. normal total serum LDH were performed using a non-parametric Chi square test. Overall survival was defined as the interval between diagnosis and death or loss to follow-up. Survival was analyzed according to the method of Kaplan and Meier and differences between survival curves were evaluated with the log-rank test for univariate analyses. Characteristics independently associated with death were identified by proportional hazards regression analysis using the Cox model. Total serum LDH, isoenzyme 2 percentages and isoenzyme 3 LDH values were considered as dichotomic variables (normal or increased). Statistical analyses were performed using Statistica software.
Results

Serum isoenzyme profiles in patients with haematopoietic malignancies

When considering the entire patient population, the analysis of LDH isoenzyme profiles showed increased percentages of isoenzyme 2 in patients with NHL, CLL and myeloproliferative syndromes, but not in samples from patients with myeloma or Hodgkin's disease (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>NHL</th>
<th>CLL</th>
<th>MPS</th>
<th>Myeloma</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>35.9 ± 7.32</td>
<td>35.5 ± 7.07</td>
<td>35.8 ± 4.56</td>
<td>32.0 ± 6.52</td>
<td>33.0 ± 6.25</td>
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<tr>
<td></td>
<td>(10^-4)</td>
<td>(10^-3)</td>
<td>(10^-4)</td>
<td>(0.56)</td>
<td>(0.51)</td>
</tr>
<tr>
<td>Pts with increased total LDH</td>
<td>39.7 ± 6.96</td>
<td>36.7 ± 11.0</td>
<td>35.8 ± 4.58</td>
<td>32.09 ± 7.12</td>
<td>28 ± 12</td>
</tr>
<tr>
<td></td>
<td>(10^-4)</td>
<td>(0.027)</td>
<td>(10^-4)</td>
<td>(0.67)</td>
<td>(0.002)</td>
</tr>
</tbody>
</table>

Table 2. Mean serum LDH isoenzyme 2 percentages in patients with haematopoietic malignancies.

Values shown concern all patients, including those with normal total serum LDH. NHL: non-Hodgkin's lymphoma; CLL: chronic lymphocytic leukaemia; MPS: myeloproliferative syndromes; HD: Hodgkin's disease. Values shown are means ± S.D. Normal serum LDH isoenzyme 2 percentages are 32.5 ± 2.5. Values in parentheses represent p values obtained when comparing the percentages of patients with normal values.

Among patients with high total serum LDH, those who had abnormally high isoenzyme 2 percentages represented 51% of NHL patients at diagnosis, 69% of NHL patients at relapse, and less than 40% of patients with other diseases.
When considering absolute values of LDH isoenzymes in patients with increased total serum LDH (Figure 1), isoenzyme 2 values were found to be increased in a majority of patients with NHL (both at diagnosis and at relapse), myeloma, and myeloproliferative diseases. Isoenzyme 1 values were found to be frequently increased in patients with NHL and myeloproliferative syndromes. Isoenzyme 3 values were increased in more than 50% of patients with NHL, myeloma, CLL and myeloproliferative diseases.

Correlation of isoenzymes with other prognostic factors in patients with NHL

As shown in Table 3, isoenzyme 3 values were found to be more frequently increased in patients with poor performance status, disseminated disease or aggressive disease. Isoenzyme 2 percentages were more frequently increased in patients with poor performance status. Of note, 94% of patients with increased isoenzyme 3 values had disseminated disease.

Figure 1. Absolute LDH isoenzyme values in patients with various hematological malignancies.

Values shown indicate the percentage of patients in each disease group who have increased absolute values of each isoenzyme. NHL: non-Hodgkin's lymphoma (diag: at diagnosis; rel: at relapse); MM: multiple myeloma; CLL: chronic lymphocytic leukaemia; MPS: myeloproliferative syndromes; HD: Hodgkin's disease.
Prognostic value of serum LDH isoenzymes in patients with NHL

In univariate analyses, total serum LDH, serum isoenzyme 2 percentages and serum isoenzyme 3 values (Figure 2, Figure 3, Figure 4) as well as performance status, tumour stage and histology (data not shown) were prognostic factors for survival in patients with NHL. High isoenzyme 2 percentages and high isoenzyme 3 values remained adversely correlated with survival when considering only patients with high total serum LDH (Figure 5, Figure 6). In a multivariate analysis taking into account all of these parameters, PS (p<10^-5), isoenzyme 3 values (p=0.007), and isoenzyme 2 percentages (p=0.04) retained prognostic value for overall survival, but not total serum LDH.

Table 3. Serum LDH isoenzyme 2 percentages and isoenzyme 3 values in NHL patients according to performance status (PS), stage and histology.

Values shown indicate the number of patients in each group. % iso2: percentage of isoenzyme 2 in total serum LDH; iso3 value: absolute values of isoenzyme 3 LDH in serum. Pts: patients.
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Figure 2. Overall survival in NHL patients according to total serum LDH values.
Dotted lines: patients with normal values; solid lines: patients with increased values.

Figure 3. Overall survival in NHL patients according to serum LDH isoenzyme 2 percentages.
Dotted lines: patients with normal values; solid lines: patients with increased values.
Figure 4. Overall survival in NHL patients according to serum LDH isoenzyme 3 values.
Dotted lines: patients with normal values; solid lines: patients with increased values.
Figure 5. Overall survival in NHL patients according to serum LDH isoenzyme 2 percentages in patients with increased serum LDH.

Dotted lines: patients with normal values; solid lines: patients with increased values.

Figure 6. Overall survival in NHL patients according to serum LDH isoenzyme 3 values in patients with increased serum LDH.
Discussion

Increased total serum LDH are commonly interpreted as reflecting high tumour burden or tumour aggressiveness. High total serum LDH carry a poor prognosis in myeloma, childhood ALL, melanoma, lung adenocarcinoma and colorectal carcinoma (2, 17-20). Increased serum LDH is a major prognostic factor in patients with NHL and total serum LDH is one of the parameters of the International Prognostic Index used in patients with NHL (3).

Although LDH is routinely measured in patients with haematopoietic malignancies, there are very little data regarding the isoenzyme content of LDH in these patients and the prognostic values of specific isoenzyme alterations. Isoenzyme 3 has been reported to be the most abundant isoenzyme in normal lymphoid tissue. Isoenzymes 3 and 4 appear to be the most abundant isoforms in maturing haematopoietic cells (21). Single oral doses of prednisolone have been shown by Ten Berge to modify the LDH isoenzyme patterns of LDH in lymphocytes in vivo, with a significant decrease of the H/M ratio in T lymphocytes (22).

Isoenzyme 1 and 2 percentages are increased in more than 75% of patients with NHL, CLL and myeloproliferative diseases. This "shift" towards the H monomer-rich isoforms of the enzymes may be due to LDH molecules produced by tumour cells in response to increased production of lactic acid. Alternatively these isoforms may be produced as a reaction of the host to disease. Increased isoenzyme 1 (1/2 "flip") is observed in non-neoplastic diseases, such as myocardial and renal infarctions. Isoenzyme 3 being the most abundant form in lymphoid tissues, it is possible that increased values of serum isoenzyme 3 directly reflects the tumour mass in patients with NHL, which would explain why their prognostic value is greater than that of total serum LDH.

We have shown that increased serum LDH isoenzyme 3 values and isoenzyme 2 percentages are independent prognostic factors for overall survival in patients with NHL. These parameters were independent of the performance status and of total serum LDH. Patients with increased isoenzyme 3 values had a 40% risk of death in the first four months of observation. In patients with relapsing NHL, the median survival was only 2 months in patients with increased serum LDH isoenzyme 3 values, as compared to 12 months in patients with normal isoenzyme 3 values (data not shown). We therefore conclude that increased isoenzyme 3 values can be used as a factor of early death in patients with NHL, even in patients with normal total serum LDH.

LDH has been showed to be a simple and useful prognostic marker in patients with NHL. We have shown that specific alterations in serum LDH profiles have prognostic value in these patients. These alterations appear to exist in other haematopoietic malignancies as well. We suggest it would be worthwhile to determine the prognostic value of serum LDH isoenzymes in diseases other than lymphoma.
References


