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Original article

Retrospective study of the prognostic role of serum thymidine kinase level in CLL patients with active disease treated with fludarabine

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Summary

Background: Previous studies have shown that the serum thymidine kinase (TK) level can be used to determine prognosis in patients with lymphoproliferative diseases, but mainly those with multiple myeloma and non-Hodgkin's lymphoma. In patients with chronic lymphocytic leukemia (CLL), TK levels may provide prognostic information independent of stage and other prognostic factors, but it is still unclear whether they can be used to predict the response to treatment and length of survival.

Patients and methods: To determine whether TK levels can be used to predict response and survival, we retrospectively examined the serum TK level in 188 previously treated and untreated patients with active or advanced CLL who were then treated with fludarabine alone or in combination with prednisone. The correlation of the TK level with other prognostic features and with outcome was then assessed.

Results: Serum TK levels were elevated in 92% of the patients, and the levels proved to associate with previous treatment, stage of disease, and other tumor-burden related features (i.e., white blood cell counts, absolute lymphocyte count, bone marrow cellularity). The levels were also directly associated with indicators of tumor cell turnover (i.e., β2-microglobulin and lactate dehydrogenase levels). Of particular importance, we found that the TK level was a significant prognostic indicator of both response to treatment and survival. Specifically, 83% of patients with a TK level of < 10 U/L responded (complete and partial response) to treatment with fludarabine, whereas only 45% of patients with a TK level of ≥ 10 U/l responded to treatment ($P < 0.01$). This difference was maintained when we separately analyzed untreated and previously treated patients, and in patients divided according to the Binet stage. The TK level also added prognostic information about response to a predictive model based on the hemoglobin and, albumin levels and the extent of prior treatment. Of further importance, the median survival rate in patients with a TK level of < 10 U/l was 65%, as opposed to a rate of 22% in patients with a TK level of ≥ 10 U/l ($P = 0.000$).

Conclusions: The serum TK level in CLL patients provides useful prognostic information regarding both response to therapy and length of survival and should be used in planning appropriate therapy. In particular, patients with a TK level of ≥ 10 U/l have a poor prognosis and should be considered for aggressive treatment.

Key words: chronic lymphocytic leukemia, prognostic factors, progression-free interval, serum thymidine kinase

Introduction

Although chronic lymphocytic leukemia (CLL) is often considered an indolent disease, it can have an extremely variable course, with the life expectancy in patients ranging from as long as that in a healthy age-matched normal population to a median of 1.5 years. Because of this variability, in the past few years, several clinical and hematological parameters have been evaluated as possible indicators of prognosis [1].

One such indicator is the thymidine kinase (TK) level. TK is a cellular enzyme involved in a salvage pathway of DNA synthesis, and its level is directly correlated with the proliferative activity of tumor cells [2, 3]. In normal cells, TK activity is present only for a short period in early S-phase, while its activity is much higher in abnormally growing cells [4]. Several studies in patients with lymphoproliferative diseases have shown the prognostic value of this enzyme. For example, in patients with non-Hodgkin's lymphoma [5–9] and multiple myeloma [10], the level of TK correlates with the grade of malignancy, the stage of disease, and the length of survival. In CLL, the TK level correlates not only with the Rai stage but also with the disease status, which in turn has allowed a distinction to be made between aggressive and indolent disease [11]. Recent studies also showed that the serum TK level can be an independent predictor of the duration of the progression-free interval in CLL [12] and it can add information to the definition of smouldering and non-smouldering in early stage CLL [13].

It remains unclear, however, whether the TK level can be used to predict response and the length of survival in patients with CLL. In the present study, we therefore measured the TK level in a cohort of patients with active

or advanced CLL treated with fludarabine, correlated the serum TK level with other presenting features, and assessed the TK level as a prognostic indicator of both response and survival.

Patients and methods

Information on the TK level was available for 188 patients subsequently treated at The University of Texas M.D. Anderson Cancer Center with fludarabine alone or in combination with prednisone. The median age of the patients was 63.5 years, and the male · female ratio was 121 : 67 CLL was diagnosed on the basis of (NCI) criteria [14], and our patient population included patients with advanced disease (Rai stage II, III, or IV) and patients with active disease in the early stage (Rai stage 0, or I). No patients with indolent disease were included.

The median time from diagnosis to treatment was 43 5 months. Fifty patients were untreated; one hundred thirty-eight had received from one to ten (median two) treatments. TK determination was performed just before the start of fludarabine therapy.

Treatment and response evaluation

Most of the patients in this study (n = 130) received fludarabine (30 mg/m^2/day) plus prednisone (30 mg/m^2/day) for five days [15] Others received fludarabine alone (30 mg/m^2) for five days (11 patients) [16], three days (7 patients) [17], or weekly (40 patients) [18].

Response to treatment was assessed according to NCI guidelines [14].

TK determination

Serum TK levels were measured using a commercially available radioenzyme assay based on the method described by Gronowitz et al. [19] that utilizes 125I-iododeoxyuridine as the substrate (Profiligen, TK REA, Sangtec, Sweden). The TK activity was expressed in units per liter, with 5 U/l as the upper level in healthy subjects.

Statistical evaluation

The distribution of the TK levels among groups of patients classified by disease stage or other characteristics was analyzed using the Kruskal–Wallis test [20]. Time to progression and survival curves were estimated by the Kaplan–Meier method [21] Associations between the TK level and the progression-free interval and overall survival were evaluated with the log-rank test [22]. Survival intervals were measured from the first day of treatment until death. Time to progression was measured from the first day of treatment to the date relapse was detected.

Results

TK levels

One hundred seventy-three of the one hundred eighty-eight patients (92%) had an elevated serum TK level, i.e., > 5 U/l. The median TK level for the entire population was 17 U/l, and the mean level was 25 ± 28 (SD) U/l (range 1.8–220.7 U/l). Previously untreated patients had a significantly lower TK level (median 9.4 U/l) than did patients who had received one or more prior treatments (median TK level 19 U/l) ($P < 0.01$).

Table 1 Relationship of TK level to stage of disease.

	Rai stage					Binet stage		
	0	I	II	III	IV	A	B	C
Number of patients	13	37	40	40	58	49	56	83
Mean TK level (U/l)	17.4	13.9	24.8	32	30.2	13.5	25.1	32.5
Standard deviation	34.7	9.4	30.3	28.5	31.8	18.9	27 9	31 3
Median TK level (U/l)[a]	7.1	10.4	11 5	20	21.8	8.3	16.8	22.2

[a] < 0.01 for both staging systems.

Table 2. Correlation between TK level and other prognostic indicators

Indicator	Number of patients	Level (U/l)	P-value
Platelets			
$< 100 \times 10^9$/l	58	21.9	
$\geq 100 \times 10^9$/l	130	14.3	0.05
Hemoglobin			
< 11 g/dl	87	22	
≥ 11 g/dl	101	10.6	< 0.01
White blood cell count			
$< 50 \times 10^9$/l	90	14.4	
$\geq 50 \times 10^9$/l	98	18.4	0.03
Lymphocyte count			
$< 21 \times 10^9$/l	62	14.6	
$21–76 \times 10^9$/l	64	16.3	0.02
$> 76 \times 10^9$/l	62	21	
Lymphocytes in bone marrow aspirate			
< 50%	34	9.0	
50%–84%	77	14 2	< 0.01
> 84%	66	22.3	
Lactate dehydrogenase			
< 350 IU/l	60	10 3	
> 350 IU/l	126	18.2	0.02
β2-microglobulin			
< 3.5 mg/l	56	10.5	
3.5–5.25 mg/l	58	19.5	< 0.01
> 5.25 mg/l	51	22 6	
IgA			
< 75 mg/dl	121	19.7	
> 75 mg/dl	56	9.55	< 0 01
Performance status			
0	87	11.9	
1, 2, 3	98	21.15	0 02
Previous treatment			
0	50	9.4	
1–3	138	19	< 0.01

Association of TK level with other variables

The TK levels were directly associated with the stage of the disease, though the distinction was more evident for Binet than for Rai staging (Table 1). The TK level was also associated with several other parameters (Table 2), such as performance status, white blood cell count (WBC), the absolute lymphocyte count and bone marrow cellularity, but not with the percentage of bone marrow lymphocytes, liver and spleen enlargement, nodal

Table 3. Relationship between TK and response to treatment according to prior treatment status.

Status group	TK level (U/l)	No of pts	Percentage of patients responding			
			CR	PR	Overall	*P*-value
Total series	< 10	60	68	15	83	0.01
	⩾ 10	128	30	15	45	
Untreated patients	< 10	27	85	11	96	0.02
	⩾ 10	23	48	21	70	
Previously treated patients	< 10	33	55	18	73	0.01
	⩾ 10	105	6	3	39	

Abbreviations: CR – complete response; PR – partial response.

Table 4. Relationship between the TK level and response according to the Binet stage.

Binet stage	TK level (U/l)	Number of patients	Percentage of patients responding			
			CR	PR	Overall	*P*-value
A	< 10	31	77	12	90	0.02
	⩾ 10	18	44	22	66	
B	< 10	18	72	16	88	0.02
	⩾ 10	38	47	10	57	
C	< 10	11	36	18	54	0.17
	⩾ 10	72	18	15	33	

Abbreviations: see Table 3.

involvement, and the serum albumin level. The TK levels were also directly associated with the β2-microglobulin and lactate dehydrogenase (LDH) levels, factors that could represent tumor cell turnover in lymphoproliferative disease. As previously noted, we observed that untreated patients had significantly lower TK levels than did previously treated patients, but no difference was found in the latter patients between those who were refractory and those who were still responsive to treatment.

Prognostic value of the TK level

We used a cutoff level of 10 U/l (twice the upper normal value) in the statistical evaluation of the prognostic value of the TK level. This cutoff point was selected because this was the same one used in studies of the TK level as a prognostic indicator in non-Hodgkin's lymphoma, including CLL [5, 8].

Roughly one-third of the patients in our series had a TK level of < 10 U/l, and the response rate to fludarabine treatment in this group (CR + PR 83%) was significantly superior to that in patients with a TK level of ⩾ 10 U/l; (CR + PR 45%) ($P < 0.01$) (Table 3). The same difference was seen between untreated and previously treated patients: 96% of 27 untreated patients with a TK level of < 10 U/l responded (CR + PR) to

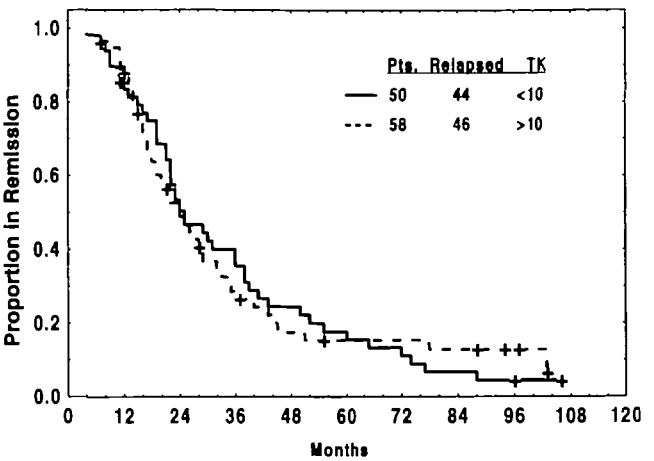

Figure 1 Time to progression of disease in patients who responded to fludarabine by thymidine kinase (TK) levels.

treatment, as opposed to 70% of 23 untreated patients with a TK level of > 10 U/l ($P = 0.02$). Among the previously treated patients, 73% of the 33 patients with a TK level of < 10 U/l responded to treatments, as opposed to 39% of the 105 patients with a TK level of ⩾ 10 U/l ($P < 0.01$) (Table 3).

We also investigated whether the TK level correlated with the response to treatment at the Binet stage, also using a TK level of 10 U/l as the cutoff (Table 4). Although patients at the same stage who had a lower TK level had a better response to fludarabine, the difference was statistically significant only for the patients in stages A and B, probably due to an unequal distribution of patients in the more advanced stage (C) group.

Keating et al. [23] recently developed a method for predicting response in previously treated CLL patients based on the hemoglobin and albumin levels and the extent of previous treatment. Using this model, we calculated the expected response rate in previously treated patients with a TK level < or ⩾ 10 U/l and found that the response rate in patients with the lower TK level was higher than expected (observed to expected ratio = 1.33), while the response rate in patients with the higher TK level was lower than expected (observed to expected ratio = 0.85).

These data collectively suggest that the TK level can serve as an additional prognostic indicator of response to treatment and can be used in addition to other already established indicators. However, a multivariate analysis has not identified the TK value as an independent prognostic indicator. In addition, our analysis showed that the TK level does not affect the duration of response (Figure 1).

In contrast, we found a highly significant difference in the percentage of patients surviving at various time intervals between patients with a TK level of < or ⩾ 10 U/l survival (65% vs. 22%; $P = 0.000$). The distinction was still significant when patients were divided into untreated ($P < 0.001$) and previously treated ($P < 0.01$) groups. Taken together, these findings support the hypothesis that, using a cutoff level of 10 U/l, the TK level is

a valuable prognostic indicator of both the probability and the duration of survival.

Discussion

The serum TK level has been extensively studied in non-Hodgkin's lymphoma [5–9] and found to correlate with the stage and grade of disease. It is also a prognostic indicator of the length of survival in such patients. Kallander et al. [11] examined the prognostic significance of the TK level in a series of 55 CLL patients and found a significant correlation between the TK level and the Rai stage. In addition, longitudinal studies showed the level of TK to increase parallel with the transition from indolent to active disease. Hallek et al. [12, 13] further found that the serum TK levels can predict the duration of progression-free survival and it can be used in the definition of smouldering CLL.

We undertook the current retrospective study primarily to determine whether the TK levels can predict the response to treatment and duration of survival in patients with CLL. Our series consisted of 188 patients with CLL treated with fludarabine in various regimens [15–18] and 92% of them had serum TK levels of $\geqslant 10$ U/l. The median TK level in our study (17 U/l) was higher than that in other reports, however, because our series included only patients with advanced disease or with progressive active early-stage disease. In fact, in comparing the mean TK level in patients in our study, we found significant differences in the mean levels between Kallander's and our study in patients with Rai stage 0 and I disease (4 vs. 17 U/l and 8 vs. 13 U/l, respectively), though not in the mean TK levels in patients with Rai, stage II and III–IV disease (27 vs. 24 U/l and 26 vs. 31, respectively) [11].

Our analysis showed that the TK level can predict both the response to fludarabine treatment and the duration of survival in both previously untreated and relapsing patients. Specifically, the patients in our series with a TK level < 10 U/l showed a higher response rate (CR + PR) than did patients with a TK level of $\geqslant 10$ U/l (83% vs. 45%), both overall and when broken down in treated and previously untreated groups. A TK level of < 10 U/l was also associated with a better survival duration in both treated and untreated patients.

We also confirmed in our study that TK levels are associated with stage of disease, especially the Binet stage. We further found that the TK level correlated with measures of tumor burden, such as the WBC, absolute lymphocyte count, and bone marrow cellularity, but not with nodal, liver, or spleen involvement. We also found a correlation with markers related to the turnover of tumor cells, such as the β2-microglobulin and LDH levels, a finding consonant with the current concept that TK passes into the serum only after disruption of the membranes of tumor cells, thus reflecting proliferative activity [2]. The TK level also added prognostic information to a statistical model that had been developed by Keating et al. [23] to predict response to fludarabine in previously treated patients. This further suggested that the TK level can serve as an important indicator of response to therapy.

In conclusion, even though the retrospective nature of the study calls for caution in data interpretation, we think we have clearly demonstrated that the TK level provides useful prognostic information in CLL patients, in terms of both response to treatment and length of survival, and should be used when planning therapeutic strategies. Because a TK level of $\geqslant 10$ U/l proved to be associated with a poor response to treatment, even with an effective therapy such as fludarabine, aggressive or experimental therapies are justified in such patients.

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