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Tritiated Thymidine Triphosphate Nuclear Labelling in 48 Patients with Acute Leukaemia

(Correlation with clinical data and response to chemotherapy)*

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Abstract—*The results of 48 patients with acute leukaemia labelled with ^{3}H-thymidine-5′-triphosphate detecting the presence of DNA polymerase and primer template DNA together with cell kinetic parameters are presented.*

In 67 patients with acute myeloid leukaemia (AML), the probability for entering remission increases the younger the patient. This was not found in acute lymphoblastic leukemia (ALL). Statistical significant correlation between mitotic index (MI) and ^{3}H-thymidine labelling index was found in patients with AML. The correlation between MI and ^{3}H-thymidine-5′-triphosphate labelling index was almost significant at the 5% level in patients with ALL. Otherwise, no correlation was found between cell kinetic parameters and these compared to response to chemotherapy or other clinical data.

INTRODUCTION

THE RESPONSE to most cytostatic drugs varies for the different types of leukaemia, but also from patient to patient with apparently the same diagnosis. In order to predict the response to chemotherapy, various studies have been done in correlating the clinical drug effect with cell kinetic studies, but only in a few studies, a large number of patients have been examined [1–3].

A new method which detects the presence of DNA polymerase and primer template DNA in leukaemic blasts cells was introduced in 1976 [4]. Up to now 83 patients with acute leukaemia have been studied, of whom 35 were previously reported [5] and the last 48 patients are published here. In addition, correlation among ^{3}H-TdR LI, MI and ^{3}H-TTP LI and their relationships to clinical data are presented.

MATERIALS AND METHODS

Thirty-eight patients with acute myeloid leukaemia and 10 patients with acute lymphoblastic leukaemia were studied. Bone marrow samples were taken at the time of diagnosis except for three patients (see legend to Table 1). Blood samples were studied in 10 of the patients. Three of the patients were studied later during the course of the disease.

Bone marrow and blood was smeared directly on the slides and stained with May–Grünwald–Giemsa. The percentage of blast cells in mitosis from late prophase to telophase (mitotic index = MI) was determined from a count of 3000–9000 blast cells in each sample. A blood leukocyte count and a differential count of 200 cells was done. The total number

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Abbreviations: ^{3}H-TTP—^{3}H-thymidine-5′-triphosphate; ^{3}H-TdR—^{3}H-thymidine; LI—Labelling index (percentage of cells labelled); MI—Mitotic index (percentage of cells in mitosis); AML—Acute myeloid leukaemia; AMoL—Acute monocytic leukaemia; AProL—Acute promyelocytic leukaemia; AUL—Acute unclassifiable leukaemia; ALL—Acute lymphoblastic leukaemia; BM—Bone marrow; B—Blood; ND—Not done.

Table 1. Clinical data, cytokinetic parameters, ^{3}H-TTP labelling and response to treatment of leukaemic blast cells

Patient	Diagnosis	Age	Sex	Leukocyte count/μl Blast count/μl	BM/B	^{3}H-TTP LI (%)	^{3}H-TDR LI (%)	MI (%)	Survival (days)	Response	Remission
KJO	AML	54	F	1300 52	BM	12.2	5.6	0.14	306	ND	ND
VEK	AML	49	F	17,000 8840	BM	33.1	14.1	0.74	295†	+	+
HOG	AML	53	M	71,000 0	BM	87.9	11.7	1.27	167	–	–
SÅO*	AML	65	M	4600 0	BM	26.9	7.0	0.58	141	–	–
CR	AML	75	M	1600 48	BM	26.4	25.4	1.70	451†	ND	ND
HPA	AML	58	M	48,000 45,600	BM B	19.2 2.1	6.0 ND	0.62	6	ND	ND
SØD*	AMoL	75	M	13,800 2208	BM	41.0	6.8	0.33	157	+	–
EB	AML	65	M	65,000 57,200	BM B	53.5 51.5	ND ND	0.36	38	+	–
MC	AML	73	F	18,150 9801	BM	50.3	9.1	0.60	38	–	–
KDA	AML	37	F	12,000 11,880	BM	18.1	8.8	0.81	39	+	–
PH	AML	54	M	6700 4800	BM	67.3	8.7	0.90	23	–	–
MJ*	AML	26	F	2500 150	BM	39.8	9.6	0.65	34	–	–
CH	AML	32	F	30,000 26,700	BM	60.3	11.6	0.75	186	+	+
HHH	AML	38	M	6950 6185	BM	76.1	0.9	0.10	435	+	+
				8300 0	BM	16.0	13.5	0.75			
JOM	AML	49	M	68,000 61,200	BM	56.6	13.8	1.16	181	+	–
SB	AML	58	F	36,300 25,410	BM B	70.5 37.3	8.0 5.0	0.90	26	+	–
HAV	AML	50	F	4750 2090	BM	37.7	17.6	1.62	153	+	–
GH	AML	63	F	4600	BM	21.6	4.2	0.85	239	+	–

NJ	AML	63	M	183,000 139,080	BM B	48.0 20.1	6.0 1.6	1.05	5	–	–
PK	AML	64	M	26,000 1300	BM	15.1	8.9	0.70	193	–	–
MHS	AProL	80	F	2600 0	BM	18.2	5.1	0.40	9	ND	ND
KI	AML	69	F	4700 3102	BM	50.0	10.2	0.68	22	–	–
ANO	AML	69	F	7700 0	BM	22.4	18.0	1.23	647†	ND	ND
CA	AML	67	M	4400 660	BM	26.3	17.9	0.75	747	ND	ND
HM	AML	73	M	3200 512	BM	27.1	13.2	1.75	1358	ND	ND
NV	AML	72	M	1100 0	BM	34.3	7.8	1.10	487	ND	ND
AAG	AML	65	F	3900 78	BM B	28.0 8.6	12.4 7.4	1.00	138	–	–
PS	AML	73	M	259,000 181,300	BM B	42.3 25.2	6.0 2.5	0.10	55	–	–
AK	AUL	76	F	23,100 4620	BM	56.5	15.3	0.58	4	ND	ND
KDI	AML	83	F	6800 5850	BM	33.0	7.9	0.69	40	–	–
GAO	AML	64	F	27,200 22,300	BM	25.4	9.5	0.58	60	+	–
JHH	AML	29	M	18,050 12,275	BM	52.1	8.5	0.64	127	+	–
AX	AML	54	M	40,200 19,700	BM	47.9	10.5	1.00	54	+	–
LC	AML	53	M	2500 225	BM	46.5	12.3	1.43	92	–	–
EC	AML	59	F	39,000 30,810	BM	26.5	5.6	1.22	141	+	–
KR	AML	23	M	11,000	BM	33.4	3.3	0.20	252	–	–
AP	AML	69	M	1700 0	BM	19.9	12.7	0.60	34	–	–
RE	AML	68	M	25,300 20,240	BM	41.5	4.7	0.95	272	+	–

*Indicates that the patients had been given prednisone or anasterone (MJ) before study.
†Indicates that the patients were alive at the time of writing. Patient HHH was, at the second study, in remission.

of blast cells per μl of venous blood was determined on basis of the mentioned parameters. Part of the sample was incubated with ^{3}H-thymidine (^{3}H-TdR) (spec. act. 2.0 Ci/mM, New England Nuclear) for 1 hr at 37°C [6]. The percentage of ^{3}H-TdR labelled cells (^{3}H-TdR LI) was determined from autoradiographs (Kodak NTB-2, exposure time 7 days) counting at least 1000 leukaemic blast cells.

Part of the remaining aliquot was smeared immediately on rinsed glass slides fixed in absolute ethanol: acetone (1:1 v/v) for 5 min at 0°C and incubated as previously reported [5]. Briefly, incubation mixture consists of 20 mM Tris (pH = 7.8), 0.135 mM each of 2-deoxyadenosine-5′-triphosphate (dATP), 2-deoxyguanosine-5′-triphosphate (dGTP), 2-deoxycytidine-5′-triphosphate (dCTP) (Sigma) respectively, 5 mmole $MgCl_2$ and Ficoll (Sigma) in a concentration of 40 g/100 ml. The final volume in each chamber was 0.5 ml to which 10 μCi ^{3}H-TTP (New England Nuclear, U.S.A., spec. act. 40–60 Ci/mmole) was added. Incubation time 1 hr at 37°C followed by rinsing, fixation and subsequent rinsing. Autoradiographs were prepared using Kodak NTB-2 dipping emulsion with an exposure time of 2 weeks. At least, 1000 blast cell nuclei were counted to determine the percentage of nuclei labelled with ^{3}H-TTP (^{3}H-TTP labelling index = ^{3}H-TTP LI).

The distribution of ^{3}H-TTP labelled blast cells with respect to their DNA content was determined by ultramicrospectrophotometry (Zeiss UMSP-I) after Feulgen staining in two patients (SB, ALN) as previously described by Ernst and Killmann [7].

The response to chemotherapy was grouped into (1) remission (less than 5% blast cells in the bone marrow and normal peripheral values), (2) response to treatment (no blast cells in the peripheral blood and normal leukocyte counts) and (3) no response to treatment.

All patients with AML were initially treated with cytosine arabinoside (i.v. bolus injection) (2.5 mg/kg BW usually for 4–6 days) and thioguanine (2 mg/kg BW usually for 4–6 days). Some patients received daunomycin (i.v. bolus injection) (1.5 mg/kg BW usually for 2–4 days). The patients with ALL all received prednisone and vincristine (i.v. bolus injection) as induction therapy, (prednisone: 1.5 mg/kg BW the first day, thereafter 0.4 mg/kg BW, vincristine 0.03 mg/kg BW). All patients had to fulfil one course of cytostatic treatment to be considered to demonstrate a response to treatment. Eighteen AML patients who failed to receive one full course of cytostatic treatment (patients in other hospitals or patients who died soon after admission to the hospital) are not included in the statistical correlation with response and remission (Table 1). Survival was determined from the time of diagnosis until death. A few patients were still alive at the time of writing (see legends to Tables 1 and 2). Correlation was done between the cell kinetic parameters and the clinical data given in Tables 1 and 2, and the previously published data [5]. The previously reported data includes the same parameters as shown in Tables 1 and 2, except the blast cell count. The statistical analysis was done by using non-parametric methods (Mann–Whitney test and the Spearmans Rank correlation coefficient test). The data were analysed separately for AML and ALL.

RESULTS

AML

In all but 3 of the 38 patients examined at time of diagnosis the ^{3}H-TTP LI of bone marrow varied from 12.2–87.9% with a median of 36%. Blood samples were studied in 6 patients and here the ^{3}H-TTP LI varied between 2.1 and 51.5%. The ^{3}H-TTP values of the bone marrow exceeded the values of the blood. The ^{3}H-TdR LI varied between 0.9 and 25.4% in the bone marrow with a median of 8.9% and between 1.6 and 7.4% in the blood. The MI values ranged from 0.10 to 1.75% with a median of 0.75%. The ^{3}H-TTP labelled blast cells with respect to their DNA content showed a distribution of cells through the entire cell cycle with a peak at 2n and a less pronounced peak at 4n (data not shown). Patient HHH was studied later in the course of the disease, when in remission (Fig. 1). The ^{3}H-TTP LI was significantly lower (16.0%) than at the time of diagnosis (76.1%), and the ^{3}H-TdR LI had increased to almost the value of the ^{3}H-TTP LI from 0.9 to 13.5%. The MI showed an increase from 0.10 to 0.75%.

The age of the patients with AML varied between 1 and 85 yr (median 63 yr). The age distribution is given in Fig. 2. Patients were grouped into response categories as described previously. Of the 67 patients with AML, 18 were indeterminate as to response. Among the remaining 49 patients there were 31 patients responding to chemotherapy, of whom 10 patients achieved complete remission. The probability for remission in patients with

Table 2. Clinical data, cytokinetic parameters, ^{3}H-TTP labelling and response to treatment of lymphoblasts

Patient	Diagnosis	Age	Sex	Leukocyte count/μl Blast count/μl	BM/B	^{3}H-TTP LI (%)	^{3}H-TDR LI (%)	MI (%)	Survival (days)	Response	Remission
ABH	ALL	20	F	56,000	BM	54.6	3.4	1.13	709*	+	+
				33,600	B	22.1	0.8				
				5300	BM	13.4	9.6	1.05			
				0							
KG	ALL	16	F	3600	BM	12.9	4.7	0.54	641*	+	+
				144							
RR	ALL	60	M	17,100	BM	19.6	0.8	0.50	4	–	–
				1710							
KDL	ALL	23	F	ND	BM	28.1	15.0	0.48	217	+	+
PFR	ALL	47	M	20,000	BM	21.7	3.9	0.50	184	+	+
				14,800							
PB	ALL	70	M	262,000	BM	24.4	6.5	0.86	57	+	+
				243,660	B	3.4	1.0				
VL	ALL	70	F	3200	BM	42.4	8.9	0.87	16	–	–
				1920							
YVE	ALL	28	F	414,000	BM	26.0	14.5	0.32	140	+	+
				378,810	B	12.0	7.2				
				16,000	BM	25.1	17.7	0.55			
				9600							
JH	ALL	30	M	57,800	BM	41.4	8.8	0.73	469*	+	+
				51,015	B	3.8	3.0				
US	ALL	26	F	32,000	BM	27.6	6.3	0.80	174*	+	+
				24,960							

*Indicates that the patients were alive at the time of writing. Patient ABH was in remission at the second study. Patient YVE was in relapse at the second study.

AML decreases with age ($P<0.05$). The age of the patients achieving complete remission varied between 15 and 74 yr with a median value of 44 yr. On the contrary, the age of the patients achieving no remission varied between 1 and 83 yr with a median value of 59 yr. Survival was closely related to response to chemotherapy as well as to remission ($P<0.001$). Clear statistical correlation was seen between ^{3}H-TdR LI and MI ($P<0.05$) (Fig. 3). However, there was no correlation between ^{3}H-TTP LI and MI or ^{3}H-TdR LI, nor these compared to age, sex, leukocyte count, blast count and survival. The ^{3}H-TdR LI, MI and ^{3}H-TTP LI had no predictive value concerning the chance of response or remission.

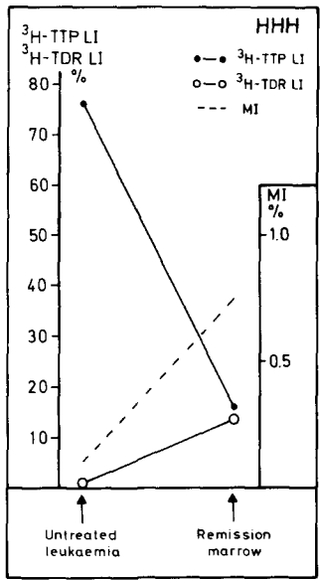

Fig. 1. ^{3}H-TTP LI, ^{3}H-TdR LI and MI at diagnosis and in remission (patient: HHH).

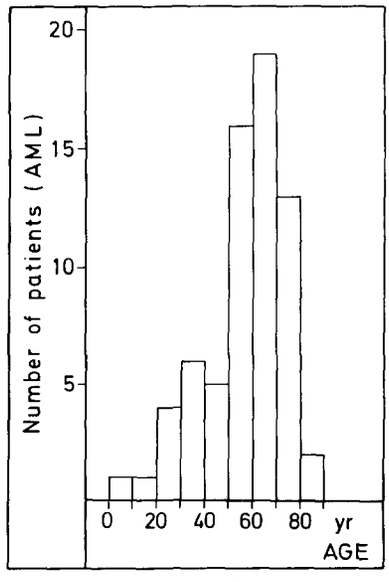

Fig. 2. Age distribution of 67 patients with AML.

ALL

In 10 patients, the ^{3}H-TTP LI of bone marrow varied from 12.9 to 54.6%, with a median of 26.8%. Blood was studied in 4 cases and ^{3}H-TTP LI in blood varied between 3.4 and 22.1%. The ^{3}H-TTP values of the bone marrow exceeded the values of the blood. ^{3}H-TdR LI varied between 0.8 and 15.0% in bone marrow with a median of 6.4% and between 0.8% and 7.2% in the blood. The MI ranged from 0.32 to 1.13% with a median of 0.64%.

Patient ABH was studied again when she was in remission. The ^{3}H-TTP LI was significantly lower (13.4%) during remission than at the time of diagnosis (54.6%), however, the ^{3}H-TdR LI value had increased from 3.4 to 9.6%. Patient YVE was studied when peripheral relapse appeared, at this time the ^{3}H-TTP value was the same as at the time of

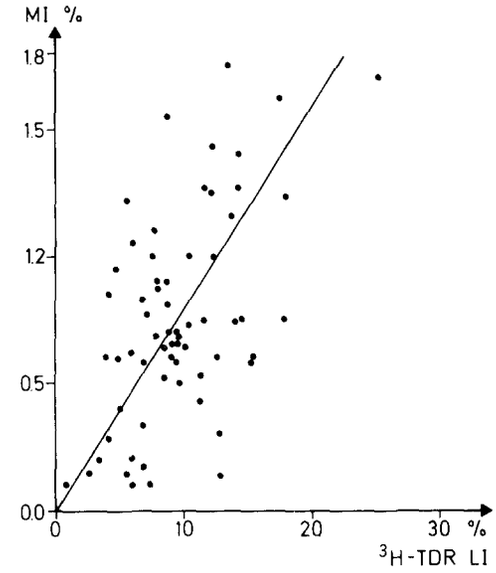

Fig. 3. Correlation between MI and ^{3}H-TdR LI in AML.

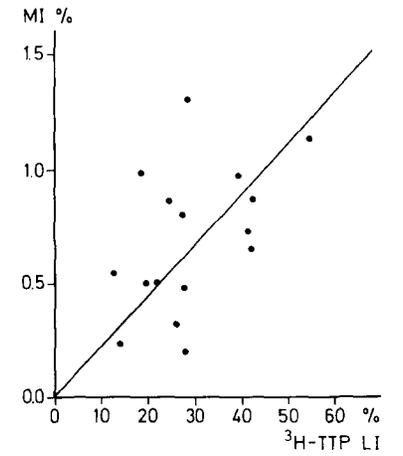

Fig. 4. Correlation between MI and ^{3}H-TTP LI in ALL.

diagnosis. Both ^{3}H-TdR LI and MI had increased moderately.

The age of the patients with ALL varied between 11 and 70 yr with a median of 32 yr. Of the 16 patients, 14 responded to chemotherapy and went into complete remission. No significant correlation was found between age and response to chemotherapy in patients with ALL. Significant correlation among survival, response to chemotherapy and remission ($P<0.02$) was seen. There seemed to be some correlation between ^{3}H-TTP LI and MI, but this was not significant at the 5% level (Fig. 4). Comparison between ^{3}H-TdR LI, MI and ^{3}H-TTP LI, and these with age, sex, leukocyte count, blast count, survival, response and remission revealed no significant correlation.

DISCUSSION

The present paper demonstrates the cell kinetic results in 48 patients with acute leukaemia, in addition nuclear labelling of leukaemic blast cells with tritiated thymidine triphosphate is demonstrated. The average and median values of the pretreatment studied parameters are consistent with the previously published 35 patients [5].

Two patients, one with AML (HHH, Fig. 1) and one with ALL (ABH, Table 2) were studied later during the course of the disease, when the patients were in remission. The ^{3}H-TTP LI had decreased, while the ^{3}H-TdR LI had increased thus achieving a ^{3}H-TTP LI/^{3}H-TdR LI ratio of 1.3:1. These results are consistent with the findings in normal bone marrow, where the ^{3}H-TTP LI values only moderately exceeded the ^{3}H-TdR LI values (1–1.6:1) [5].

In one patient with ALL (YVE, Table 2) studied at the time of relapse, the ^{3}H-TTP LI and ^{3}H-TdR LI were almost identical with pretreatment values, but the MI had increased at relapse. The ^{3}H-TdR LI in ALL patients at diagnosis and at relapse were almost identical as observed by Karle *et al.* [8] using *in vivo* ^{3}H-TdR and by Gavosto and Masera using *in vitro* labelling [9]. Inconsistent with these results are the previous observations with higher ^{3}H-TdR LI at early relapse as compared to diagnosis [10–12].

To our knowledge, statistical analysis on cell kinetic parameters have not been studied so far. In patients with AML, a clear correlation between the ^{3}H-TdR LI and MI is evident as seen in Fig. 3. This finding is expected, as a cell entering S-phase is bound to enter mitosis some time. In ALL, an almost significant correlation between ^{3}H-TTP LI and MI was seen (Fig. 4), whereas correlation between ^{3}H-TdR LI and MI was not achieved. The difference between ^{3}H-TdR LI and ^{3}H-TTP LI in patients with ALL is not as pronounced as the difference between these two parameters seen in patients with AML.

Patients with acute leukaemia entering remission live significantly longer than those who fail to respond to chemotherapy. This was also clearly supported by the present investigation. Furthermore, the probability for entering remission was found higher with younger age in patients with AML as observed by Hart *et al.* [2] although Gunz *et al.* found no significant correlation between induction of remission and age [13]. It seems that at an age over 70 yr, the induction of remission is very low [13, 14]. This is in agreement with the present results. However, no correlation between response to treatment and age was observed. The literature on this finding is inconsistent.

There was no correlation between age (median 32 yr) and remission or response to chemotherapy in patients with ALL (Table 2). This is consistent with the work by Willemze *et al.*, who studied 41 patients with a median age of 19 yr and found no difference in duration of complete remission obtained in patients between 14 and 20 yr of age and older patients [15]. In another work by Gee *et al.*, better results in remission rate, remission duration and survival of children as compared to adults on the same drug regime were found [16]. But in this work, the median ages were 4 and 23 yr, respectively, and cannot be compared to our data or the work by Willemze *et al.* [15].

Several investigators have demonstrated a better response to chemotherapy in patients who had a high initial ^{3}H-TdR LI [2, 17]. An increase in ^{3}H-TdR LI after various cytostatic drugs have also been demonstrated to be correlated with response to treatment [18, 19]. In this study, where 67 patients with AML were examined, there was no significant correlation between the pretreatment cell kinetic parameters, ^{3}H-TTP LI and the response to cytostatic treatment. This is consistent with the results of Vincent *et al.* [20] and partly consistent with what Vogler *et al.* [3] found.

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