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Mitotic Activity and Survival in Advanced Non-Hodgkin's Lymphoma of Unfavourable Histology

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Abstract—*The number of mitoses per high power field (mitotic index, MI) was assessed in* 2 μm *sections of lymph node biopsies from* 58 *adults with non-Hodgkin's lymphoma. All had diffuse nodal lymphomas of unfavourable histology and stage II–IV disease. The patients were treated with chemotherapy and followed for a minimum of* 3 *years or until death. None out of* 29 *patients with a MI* ≥ 3.0 *survived for* 3 *years after diagnosis whereas* 13 *out of* 29 *other patients with MI* < 3.0 *became long-term survivors* (P = 0.00002). *Differences in age, sex or clinical stage between short- and long-term survivors were negligible. The initial chemotherapy regimens were not more intense for the long-term survivors. Twenty-nine patients were given an equivalent initial treatment with CHOP or CHOP plus methotrexate. The association between MIs and survival was evident also in this subgroup. The results indicate that survival is extremely poor for patients with advanced diffuse nodal lymphomas of unfavourable histology and a high mitotic count. It seems especially important to evaluate alternative chemotherapy regimens, suggested to be more effective than current programmes, in this subset of patients.*

INTRODUCTION

MANY patients with advanced non-Hodgkin's lymphoma (NHL) of unfavourable histology may achieve long-term survival following combination chemotherapy whereas others die with progressive disease within a few years despite similar treatment. An accumulating amount of data indicates that in NHL the proliferative activity of the lymphoma cells is of great prognostic importance [1–9].

There is a strong correlation between thymidine labelling indices in lymphoma cell suspensions and the number of mitoses in thin section of biopsies prepared for diagnosis and histopathologic classification [8]. The assessment of a mitotic index may therefore give good information on the proliferative activity of lymphomas. In the present study of 58 patients with advanced NHL of unfavourable histology, mitotic indices were assessed in lymph node biopsies obtained at diagnosis. All patients were treated with chemotherapy and the impact of mitotic activity on survival was evaluated through follow up for a minimum of 3 years or until death.

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MATERIALS AND METHODS

Patients

Fifty-eight adults, 36 men and 22 women (median age 63 years) with NHL were studied. A lymph node biopsy was used for histopathologic classification in all patients and the lymphomas were classified according to the Kiel classification [10]. Patients with high grade lymphomas, large cell centrocytic and diffuse centroblastic–centrocytic lymphomas were studied. Although not classified as high grade malignant lymphomas, the latter two histologic subgroups were included because previous studies have indicated that they might be prognostically unfavourable [11, 12]. Patients with unspecified diffuse high grade lymphomas and true histiocytic lymphomas were also accepted for the study. All patients had clinical stage II–IV disease according to the Ann Arbor system [13]. All patients were initially treated with chemotherapy. Each patient was followed for a minimum of 3 years or until death.

Histopathologic examination and assessment of mitotic activity

Biopsies from enlarged lymph nodes were used for diagnosis and classification as described previously

[8]. The mitotic counts were assessed in thin 2 μm sections stained with H&E. In all cases the number of mitotic figures was determined in a Zeiss microscope equipped with a WF 12.5× eyepiece and a 40× objective giving a high power field of 0.16 mm^2. The number of mitoses was recorded in 10 high power fields and divided by 10. The quotient was designated mitotic index (MI). The diagnoses, classifications and determination of MIs were performed by one pathologist (M.Å.) who was unaware of the outcome for the patients.

Treatment

Initial treatment for 23 patients was CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), for six patients CHOP + methotrexate, for 12 patients MEV (methotrexate, cyclophosphamide and vincristine), for one patient COMLA (cyclophosphamide, vincristine, methotrexate and cytosine arabinoside), for one patient a combination of daunorubicin, vincristine, cyclophosphamide, l-asparaginase, cytosine arabinoside, 6-mercaptopurine and prednisone, and for two patients CVP (cyclophosphamide, vincristine and prednisone). Thirteen patients, mainly elderly with a poor performance status, were treated with prednimustine, a chlorambucil ester of prednisolone.

RESULTS

The mitotic indices and histopathologic classifications for all 58 patients are shown in Fig. 1. Twenty-three patients died within 1 year after diagnosis (median 7 months). Their median MI was 4.9 (mean 5.3). Twenty-two patients who died 1–3 years (median 20 months) after diagnosis had a median MI of 3.6 (mean 3.4) and 13 patients who have survived for more than 3 years (median > 4.5 years) had a median MI of 1.4 (mean 1.4). The difference in MIs between patients surviving less than one year and those who died 1–3 years after diagnosis is significant ($P = 0.015$, Student's t test). The difference between the MIs for those who died 1–3 years after diagnosis and the long-term survivors is also significant ($P = 0.005$).

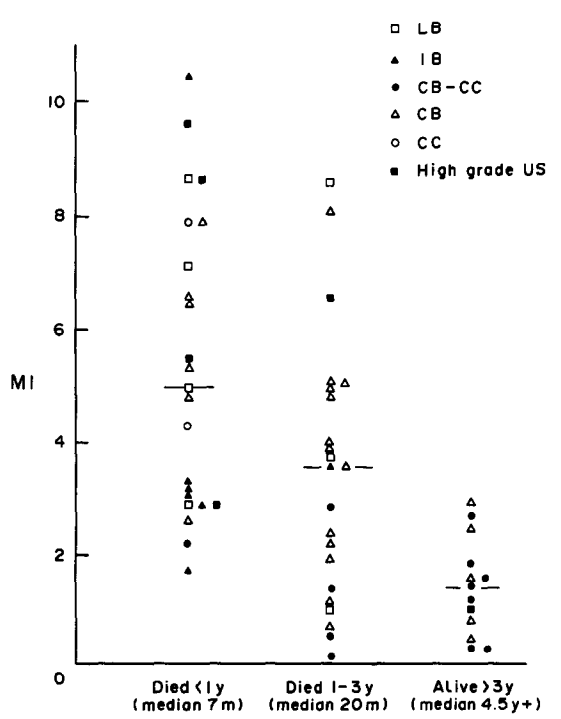

Fig. 1. Mitotic indices in 58 patients surviving < 1 year, 1–3 years or > 3 years. LB = lymphoblastic, IB = immunoblastic, CB = centroblastic, CB-CC = centroblastic centrocytic, CC = centrocytic, large cell, US = unspecified high grade lymphoma.

None of the 29 patients with MI ≥ 3.0 was alive 3 years after diagnosis whereas 13 out of 29 patients with MI < 3.0 have survived this period of time. The difference in survival between patients with MIs ≥ 3.0 and MIs < 3.0 is highly significant ($P = 0.00002$, Fisher's exact test).

There was an association between histopathologic classification and survival. Thus seven out of 14 patients (50%) with large cell centrocytic (CC) or diffuse centroblastic–centrocytic (CB-CC) lymphomas were long-term survivors. Only six out of 44 patients (14%) with high grade lymphomas, i.e. lymphoblastic (LB), immunoblastic (IB), centroblastic (CB) or unspecified high grade (US) lymphomas, survived for more than 3 years. The difference is significant ($P = 0.009$). Excluding the CC and CB-CC lymphomas the prognostic impact of MIs is still evident. Thus none out of 27 patients with high grade lymphoma and MI ≥ 3.0 survived for 3 years whereas six out of 17 patients with MIs < 3.0 became long-term survivors ($P = 0.002$).

The median age of the patients who died within 3 years after diagnosis and the long-term survivors were similar, 63 years (range 33–84) and 62 years (range 46–76) respectively. The sex distribution in these two groups was also comparable; 29 out of 45 short-term survivors were men (64%) compared to seven out of 13 long-term survivors (54%). The distribution of clinical stage was also similar in the two groups. Thirty-nine out of 45 short-term survivors (87%) and all long-term survivors had stage III–IV disease. The initial treatment was not less intense for the short-term survivors than for those who survived more than 3 years. Thus CHOP, CHOP-M, MEV or other multidrug regimens were given to 32 (71%) of the short-term survivors. Thirteen patients (29%) had a less intense initial treatment with CVP or prednimustine. The corresponding figures for the 13 long-term survivors were 11 (85%) and two (15%). The difference is not significant ($P = 0.28$). In Fig. 2 the MIs are shown for 29 patients who were initially treated with CHOP or CHOP-M. Survival following treatment of unfavourable lymphomas with these two regimens has been found to be equivalent [21]. None out of 15 patients with MIs ≥ 3.0 survived for 3 years whereas seven out of 14 with MIs < 3.0 became long-term survivors ($P = 0.002$).

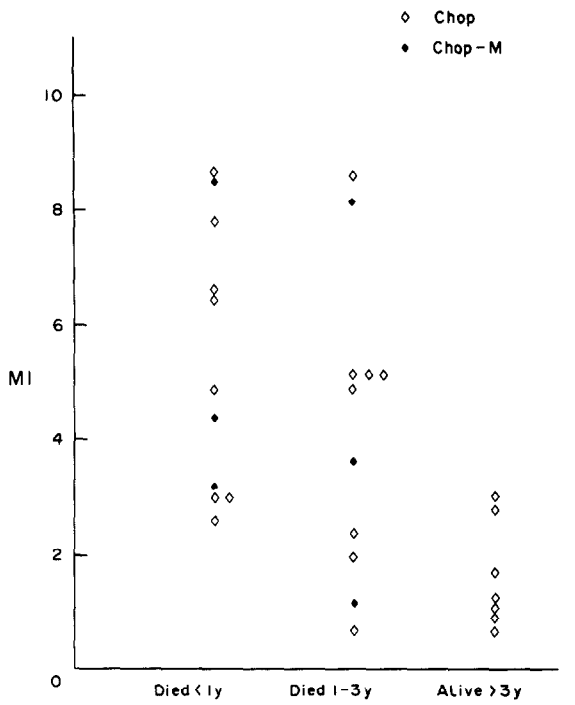

Fig. 2. Mitotic indices and survival in 29 patients initially treated with CHOP or CHOP + methotrexate.

DISCUSSION

The present results confirm our previous finding that the assessment of mitotic activity in NHL has great prognostic impact in addition to other parameters such as histopathologic classification, stage, age, sex and treatment [8]. Compared to other methods used to evaluate cell proliferation in NHL, i.e. thymidine labelling indices or S-phase estimations in flow cytometry, the assessment of mitotic index is a simple and rapid method. Results of repeated determinations are fairly constant [8] and interobserver variations are small [14]. Previous investigations have indicated an association between a high mitotic activity and a poor prognosis in NHL [9, 15–18] and our findings are in line with these results. It must be emphasized that the strong prognostic impact of MIs found in the present study applies only to patients with nodal NHL and that our results are based entirely on MIs assessed in lymph node biopsies. Likewise Donhuijsen [9] used lymph node biopsies for the demonstration of a strong prognostic importance of MIs in NHL. Many high grade lymphomas are primarily located extranodally. In a preliminary investigation of gastric lymphomas we have observed that high MIs may be compatible with long-term survival (unpublished). A separate study of the relation between proliferative activity in extranodal lymphomas and prognosis may therefore be useful. This suggestion is supported by results reported by Warnke *et al.* [19] who failed to demonstrate a significant relation between mitotic activity and prognosis in a study of consecutive patients with biopsies from various sites. It should also be noted that follicular lymphomas were not included in the present study. For such lymphoma types no prognostic significance of mitotic activity was found by Ellison *et al.* [14].

In the present study the importance of mitotic activity was evaluated in a special clinical setting, i.e. advanced NHL of unfavourable histology. Although the results of various chemotherapy regimens may differ in these lymphomas, the survival curves generally decline steeply during the first 2–3 years after diagnosis. Thereafter, there is a flattening of the curves representing patients with long-term survival [20]. It was therefore considered meaningful to compare patients who died within 3 years with those surviving for a longer period of time. This comparison showed that differences between short- and long-term survivors were negligible regarding age, sex and stage. Nor was there any indication that long-term survivors had initially been treated more intensely than those who died within 3 years. The most pronounced difference observed between short- and long-term survivors was the mitotic activity. Thus half of the patients had MIs ≥ 3.0 and all these patients died within 3 years after diagnosis. In contrast a considerable number of patients with MIs < 3.0 became long term survivors.

Histopathologic classification had some bearing on prognosis. Although large cell CC and diffuse CB-CC lymphomas are classified as low grade according to the Kiel classification [10], it has been proposed that such lymphomas are prognostically unfavourable [11, 12], and they were therefore included in the present study. Our results support the concept that these lymphomas are less aggressive than the high grade lymphomas. A separate analysis of patients with high grade lymphomas was therefore performed and confirmed that the mitotic activity was of utmost importance for the outcome. Thus all patients with high grade lymphomas and MIs ≥ 3.0 died within 3 years whereas more than a third of the patients with MIs < 3.0 became long-term survivors.

The aim of multiple drug chemotherapy in advanced NHL of unfavourable histology is to achieve long-term survival and possibly cure. The chemotherapy regimens used by us were not followed by long-term survival for any patient with a MI ≥ 3.0. CHOP and CHOP plus methotrexate are known to be equivalent therapies in terms of survival in NHL of unfavourable histology [12]. Half of our patients were treated with either of these two regimens. When this subgroup of patients was analysed the pattern of survival was similar to that observed in the whole material, i.e. no patient with MIs ≥ 3.0 survived for 3 years whereas about half of the patients with lower MIs became long-term survivors. An assessment of the mitotic activity at diagnosis may have implications for the choice of alternative treatments. Results of chemotherapy

regimens introduced more recently suggest that some of them may be associated with longer survival compared with CHOP [20]. It seems appropriate to consider such alternative programmes for patients with advanced high grade NHL and high MIs. In the present material the outcome for these patients was extremely poor; about half of them died within 1 year and all were dead within 3 years. The possible superiority of some alternative regimen should therefore become apparent in a relative short period of time.

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