Eur J Cancer Clin Oncol, Vol. 18, No. 7, pp. 611–616, 1982.
Printed in Great Britain.

0277-5379/82/070611-06$03.00/0

Polyamines as Biological Markers in Malignant Lymphomas

A. THYSS, G. MILANO,* C. CALDANI, G. LESBATS, M. SCHNEIDER and C. M. LALANNE
Centre Antoine Lacassagne, 36 Voie Romaine, 06054 Nice Cédex, France

Abstract—*The urinary polyamines putrescine (PU) and spermidine (SPD) were measured for 67 patients with lymphomas: 49 non-Hodgkin (NHL) and 18 Hodgkin's disease (HD). Monthly repeat measurements were obtained over an average follow-up period of 12.5 months. For NHL, a good association was observed between polyamine levels and the severity of the stage; the distribution of values between nodular and diffuse forms was also significantly different. No such correlations were seen for HD. The evolution of PU values in time has been shown to reflect disease activity for NHL. Non-specific fluctuating PU values were found in the case of HD. In the case of 20 patients with NHL undergoing chemotherapy, comparison of polyamine levels before and after chemotherapy allowed differentiation between the population of complete responders to treatment and those with only partial or no response.*

INTRODUCTION

THE POLYAMINES putrescine (PU) and spermidine (SPD) are organic polycations associated with the crucial events governing cell multiplication and growth [1–6]. Tissues undergoing rapid growth, and especially tumoral tissue, are those which accumulate and excrete the greatest amounts of polyamines, with PU reflecting proliferative activity and SPD corresponding primarily to the intensity of cell lysis, either spontaneous or induced [7–9]. These properties have been the object of numerous studies in experimental [10–12] and clinical oncology [13–17], and the initial encouraging results obtained by Russel have recently been confirmed [18–21]. Our own experience has led us to consider PU as a disease activity marker correlated with the initial stage which provides information on lesion evolution in time, and to treat SPD as a parameter of pharmacodynamic evaluation whose modifications under chemotherapy allow short-term appraisal of the response to treatment. These properties were recently discussed in a preliminary study on lymphomas [22]. Our promising results led us to continue our study in an attempt to define not only correlations with the initial stage, but also with the histological type (nodular or diffuse) and the histoprognostic groups proposed by Rappaport and the research team at Stanford (personal communication) which at the moment seems to have obtained a wide consensus [23].

Accepted 2 February 1982.
*To whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

Patients

Our study included 67 patients with lymphomas: 49 non-Hodgkin lymphomas (NHL) (mean age 64.7 years limits 23–84 years) and 18 Hodgkin's disease (HD) (mean age 34 years, limits 8–68 years). A total of 272 urinary polyamine measurements were performed monthly over an average follow-up period of 12.5 months (limits 3–37 months).

Diagnosis was always based on histological evidence, and the histoprognostic classification system of Rappaport (personal communication) was employed (see Table 1).

Evaluation of patient staging was based on clinical investigations, including Waldeyer's ring, chest X-ray, bilateral lymphography, echography and/or liver and spleen scan, bone marrow biopsy, biological liver function tests (gamma GT, alkaline phosphatases), and transparietal liver biopsy or liver biopsy during laparotomy, when doubt existed as to the

Table 1. Histoprognostic classification of non-Hodgkin lymphomas [The non-Hodgkin lymphoma pathology classification project, National Cancer Institute. Sponsored study of classifications of non-Hodgkin lymphomas. Summary and description of working formulations for clinical use. J Natl Cancer Inst *(in press)]*

1. Low grade	Small lymphocytic
	Follicular, predominantly small cleaved cells
	Follicular, mixed small cleaved or large cells
2. Intermediate grade	Follicular, predominantly large cells
	Diffused, small cleaved cells
	Diffused, mixed small and large cells
	Diffused, large cells
3. High grade	Diffused large cells, immunoblastic
	Lymphoblastic
	Small non-cleaved cells (Burkitt or non-Burkitt)

presence of liver lesions. Evaluation parameters were as follows:

Active state: unchanged or large lesions detectable clinically, radiologically, by a scan or by echography.

Remission: complete: disappearance of all clinical or paraclinical evidence of disease, with a minimum follow-up period of 2 months.
partial: persistence of residual lesions with the remaining tumor less than or equal to 50% of the initial involvement.

Appraisal of the efficacy of chemotherapy was based on clinical or paraclinical examinations after a minimum follow-up period of 2 months after treatment completion.

Complete response: obtention of complete remission.
Partial response: obtention of partial remission.
No response: persistence of active state.

The following chemotherapy protocols were employed:
HD: MOPP [24]

NHL: main protocol (polychemotherapy): day 1, adriamycin, 40 mg/m^2; day 2, VM26, 40 mg/m^2, bleomycin, 10 mg/m^2; days 3 and 4, cyclophosphamide, 400 mg/m^2 with prednisone; monochemotherapy: vindesine, 2 mg/m^2.

Polyamine analysis

An automatic amino acid analyser (LIQUIMAT III, Kontron) was used for polyamine analysis as previously described by Milano *et al.* [25]. The procedure involved continuous fluorescent detection of the chromotographed amines by u.v. detection of the *o*-phthalaldehyde derivatives. Reproducibility was evaluated by repeat analysis of a pooled urine sample; the coefficient of variation for intra-assay reproducibility was 2.8% for PU and 0.8% for SPD. Interassay reproducibility was 4.5% for PU and 5.0% for SPD.

Polyamines were measured in 24-hr urine samples. The samples were stored at 4°C between each collection. An aliquot of each sample was stored at −20°C until analysis. In addition, for 20 patients with lymphosarcomas, putrescine (*P*) and spermidine (*S*) concentrations were determined 24 hr before (*P*1, *S*1) and after completion of a course of chemotherapy (*P*2, *S*2).

A control group of 22 healthy adults was used to set the upper measurement limits (mean ± 2 S.D.): PU, 2.00 μg/mg creat.; SPD, 1.60 μg/mg creat. Comparison of distributions for men and women (μg/mg creat.) did not reveal any significant differences: men (14): PU, 1.24 ± 0.42; SPD, 1.00 ± 0.37; women (8): PU, 1.31 ± 0.32; SPD, 0.90 ± 0.16.

RESULTS

Table 2 gives the distribution of median and extreme urinary PU and SPD values for the NHL population. As a function of the staging, the distributions of the values for stages I and II can be seen to differ significantly from those for stages III and IV, both for PU and SPD. The histological notion of nodular or diffuse disease is also accompanied by significantly different distributions for PU and SPD: whereas nodular disease is associated with low PU and SPD values comparable to those of healthy individuals, diffuse disease is accompanied by pathological PU and SPD levels. Likewise, the median polyamine values follow the increasing grades of the histoprognostic groups defined by Rappaport (personal communication).

As concerns Hodgkin's disease, Table 3 shows that polyamines are less useful for the definition of the severity of the stage. In contrast, a good correlation exists between SPD levels and the absence (group A) or the presence (group B) of general clinical signs.

Table 2. Distribution of values for urinary putrescine and spermidine in non-Hodgkin lymphomas

		Staging I, II($n = 9$) III, IV($n = 22$)			Histology N($n = 10$) D($n = 23$)			Histoprognostic group I($n = 9$) II($n = 10$) III($n = 16$)				
Putrescine (μg/mg creat.)	Lower limit	1.05		1.40	1.15		1.05	1.35		1.05		1.30
	Median	1.55	*	3.75	1.90	*	3.35	1.72	†	3.45	†	3.10
	Upper limit	3.10		10.80	4.80		10.80	4.25		6.40		10.80
Spermidine (μg/mg creat.)	Lower limit	0.60		0.70	0.55		0.75	0.60		0.65		0.95
	Median	1.15	*	3.10	1.35	*	2.75	1.38	*	2.30	†	3.15
	Upper limit	1.70		20.60	5.10		20.60	3.70		5.50		20.60

*The two adjacent groups of patients are significantly different, with a threshold of $P < 0.05$ (Student's t-test).
†The two adjacent groups of patients are not significantly different, with a threshold of $P < 0.05$. n, Number of cases; N, nodular disease; D, diffuse disease.

Table 3. Distribution of values for urinary putrescine and spermidine in patients with Hodgkin's disease

		Staging I,II($n = 5$) III, IV($n = 8$)			A ($n = 5$)		B ($n = 6$)
Putrescine (μg/mg creat.)	Lower limit	0.78		1.03	0.78		1.03
	Median	1.43	†	1.50	1.24	†	1.73
	Upper limit	1.91		3.95	1.91		7.09
Spermidine (μg/mg creat.)	Lower limit	0.39		0.88	0.39		1.14
	Median	1.12	†	2.00	0.88	*	2.45
	Upper limit	1.80		3.90	1.12		4.53

*The two adjacent groups of patients are significantly different, with a threshold of $P < 0.05$ (Students's t-test).
†The two adjacent groups of patients are not significantly different, with a threshold of $P < 0.05$. n, Number of cases; A, presence of general signs (intermittent fever, night sweats); B, absence of general signs.

The evolution of urinary PU values in time as a function of disease activity during NHL is illustrated in Table 4. Patients 1–3, whose disease always remained active, exhibited high PU values, indicative of the presence of lesions which were not responding to treatment. For patients 4–9, passage from the active stage to remission was accompanied by a significant drop in urinary PU levels. Moreover, in the case of relapse, the PU levels systematically rose back up to pathological values. Use of polyamines to follow up patients with HD was found less satisfactory since the values fluctuated independent of disease activity.

In the case of 20 patients with NHL undergoing chemotherapy, the polyamines putrescine (P) and spermidine (S) were measured 24 hr before the start ($P1$, $S1$) and after completion of treatment ($P2$, $S2$). The ratio $S2/P2 : S1/P1$, described previously [20], was employed to take the respective variations of each polyamine into account. In theory, in the case of a

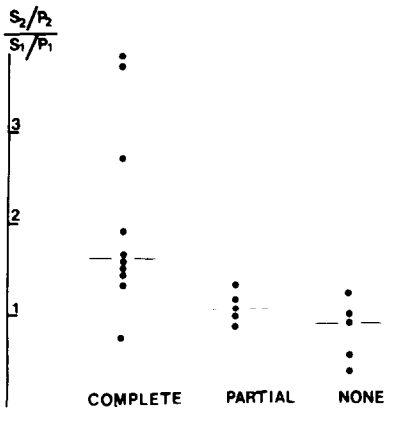

Fig. 1. Distribution of the ratio S2/P2 : S1/P1 as a function of the type of response to chemotherapy for patients with HNL (horizontal line = median).

favorable response and thus tumor lysis, $S2$ is greater than $S1$ and $P2$ is lower than, or unchanged with respect to, $P1$. The $S2/P2 : S1/P1$ ratio is thus high. The opposite is true when there is no response. Figure 1 represents the respective distributions of this ratio for the three response categories we defined. Except for a single value, there was no cross-over between the population of complete responders and those with a partial or no response.

DISCUSSION

In an initial report on malignant lymphomas [22], we showed that there was a good correlation between polyamine levels and the severity of the evolutionary stage of the disease. In the present, more complete study, we have confirmed our previous results and completed our investigations by showing that there is a close correlation between the low initial urinary levels for NHL patients with histologically nodular disease and the abnormally high levels found for diffuse disease. Since the evolution of nodular NHL is better than that for diffuse NHL, the prognostic value of polyamines is evident. This would justify the obtention of polyamine measurements as part of pretherapy work-ups.

During the follow-up of NHL patients, PU excretion appears to be a faithful indicator of disease evolution. Whereas low values at the limit of the normal range (less than 2.00 μg/mg creat.) were found only for patients in remission, pathological values were associated with an active initial stage and with relapses. This observation concurs with the recent conclusions by Uehara *et al.* [26] concerning repeat measurements of erythrocytic spermidine and spermine in patients with malignant lymphomas. The evolutionary pattern for HD generally differs from that for NHL: whereas the host/tumor relationship and marked immunodeficiency in HD appear to play an important role in the final outcome [27], the major risk in NHL is often lesion evolution. Observation of dispersed polyamine values during HD evolution is thus not surprising.

Finally, this study confirms the results of our

Table 4. Variations in urinary putrescine levels (μg/mg creat.) during follow-up of NHL patients

Case No./ age/sex	Stage/ histology	Follow-up period	I	Active disease	Treatment Chem. Rx	Remission	Relapse
1	IV	22/9/78	1	2.20	+ +		
79 yr		7/2/79	4.52		+		
M	Diffuse		3.66		+		
				2.97	+		
				2.50	+		
2	IV	30/10/78	2	5.65	+		
46 yr		16/7/79		5.70	+		
M	Diffuse			4.25	+		
				3.90	+		
3	IV	21/8/78	3	2.36	+ +		
62 yr		26/9/80		2.04	+		
F	Diffuse			3.65	+		
				3.50	+		
				2.40	+		
				2.75	+		
				2.90	+		
4	III	13/3/78		2.41	+		
84 yr		15/9/78				0.70	
F	Diffuse					1.45	
						1.47	
						0.56	
							2.40
5	II	9/2/79	1	2.88	+ +		
57 yr		19/4/79			+	1.20	
M	Diffuse				+	1.96	
					+		
					+		3.44

Table 4. (*Contd.*)

Case No./ age/sex	Stage/ histology	Follow-up period		Active disease	Treatment Chem. Rx	Remission	Relapse
6	III	23/7/79	1	4.00	+		
65 yr		15/2/80			+	1.85	
F	Nodular				+	2.93	
					+	1.86	
					+		2.86
					+		3.49
							4.12
7	IV	3/10/80	1		+	1.65	
65 yr		20/3/81			+	1.10	
M	Diffuse				+	1.54	
							2.53
8	III	30/10/78	2	2.07			
64 yr		5/6/81			+ +	1.76	
F	Nodular				+	2.22	
					+	2.20	
					+	2.05	
					+	1.85	
							2.92
9	III	25/4/76	1	2.31	+ +		
65 yr		28/12/78			+	2.49	
F	Diffuse				+	2.74	
					+	2.71	
					+	2.60	
					+		5.33
					+		3.85
					+		4.71
		Total mean		3.62	*	1.84*	3.66
		S.D.		0.31		0.65	0.27

I, Time interval between determinations (in months); Chem, chemotherapy; Rx; radiotherapy.
*Significantly different at a level of $P < 0.05$ (Student's t-test).
For definitions of active disease, remission and relapse see Materials and Methods.

previous work on the pharmacodynamic properties of polyamines, as illustrated in Fig. 1. Other teams have recently reported similar results [21, 28] and the original hypothesis of Russel [29] thus appears to fulfill its promises during practical applications. In our opinion, this last characteristic of polyamines represents the most interesting feature due to the time it can save the oncologist and the increased benefits for patients as the result of rapid adoption of an effective therapy program.

REFERENCES

1. WILLIAMS-ASHMAN HG, CANELLAKIS LN. Polyamine in mammalian biology and medicine. *Biol Med* 1979, **22**, 421–453.
2. STURMAN JA, GALL GE. Polyamine biosynthesis in human fetal liver and brains. *Pediat Res* 1974, **8**, 231–237.
3. RUSSEL DH, BYUS CU, MANEM CA. Proposed model of major sequential biochemical events of a trophic response. *Life Sci* 1976, **19**, 1972.
4. RAINA A, JANNE J, SIIMS M. Stimulation for polyamine synthesis in relation to nucleic acids in regenerating rat liver. *Biochim Biophys Acta* 1966, **123**, 197–201.
5. WOO KB, PERIN F, SADOW J, SULLIVAN C, FONKHOUBER W. Polyamine content and release during the proliferation of Burkitt's lymphoma cells *in vitro*. *Cancer Res* 1979, **39**, 2429–2435.
6. ANEHUS S, BENGTSSON G, ANDERSON G *et al.* Urinary putrescine and plasma lactate dehydrogenase as markers of experimental adenocarcinoma growth. *Eur J Cancer* 1981, **17**, 511–518.

7. BRANDT JT, PERCE DA, FAUSTO N. Polyamines and growth hormone. *Biochim Biophys Acta* 1972, **279**, 184–193.
8. RUSSEL DH. Effect of MTX and cytosine arabinoside on polyamine level of L1210 ascites cells. *Cancer Res* 1972, **32**, 2459–2462.
9. RUSSEL DH, LOOREY WB, KOVACO CJ *et al.* Polyamine depletion of tumor tissue and subsequent elevation of spermidine in the sera of rats with 3924 A hepatomas after 5-fluorouracyl administration. *Cancer Res* 1974, **34**, 2382–2385.
10. ABDEL-MOHEM MM, OHNO K. Polyamine metabolism III, urinary acetyl polyamines in human cancer. *J Pharm Sci* 1978, **67**, 1671–1674.
11. FUJITA K, NAGATSU T, MARUTA K, IBO M, SENBA H, MIKI K. Urinary putrescine, spermidine and spermine in human blood and solid cancers and in an experimental gastric tumor of rat. *Cancer Res* 1976, **36**, 1320–1324.
12. O'BRIEN TG, SINSIMAN RC, BOUTWELL RK. Induction of the polyamine biosynthetic enzymes in mouse epidermis by tumor-promoting agents. *Cancer Res* 1975, **35**, 1662–1670.
13. RUSSEL DH. Increased polyamine concentrations in the urine of human patients. *Nature (Lond)* 1971, **233**, 144.
14. LIPTON A, SHUMAN LM, KESSLER GF. Urinary polyamine levels in human cancer. *Cancer* 1975, **35**, 464–468.
15. LIPTON A, SHUMAN L, MORREL R, HARVEY IA. Urinary polyamine levels in patients with localized malignancies. *Cancer* 1976, **38**, 1344–1347.
16. RUSSEL DH. Clinical relevance of polyamine as biochemical markers of tumor kinetics. *Clin Chem* 1977, **23**, 22–27.
17. JÄNNE J, POSO H, RAINA A. Polyamines in rapid growth and cancer. *Biochim Biophys Acta* 1978, **473**, 241–293.
18. HARAK SI, SULTON CH. Putrescine as a biochemical marker of malignant brain tumors. *Cancer Res* 1979, **39**, 5010–5015.
19. SAVORY J, STRIDLER CE, SHIPE JF, WILSON MR. Erythrocyte polyamine determination in cancer. *Clin Chem* 1980, **26**, 1041–1046.
20. MILANO G, VIGUIER E, LALANNE CM *et al.* The clinical value of urinary polyamine analyses in cancer patients. *Oncodev Biol Med* 1980, **1**, 215–225.
21. ROMANO M, CECCO L, CERRA M, MONTUORI R, DE ROSA C. Polyamines as biological markers of the effectiveness of therapy in acute leukemia. *Tumori* 1980, **66**, 677–687.
22. MILANO G, CASSUTO JP, SCHNEIDER M *et al.* Polyamines in lymphomas: their value in disease staging and monitoring of therapy efficiency. *Nouv Rev Fr Hematol* 1980, **22**, 249–255.
23. WEIL M, JACQUILLAT C, DUMONT J. Lymphomes non Hodgkiniens. *Nouv Presse Med* 1981, **27**, 2255–2256.
24. DE VITA VT, SERPICK AA, CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Int Med* 1970, **73**, 881–887.
25. MILANO G, SCHNEIDER M, CAMBON P *et al.* An improved method for routine analysis of polyamines in biological fluids with a conventional amino acid analyzer. *J Clin Chem Clin Biochem* 1980, **18**, 157–162.
26. UEHARA N, KITA K, SHIRAKAWA S, UCHINO H, SACKI Y. Elevated polyamine content in erythrocytes of maligant lymphoma cells. *Gann* 1980, **71**, 393–397.
27. SCHNEIDER M, MATHE G, SCHWARTZENBERG L *et al.* Non-specific immune responses in hamatosarcomas and acute leukemias. *Recent Results Cancer Res* 1974, **47**, 42–55.
28. BAKOWSKI ME, TOSELAND PA, WICKS JFC, TROUNCE JR. A rapid gas chromatographic method for the determination of plasma polyamines and its applications to the prediction of tumour response to chemotherapy. *Clin Chim Acta* 1981, **110**, 273–286.
29. RUSSEL DH, DURIE BLM, SALMON SE. Polyamines as predictors of success and failure in cancer chemotherapy. *Lancet* 1975, **ii**, 797–799.