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Estimation of Differential *In Vitro* Sensitivity of Non-Hodgkin Lymphomas to Anticancer Drugs*

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Abstract—*The sensitivity to adriamycin, prednisolone, bleomycin and vincristine of human lymphoma cells in short-term culture was studied in* 30 *non-Hodgkin lymphomas (NHL). As indicators of drug action, the interference on* [^{3}H]*thymidine and* [^{3}H]*uridine incorporation was studied. The* in vitro *sensitivity to the drugs was then compared with the clinical response to treatment. On the basis of the retrospective analysis the* in vitro *sensitivity indexes were defined taking into account the biologic aggressivity of the tumor. A statistically significant correlation between the* in vitro *sensitivity to adriamycin and prednisolone and complete remission, relapse-free time and survival was observed in highly proliferative NHL, while in low proliferative NHL the* in vitro *sensitivity was statistically associated only to relapse-free survival within* 22 *months.*

INTRODUCTION

CHEMOTHERAPY has substantially improved the clinical results in the treatment of non-Hodgkin lymphomas (NHL) [1], although the long-term complete remission is still relatively low in high proliferating lymphomas [2] and controversial in unfavorable histologies [3, 4]. It appears that further improvement would also require information on the chemosensitivity of individual NHL, since they show a wide range of sensitivity to drugs even within the same histologic type. The search for predictive techniques which allow the selection of effective chemotherapy for individual cancer patients is a problem for all cancer chemotherapy. So far the results are meagre because of the choice of morphologic, non-quantitative criteria of *in vitro* response [5, 6] or due to the lack of the assessment of the maintenance of the original metabolic conditions of tumor tissue [7–9] or the non-standardization of the cell culture conditions used [10]. Some authors have more recently utilized more useful antimetabolic techniques on lung tumors [11] and non-lymphoblastic leukemia [12, 13] and more interesting correlations between *in vitro* and *in vivo* chemosensitivities have been reported. More recently, it was found that if a colony-forming assay of tumor stem cells is utilized to test the sensitivity of the tumor to anticancer drugs, the *in vitro* cytocidal effect strictly correlates with the clinical response of patients with myeloma and ovarian carcinoma [14]. Unfortunately, colonies cannot be obtained for all tumors, and therefore it seems worthwhile to explore the possibility of developing bioassays based on biological markers other than colony growth. A central metabolic event, such as the incorporation of nucleic acid precursors into DNA and RNA, may be a good marker of drug efficacy.

In this study, the revelance of the incorporation of [^{3}H]thymidine and [^{3}H]uridine in short-term cultures of NHL as an indicator of the therapeutic effect of the drugs was evaluated. The main advantages of such a test are that it can be used on all tumors and the results are available within a few days.

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MATERIALS AND METHODS

Cell suspensions

Lymph nodes obtained by surgical excision were divided into a portion for histologic examination and a portion for autoradiographic and biochemical determinations. For the latter analyses, the lymph node was minced and then washed in complete medium (F10 medium with 20% fetal calf serum and antibiotics: 100 U penicillin and 100 γ/ml streptomycin). The medium containing fragments was transferred to a conical flask, which was then placed on a magnetic stirrer in the cold. The cell suspensions, collected after three successive washes (for 5 min each at 4°C, continuously stirred), were filtered through sterile gauze to remove cell clumps and centrifuged at 700 ***g*** for 5 min at 4°C [15]. The pellets were resuspended in complete medium, and the viability was determined by the Trypan blue dye exclusion test. The percentage of viable cells before *in vitro* treatment ranged from 85 to 95%. Both autoradiographic and chemosensitivity studies were performed on the whole lymph node population.

Autoradiographic determinations

For each NHL the labeling index (LI) was determined on cell suspensions. Samples of 6 to 10×10^6 cells were incubated in 2 ml of complete medium with 2 μCi/ml [^{3}H]-thymidine (sp. act. 5 Ci/mmol) for 1 hr. At the end of the incubation period, the samples were centrifuged at 700 ***g*** for 5 min at 4°C. The pellets were resuspended in a small volume of 0.9% NaCl solution and smeared on slides. The smears were fixed in Bouin's solution for 1 hr, left in 80° alcohol overnight, and submitted to Kodak AR10 ARGraphic stripping film [16]. After an exposure time that ranged from 3 to 5 days, the ARGraphies were developed in Kodak D19b (at 18°C for 5 min), fixed in Kodak F5, and stained with hematoxylin and eosin. The LI was evaluated as the percentage of labeled cells of the whole cell population on at least 5000 cells.

In vitro *drug assay*

For all assays, adriamycin (Adriblastina, Farmitalia, Milan, Italy) and prednisolone (Endo Prenovis, Vister, Milan, Italy) were tested; in some cases, bleomycin (Nippon Kayaku Co., Tokyo, Japan) and vincristine (Eli Lilly, Indianapolis, IN, U.S.A.) were tested. The tests for each patient were performed simultaneously on aliquots from the same biopsy at the time of diagnosis or at relapse. The drugs were tested at different concentrations. The basic concentration (n) was the calculated clinical dose (3 γ/ml adriamycin, 4 γ/ml prednisolone, 0.05 γ/ml vincristine, 0.6 γ/ml bleomycin) [10]; in addition, $n/2$ and $2n$ concentrations were tested for both adriamycin and prednisolone and $2n$ and $4n$ for both bleomycin and vincristine. Samples of 6 to 10×10^6 viable cells in triplicate were incubated for 3 hr in the presence of the freshly prepared, different drug concentrations. During the last hour of incubation, both labeled precursors were added to the same sample (4 μCi/ml [^{3}H]thymidine, sp. act. 400 mCi/mmol, and 4 μCi/ml [^{3}H]-uridine, sp. act. 120 mCi/mmol; Radiochemical Centre, Amersham, U.K.). At the end of incubation, the samples were stored at −18°C or immediately processed. RNA and DNA were extracted according to the method of Schneider [17], which was opportunely modified to obtain the optimal separation of the RNA and DNA phase; the maximal contamination of the two phases was 10% of the incorporation values [15]. Aliquots of 0.2 ml of RNA and DNA extracts were dissolved in 10 ml of emulsifier scintillation fluid (Packard Instruments), and the radioactivity was determined in a Packard Tricarb 2635 liquid scintillation counter. The counting efficiency was about 40%. The c.p.m. values of [^{3}H]thymidine and [^{3}H]-uridine incorporation in the control samples among the tumors tested ranged, respectively, from 380 to 13,300 and from 220 to 13,400. The *in vitro* effects of the drugs on nucleic acid precursor incorporation were expressed as percentage variation from control values. The maximal variation of the incorporation among triplicate samples was 20%.

Patient characteristics

The population studied consisted of 30 adult lymphoma patients (18 males and 12 females, with a median age of 56) in whom the initial lymph node biopsy was performed at the Istituto Nazionale Tumori of Milan. The histologic diagnosis was made according to the Rappaport classification [18], and the pathologic stage (PS) was assessed according to the Ann Arbor criteria [19]. The series is not consecutive, since the large majority of patients referred to the Institute have had their diagnostic biopsy performed elsewhere, and in a few cases the biopsy material was inadequate to carry out chemosensitivity tests.

Case admission was started in April, 1975, and follow-up information was available up to November, 1979. The median follow-up time is 18.5 months (range 4–52 months). Patients staged as PS I were excluded from this study when they had undergone initial curative radiotherapy. Chemotherapy regimens adopted were related to stage: all patients underwent combination chemotherapy with cyclophosphamide, vincristine, prednisolone, adriamycin and bleomycin in different combinations according to various treatment schedules (e.g., CVP alternated with ABP and BACOP) as described in previous publications [20]. A few patients did not receive steroid therapy for special clinical reasons. Involved field irradiation was given after chemotherapy in patients with PS II disease and only in one patient with a PS III lymphoma. Most patients were untreated at the time of admission to the study, and all the 6 previously treated patients (3 previously treated with irradiation and 3 with chemotherapy) had received inadequate treatment and were showing progressive disease at the time of the chemosensitivity test. Clinical assessments were performed after 3 cycles of chemotherapy in stages I and II, and after 6 cycles in stages III and IV. The overall chemotherapeutic treatment consisted of at least 12 cycles, one cycle every 20 days. Assessment of response was established according to pathologic restaging with peritoneoscopy [21] plus bone marrow biopsies.

Data analysis

For a comparison between the *in vitro* effect of the drugs on nucleic acid precursor incorporation and the clinical response, the *in vitro* effect of the calculated clinical dose (n) was considered. On the basis of a retrospective comparative analysis of *in vitro–in vivo* chemosensitivity, tumors were considered 'sensitive' when inhibitions more than 40% for low proliferating NHL ($LI \leqslant 4\%$) and more than 70% for high proliferating NHL ($LI > 4\%$) were observed. Statistical association of the *in vitro* and *in vivo* results was studied with the kappa test [22], that also indicates the degree of agreement between *in vitro* and *in vivo* data. The actuarial life method was used to summarize relapse-free survival (RFS), and survival distribution and the statistical significance of differences observed was assessed by the log-rank test. Both RFS and survival times were evaluated from the beginning of therapy.

RESULTS

In vitro *chemosensitivity*

In agreement with that observed in a preliminary study [15], similar dose-effect plots of [^{3}H]thymidine and [^{3}H]uridine incorporation were observed for the tested drugs in each tumor; thereafter, only the [^{3}H]-thymidine plots are reported. The interference of different doses of adriamycin, prednisolone, vincristine and bleomycin on [^{3}H]-thymidine incorporation is reported in Figs. 1–4, respectively. The chemosensitivity tests were carried out on 51 cases, but only the 30 cases treated with the same drugs tested *in vitro* and then considered for the comparative analysis between the *in vitro* and *in vivo* response of the same tumor are reported.

Adriamycin. Dose-effect plots for adriamycin on 30 NHL *in vitro* are shown in Fig. 1. The wide variations among inhibition values of different tumors indicated a wide range of sensitivity to the drug. A characteristic dose-related effect was usually observed for this drug.

Prednisolone. Dose-effect plots for prednisolone on 30 NHL are shown in Fig. 2. In all cases in which an inhibitory effect was obser-

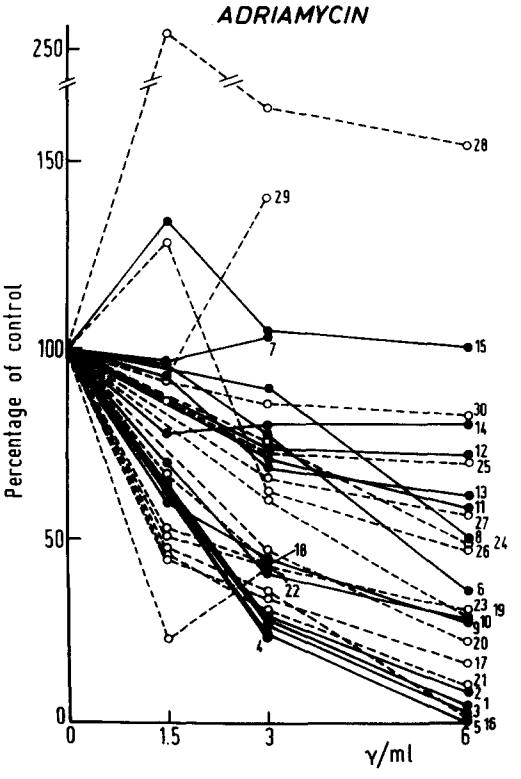

Fig. 1. Effect of adriamycin on [^{3}H]thymidine incorporation in high (solid line) and low (broken line) proliferating NHL. The values are expressed as percentage variations from control values. Plots for individual patients are designated with the identification numbers shown in Tables 1 and 2.

ved, prednisolone caused an initial inhibition of [^{3}H]thymidine incorporation and a leveling off of the effect at the higher doses.

Vincristine. The effect of vincristine (Fig. 3) was tested on 18 tumors *in vitro*. Poor antimetabolic effects were observed, and only in a few cases (Nos. 2, 3, 4, 15) did they exceed the experimental error.

Bleomycin. This drug was tested on 9 tumors (Fig. 4), and the antimetabolic effect only exceeded the experimental error in 1 case (No. 2).

An enhancement of [^{3}H]thymidine incorporation was occasionally observed at the lower or at all the doses tested. The shape of the dose-effect plots for each drug is similar for both low and high proliferating tumors, and no relationship was observed between the degree of antimetabolic effects and the proliferative activity of the cell population.

Clinical features and in vitro *sensitivity*

When tested, the *in vitro* effects of adriamycin and prednisolone, as well as of vincristine and bleomycin, were compared with the response of the same patients to polychemotherapy that included these drugs. As already stated, the effect of the drugs on [^{3}H]-

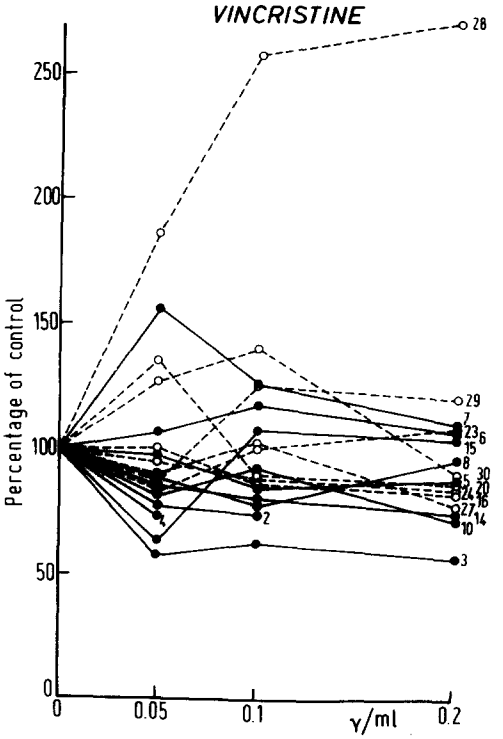

Fig. 3. Effect of vincristine on [^{3}H]*thymidine incorporation in high (solid line) and low (broken line) proliferating NHL. The values are expressed as percentage variations from control values. Plots for individual patients are designated with the identification numbers shown in Tables 1 and 2.*

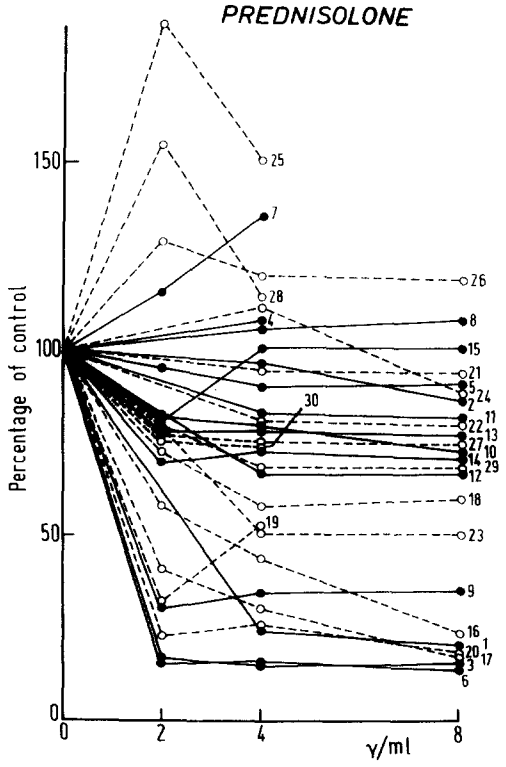

Fig. 2. Effect of prednisolone on [^{3}H]*thymidine incorporation in high (solid line) and low (broken line) proliferating NHL. The values are expressed as percentage variations from control values. Plots for individual patients are designated with the identification numbers shown in Tables 1 and 2.*

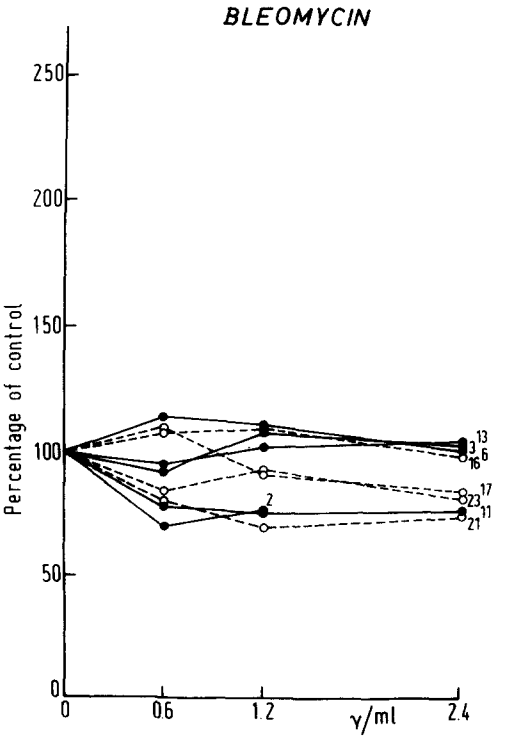

Fig. 4. Effect of bleomycin on [^{3}H]*thymidine incorporation in high (solid line) and low (broken line) proliferating NHL. The values are expressed as percentage variations from control values. Plots for individual patients are designated with the identification numbers shown in Tables 1 and 2.*

Table 1. Clinical features and in vitro *sensitivity of high proliferative NHL (LI > 4%) to anticancer drugs*

Case	Status when studied	Histology	Pathologic stage	L.I. (%)	*In vitro* [^{3}H]Thymidine–[^{3}H]uridine (% inhibition) A [^{3}H]t	A [^{3}H]u	P [^{3}H]t	P [^{3}H]u	V [^{3}H]t	V [^{3}H]u	B [^{3}H]t	B [^{3}H]u	Sensitivity*	*In vivo* Time to complete remission (CR)	Free time	Survival (months)
1	In progression after RT, to begin CVP	DH	II EA	14.2	−72	−78	−75	−71	NT		NT		Sensitive	CR (1 month)	44+	44+
2	Untreated, to begin CVP–ABP	DM	II A	6.7	−71	−68	−4	−1	−22	−20	−30	−20	Sensitive	CR (2 months)	7+	7+
3	Untreated, to begin BACOP	DM	III A	10.6	−72	−84	−85	−75	−43	−25	−8	−7	Sensitive	CR (3 months)	50+	50+
4†	Relapse after ABP–CVP, to begin ABP–CVP	DM	III SA	6.1	−76	−46	+7	+2	−26	−33	NT		Sensitive	CR (5 months after relapse)	11+	11+
5	Untreated, to begin CV–AB	DLPD	III B	8.7	−75	−82	−10	−18	−2	−29	NT		Sensitive	CR (5 months)	30+	30+
6	Untreated, to begin CVP–ABP	DM	IV A	17.0	−22	−54	−84	−82	+7	−20	+14	−6	Sensitive	CR (10 months)	46	46+
7	Untreated, to begin BACOP	DLPD	I EA	7.8	+4	−11	+35	+30	+56	+39	NT		Non-sensitive	CR (2 months)	13	17
8	Untreated, to begin CVP–ABP	DH	IV A	30.0	−10	+14	+6	+5	−12	−16	NT		Non-sensitive	CR (4 months)	14	18+
9	Untreated, to begin CVP–ABP	DH	III B	15.0	−55	−44	−65	−52	NT		NT		Non-sensitive	Progression	—	4
10	Untreated, to begin CVP	NLPD	IV B	7.6	−57	−49	−20	−21	−17	−6	NT		Non-sensitive	Progression	—	7
11	In progression after CHOP, to begin CVP–ABP	DU	III A	22.0	−28	−6	−17	−7	NT		−22	0	Non-sensitive	Progression	—	13
12	Untreated, to begin CVP–ABP	NLPD	IV A	13.8	−27	−26	−33	−27	NT		NT		Non-sensitive	Progression	—	6
13	In progression after RT, to begin CVP–ABP	NM	II A	14.0	−31	−38	−22	−4	NT		−5	+5	Non-sensitive	Progression	—	7
14	Untreated, to begin CVP–ABP	DLPD	IV A	14.2	−20	−33	−27	−16	−14	−4	NT		Non-sensitive	Progression	—	6
15	Untreated, to begin CVP–ABP	DM	III B	6.1	+5	−27	0	+4	−36	−8	NT		Non-sensitive	Progression	—	7

*Sensitive, tumors which showed an inhibition of more than 70% of [^{3}H]thymidine incorporation by adriamycin (A) or prednisolone (P). Non-sensitive, tumors which showed an inhibition of less than 70% of [^{3}H]thymidine incorporation by adriamycin or prednisolone.

†Same patient as No. 28, Table 2.

Table 2. Clinical features and in vitro *sensitivity of low proliferative NHL (LI < 4%) to anticancer drugs*

Case	Status when studied	Histology	Pathologic stage	L.I. (%)	*In vitro* [^{3}H]Thymidine–[^{3}H]uridine (% inhibition) A [^{3}H]t–[^{3}H]u	P [^{3}H]t–[^{3}H]u	V [^{3}H]t–[^{3}H]u	B [^{3}H]t–[^{3}H]u	Sensitivity*	*In vivo* Time to complete remission (CR)	Free time	Survival (months)
16	In progression after RT, to begin CVP	DLPD	II A	0.45	−64 −71	−56 −26	0 0	+8 +15	Sensitive	CR (3 months)	39+	39+
17	Untreated, to begin CVP–ABP	NLPD	IV A	0.5	−66 −51	−69 −31	NT	+10 −4	Sensitive	CR (5 months)	46+	46+
18	Untreated, to begin CVP–ABP	NLPD	III SA	0.3	−56 −64	−42 −27	NT	NT	Sensitive	CR (7 months)	25	26+
19	Untreated, to begin CVP–ABP	NLPD	IV A	1.2	−40 −63	−68 −81	NT	NT	Sensitive	CR (7 months)	19+	19+
20	Untreated, to begin CVP–ABP	Lennert	IV A	1.8	−69 −56	−74 −67	+35 +5	−21 −10	Sensitive	CR (9 months)	33	52+
21	Untreated, to begin CVP–ABP	NLPD	IV B	2.8	−47 −38	−6 +15	NT	NT	Sensitive	CR (9 months)	34+	34+
22	Untreated, to begin CVP–ABP	NLPD	III A	2.6	−57 −41	−19 −40	NT	NT	Sensitive	CR (10 months)	39+	39+
23	Untreated, to begin CV–AB	DM	IV A	2.6	−58 −60	−49 −51	−16 −30	−16 −38	Sensitive	CR (10 months)	14+	14†
24	In relapse, previous treatment with CVP–ABP, to begin CVP	DLPD	IV A	0.6	−25 −58	+11 −7	−5 +11	NT	Non-sensitive	No response	—	15+
25	Untreated, to begin CVP–ABP	DLPD	IV B	0.1	−27 +35	+50 +40	NT	NT	Non-sensitive	Progression	—	11
26	Untreated, to begin CV–AB	NM	III A	2.8	−38 −10	+19 +8	NT	NT	Non-sensitive	CR (2 months)	9	13
27	Untreated, to begin CVP–ABP	NM	III A	3.6	−34 −35	−25 −52	−10 −5	NT	Non-sensitive	CR (3 months)	31+	31+
28‡	Untreated, to begin CVP–ABP	NM	III SA	2.1	+64+180	+14+121	+86 +70	NT	Non-sensitive	CR (4 months)	16	28+
29	Untreated, to begin CVP–ABP	NM	III A	0.4	+40 +50	−32 +70	−14 +40	NT	Non-sensitive	CR (5 months)	22+	22+
30	Untreated, to begin CVP–ABP	NM	IV A	0.2	−15 −14	−25 −5	+27 +11	NT	Non-sensitive	CR (10 months)	22	34+

*Sensitive, tumors which showed an inhibition of more than 40% of [^{3}H]thymidine incorporation by adriamycin (A) or prednisolone (P). Non-sensitive, tumors which showed an inhibition of less than 40% of [^{3}H]thymidine incorporation by adriamycin or prednisolone.

†Death unrelated to the disease.

‡Same patient as No. 4 in Table 1.

thymidine was generally similar to that on [^{3}H]uridine. However, when a discrepancy existed the effect on the DNA precursor appeared to be more closely related to the clinical effect of the drugs, and then it was considered for the comparison of *in vivo–in vitro* results. From a retrospective analysis of the levels of antimetabolic *in vitro* effects that correlated to the clinical response, it was observed that different criteria of *in vitro* responsiveness must be adopted in relation to the proliferative activity of the tumors. In fact, a higher *in vitro* inhibition (>70%) for high proliferating NHL and a lower *in vitro* inhibition (>40%) for low proliferating NHL correlated with a positive therapeutic effect. This is in complete agreement with the previous finding of a different biologic aggressiveness [23, 24] and clinical [2] behavior for NHL with different labeling indexes.

The *in vitro* inhibition of [^{3}H]thymidine and [^{3}H]uridine incorporation at the calculated clinical dose and the clinical features after treatment for each patient are reported in Tables 1 and 2. The results for 15 highly proliferating NHL are reported in Table 1. Eleven patients were untreated, and three were in progression and one in relapse after previous treatment. Different values of inhibition of [^{3}H]thymidine incorporation *in vitro* were observed for the different drugs tested and also for the same drug on NHL of the same histologic type. For all 6 tumors in which an inhibition of more than 70% on [^{3}H]thymidine incorporation was observed by both adriamycin and prednisolone or by only one of these drugs, a complete remission was reached followed by a long survival of the patients. Of the 9 NHL for which an inhibition of less than 70% was observed, 7 progressed and 2 reached complete remission followed by a quick relapse; all but one died within 17 months. When tested, the antimetabolic effect by vincristine and bleomycin did not correlate with any clinical feature. Statistical analysis showed a significant association between *in vitro* sensitivity and *in vivo* response to therapy in terms of complete remission ($P=0.003$). The actuarial analysis of RFS (Fig. 5) and survival (Fig. 6) in relation to the *in vitro* sensitivity showed a significant difference in both RFS ($P=0.03$) and survival ($P<0.005$) between *in vitro*-sensitive and non-sensitive tumors.

The *in vitro* sensitivity and clinical features for low proliferating NHL are reported in Table 2. Of the 15 patients studied, 13 were untreated and 1 in progression and 1 in

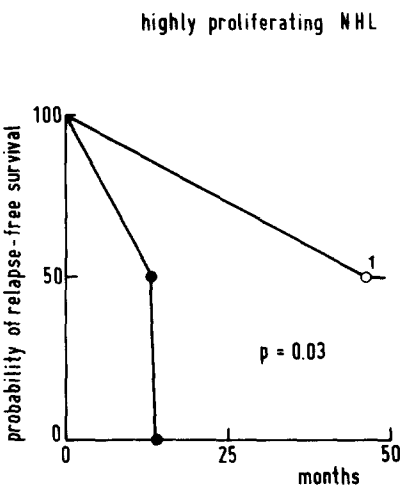

Fig. 5. Relapse-free survival of patients with high proliferating NHL-sensitive (○—○, 4 *cases*) *and non-sensitive* (●—●, 2 *cases*) in vitro.

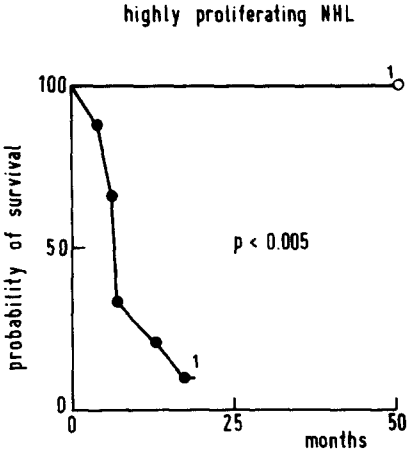

Fig. 6. Survival of patients with high proliferating NHL-sensitive (○—○, 6 *cases*) *and non-sensitive* (●—●, 9 *cases*) in vitro.

relapse after previous treatment. For this kinetic group, in the present study, long survivals and frequent complete remissions, both in nodular and in diffuse pattern, were observed, in agreement with the result previously reported on a larger number of cases [2, 23]. Only 2 of 15 NHL did not reach complete remission, and 2 patients died from their disease. The statistical analysis showed no significant differences in complete response and survival between *in vitro*-sensitive and non-sensitive tumors. For analogy to the clinical criterion adopted for low-aggressive nodular NHL [25], RFS was considered the most significant indicator of therapeutic efficacy. The analysis of *in vitro* sensitivity in relation to RFS showed that of the 8 NHL, in which an inhibition of more than 40% on

[^{3}H]thymidine incorporation was observed by both adriamycin and prednisolone or by only one of these two drugs, 6 patients are in complete remission from 14+ to 46+ months, and 2 relapsed after more than 2 years. On the contrary, among the 7 NHL, for which the inhibition was less than 40%, 2 patients progressed, 3 relapsed, after an initial clinically complete remission, within the first 2 years (9, 16 and 22 months), and only 2 reached complete remission and are still disease-free at 22 and 31 months. Actuarial analysis showed that the two curves of RFS for *in vitro*-sensitive and non-sensitive tumors were significantly different ($P<0.025$) within 22 months and no longer (Fig. 7). The inhibition by vincristine and bleomycin, when tested, showed no correlation with any clinical feature, also in this kinetic group.

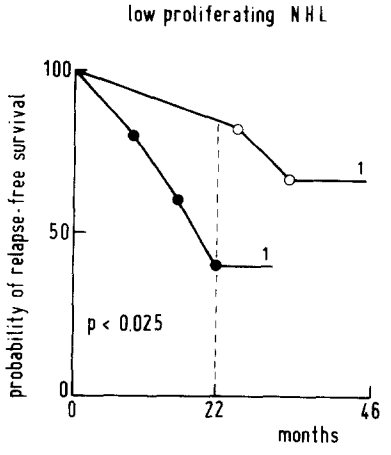

Fig. 7. Relapse-free survival of patients with low proliferating NHL-sensitive (○—○, 8 *cases*) *and non-sensitive* (●—●, 5 *cases*) in vitro.

DISCUSSION

The critical point of the *in vitro* chemosensitivity tests is the definition of sensitivity indexes, that is, what degree of *in vitro* effect corresponds to the therapeutic effect. The object of chemosensitivity tests, based on the interference of drugs on metabolic activity of the cells, is to identify the metabolic events more indicative of clinical effects by the drugs, and to define by a retrospective analysis the degree of inhibition that corresponds to a clinical response. It is possible that by increasing the number of cases, the sensitivity indexes of 70% and 40% as proposed in our text for high and low proliferating NHL, respectively, will somewhat change and border line values will be more accurately defined.

The complete approach requires that all the drugs used in clinical trials be tested *in vitro*, but in our *in vitro* study cyclophosphamide was not tested due to the difficulty in obtaining *in vitro*-active metabolites; at present, both 4-hydroperoxycyclophosphamide and different alkylating agents, as reported in other *in vitro* studies [14], are used instead of the *in vitro*-inactive drug.

Analysis of the relationship between clinical response and *in vitro* effect by adriamycin, prednisolone, bleomycin and vincristine showed that the *in vitro* effect of the last two drugs, for which only occasionally the antimetabolic effect, exceeded the experimental error, gave no further indication of predictivity above that obtained by adriamycin and prednisolone. Thus, although it is possible that vincristine and bleomycin are responsible for a clinical effect in addition to that exerted by adriamycin and prednisolone, clinical features were analysed in relation to the *in vitro* effect of the latter drugs, which were systematically tested.

The retrospective clinical study showed a correlation when different sensitivity indexes in relation to proliferative activity were used. This finding is in agreement with the different clinical course observed for the different kinetic groups [2, 23, 24] and is consistent with the hypothesis that a smaller antimetabolic effect is sufficient to maintain or repress the indolent proliferative activity of low proliferating NHL, while a higher effect is necessary to counteract the higher growth fraction of high proliferating NHL. In this group the *in vitro* inhibition of [^{3}H]thymidine by adriamycin alone or by both adriamycin and prednisolone was significantly related to complete remission, RFS and survival. Similar clinical results were obtained also for the patient whose tumor showed *in vitro* sensitivity to only prednisolone. However, the demonstration of a determinant effect of prednisolone alone on the clinical course of the disease must be substantiated by an adequate number of findings. In low proliferating NHL, the *in vitro* sensitivity appeared to be statistically related only to RFS within 22 months from the beginning of therapy, whereas complete remission and long survival times were generally observed, regardless of *in vitro* sensitivity to adriamycin and prednisolone. Perhaps cyclophosphamide, which shows a high response rate in the treatment of nodular NHL [26], is responsible for the frequent complete remission of this kinetic group.

In conclusion, these findings from the retrospective clinical study suggest the potential usefulness of the antimetabolic tests through the identification of the sensitivity indexes for each drug. The evidence of a broad variability of clinical response for tumors with similar clinical, pathologic and morphologic features and the finding of a lack of relevance of proliferative activity in predicting drug sensitivity, contrary to that observed in experimental tumors [27], supports the hypothesis of the existence of an intrinsic cellular chemosensitivity as already supposed by other authors [28], and emphasizes the opportunity and the usefulness of individual selection of drugs for a more successful management of patients.

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