Are Activated Cytoxic T Cells in Hodgkin’s Disease Biopsies a Poor Prognostic Marker?

To the Editor:

In their recent report, Oudejans et al1 studied 80 lymph node biopsies from patients with Hodgkin’s disease (HD). In 10 biopsies, greater than 15% of the T cells contained granzyme-B (GrB). Using the Cox-proportional hazards model for multivariate analysis, the investigators found the percentage of GrB+ lymphocytes to be the strongest prognostic marker in patients with stage II disease (n = 44). Against what one may have expected, the investigators concluded that the presence of a high percentage of activated cytotoxic T cells in biopsy material of HD patients is a strong indicator for an unfavorable clinical outcome.

Many issues in this study raise the possibility that this conclusion is premature, if not inaccurate. (1) The study was performed on selected specimens and was not performed prospectively. No information was provided on what kind of therapy those patients received. (2) Relapsed patients were known before the immunohistochemical staining studies. Was the interpretation performed blindly? (3) How many slides from each lymph node were studied and were the results concordant? (4) Was there any difference between GrB+ cells in biopsies at diagnosis and at the time of relapse? (5) Finally, and most importantly, the investigators used 9 variables in their multivariable assessment to analyze 80 patients, of whom 12 died of disease (only 3.17% of the patients died of disease). This finding is important, especially now that we have shown that Fas ligand expression is also decreased in T cells of HD biopsies.

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REFERENCES


Response

To the Editor:

In response to Dr Younes’ comments concerning the validity of the conclusions drawn by us in our recent report,1 we would like to add the following.

Our conclusion that a high percentage of activated CTLs is strongly related to a poor clinical outcome is suggested to be inaccurate by Younes on the basis of an inappropriate statistical procedure. However, in contrast to his suggestion, we came to our conclusion primarily on the basis of univariate analysis, using the log-rank test. This test clearly showed that, irrespective of stage, the presence of greater than 15% activated CTLs is strongly correlated with a poor clinical outcome (P < .0001). The Cox-proportional hazards model was used to show that, in stage II patients, the presence of many activated CTLs was a prognostic marker independent from clinical parameters as age or the presence of B symptoms. It was not mentioned clearly in our article that, when only the three variables that were significant in univariate analysis (age, presence of B symptoms, and percentage of activated CTLs) were included in the Cox model, identical results were obtained. There is therefore no “violation” of any rule of thumb.

Our study was performed retrospectively on a group of patients receiving different therapy regimens due to the fact that patients participating in different clinical trials were entered. Therefore, our study should be repeated prospectively in a group of patients, all participating in a single clinical trial.

Furthermore, the percentage of activated CTLs in primary tumors could be compared with biopsy specimens of relapsed tumors in only 7 cases. These numbers are too small to draw any (preliminary) conclusions. In addition, the immunohistochemical studies and the quantification of the number of activated CTLs were performed before analysis of clinical data. We thought that this was so obvious that it was not necessary to mention it.

Identical results were obtained when different slides from one lymph node were investigated. Moreover, the computer-assisted quantification of numbers of activated CTLs included a random selection of fields, excluding the possibility that only selected areas of the biopsy specimens were analyzed.

Finally, Younes suggests that our data merely strengthen their notion that the absence of many activated CTLs in the majority of cases explains why Reed-Sternberg cells can survive within the presence of a high number of T cells. This hypothesis would imply that cases with many activated CTLs would have a relatively favorable clinical outcome. However, our data strongly indicate that rather the opposite is true.

In our opinion, our results are best explained by the hypothesis that, in cases with many activated CTLs, only those Reed-Sternberg cells can survive that are resistant to apoptosis induced by activated CTLs, but also to apoptosis induced by either radiotherapy or chemotherapy. Functional
Valproic Acid and Augmentation of Fetal Hemoglobin in Individuals With and Without Sickle Cell Disease

To the Editor:

Increased synthesis of fetal hemoglobin may ameliorate the clinical severity of sickle cell disease; both chemotherapeutic and nonchemotherapeutic agents have been shown to augment fetal hemoglobin synthesis in selected populations of affected patients. Some studies have suggested that the fatty acid analogue valproic acid (n-dipropylacetic acid) may increase the synthesis of fetal hemoglobin. A study of 36 patients with epilepsy treated with valproic acid (10 to 46 mg/kg body weight per day) reported that the percentage of red blood cells containing fetal hemoglobin (F-cells) was significantly higher in these individuals than in 293 patients not receiving this therapy; parallel changes in fetal hemoglobin were not reported.

A study of four adults with sickle cell disease treated with valproic acid (15 to 40 mg/kg/d) reported a threefold increase in fetal hemoglobin in three patients, with no corresponding decrease in the frequency of vaso-occlusive crises, over 2 to 13 weeks. Finally, a study in which 10 patients with sickle cell disease received treatment with hydroxyurea followed by valproic acid (20 mg/kg/d) reported no difference in the concentrations of fetal hemoglobin, frequency of vaso-occlusive crises, or adverse drug effects in patients treated with either agent.

We examined the changes in fetal hemoglobin synthesis in individuals without sickle cell disease and receiving valproic acid for epilepsy to determine whether previously reported valproic acid-induced changes in F-cells are associated with measurable increases in fetal hemoglobin. In parallel, we evaluated laboratory and clinical responses associated with valproic acid therapy in seven patients with sickle cell disease.

One hundred and six consecutive patients attending the Neurology Clinic at The Hospital for Sick Children, Toronto, with a diagnosis of seizure disorder, none of whom had sickle cell disease or trait, were studied. Fifty-seven patients, aged (mean ± SD) 9.3 ± 4.4 years, were receiving therapy with carbamazepine (Tegretol), whereas 49 patients, aged 9.9 ± 5.2 years, had been treated with valproic acid (15 to 50 mg/kg/d) for 4.0 ± 0.5 years. Although a significant mean increase in mean red blood cell volume (from 84 ± 5 fL before treatment with valproic acid to 90 ± 5 fL; \( P < 0.01 \)) was observed in the latter group, the percentage of fetal hemoglobin (mean ± SEM, 0.9% ± 0.1%) in patients with therapeutic serum drug concentrations of valproic acid did not differ significantly from that of the carbamazepine-treated patients (0.8% ± 0.1%; \( P = \text{NS} \)).

In parallel, seven patients, aged 16.5 ± 10 years, with severe sickle cell disease, defined as three or more hospitalizations in the year before treatment, were offered valproic acid at 15 ± 3 (range, 9 to 20) mg/kg/d. Complete blood counts were obtained twice monthly. Patients were reviewed by a physician twice a month and questioned regarding adverse effects of treatment, sickle cell disease-related pain, admissions to hospital, and compliance with therapy. Annual rates of vaso-occlusive crises were calculated as previously described by dividing the number of crises by the number of years of therapy (for example, 2 crises in 6 months = 4 crises per year). The number of days spent in hospital and the number of red blood cell transfusions administered were calculated in a similar manner. Compliance with valproic acid was monitored by the Medication Event Monitoring System, a bottle with a computer chip in the lid that determines the timing and frequency of bottle openings, and by determinations of concentrations of serum valproic acid obtained at clinical visits.

The changes observed during therapy with valproic acid over 5.8 ± 0.9 (3.2 to 9.0) months are shown in Table 1. Data are presented as the mean ± standard deviation. Compliance with valproic acid was 93% ± 7% drug taken of that prescribed. Mean trough serum levels (561 ± 115 µmol/L) were within the therapeutic range (350 to 750 µmol/L, 50 to 100 µg/mL). No changes in liver function tests were observed; in one patient, the platelet count decreased to 98 × 10^9/L, prompting discontinuation of valproic acid. Review of this patient’s medical records showed that he had chronic low-grade thrombocytopenia before the initiation of valproic acid; this finding has persisted after withdrawal of valproic acid. Bone marrow aspiration showed abundant megakaryocytes. The relationship between thrombocytopenia in this patient and valproate therapy, acknowledged to induce thrombocytopenia in 21% to 60% of treated patients who have serum concentrations exceeding 100 to 140 µg/mL, is unclear.

Because of the lack of clinical improvement and frequent hospital admissions during valproic acid, one patient requested discontinuation of treatment. No differences in annual rates of vaso-occlusive crises, or hospitalization were observed in patients treated with either valproic acid or carbamazepine. Mean cell volume (fL) 83 ± 8.0 increased significantly from the baseline value of 80 ± 7.0 fL in the carbamazepine-treated patients (\( P < 0.01 \)).

Table 1. Clinical and Laboratory Characteristics of Seven Patients With Sickle Cell Disease Treated With Valproic Acid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Valproic Acid</th>
<th>Post-Valproic Acid</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.3 ± 0.9</td>
<td>9.3 ± 1.2</td>
<td>.05</td>
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<tr>
<td>Hemoglobin F (%)</td>
<td>6.4 ± 2.6</td>
<td>8.4 ± 4.3</td>
<td>.05</td>
</tr>
<tr>
<td>Hemoglobin F (g/dL)</td>
<td>5.4 ± 2.2</td>
<td>7.9 ± 4.5</td>
<td>.05</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>83 ± 8.0</td>
<td>86 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Annual rate of</td>
<td>Vaso-occlusive crises</td>
<td>3.1 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>In-patient hospital days</td>
<td>31 ± 28</td>
<td>37 ± 53</td>
<td>NS</td>
</tr>
</tbody>
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Data obtained at evaluation before beginning valproic acid were compared with those at the completion of the study using the Student’s \( t \)-test for paired data. All tests were two-tailed; a significance level of .05 was used to indicate statistical significance.

REFERENCE