[CANCER RESEARCH 44, 4675–4678, October 1984]

Relationship of Urinary Polyamines to Tumor Activity and Tumor Volume in Patients[1]

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ABSTRACT

Serially obtained urinary polyamine levels were determined for 192 patients during a specified time period. The number of patient urine samples totaled 938. The patients had tumors of either the breast, stomach, prostate, or female genital tract, or metastatic carcinomas of unknown origin. Tumor activity and tumor volume, along with other clinical information, were also recorded during the time period. Possible associations between tumor activity and tumor volume on one hand, and polyamine levels on the other hand, were explored via different statistical analyses. For each tumor type, statistically significant group differences were found in polyamine levels between patients with nonactive tumors and patients with active large tumors. Predictive values of polyamine assays for change in disease activity and stability in disease nonactivity for tumors of the breast, female genital tract, and prostate were also computed. For breast tumors, these predictive values do not support the clinical utility of the use of polyamine levels to monitor disease states. For tumors of the female genital tract and prostate, these predictive values yield an indeterminant conclusion.

INTRODUCTION

Continued efforts to define the utility of monitoring polyamine levels in biological fluids as indicators of the progression or regression of tumor growth have revealed a variety of interesting results, biological insights, and the beginnings of what may eventually be defined clinical utility (3). Since many published studies unfortunately have not sufficiently integrated clinical data (tumor staging, activity, therapy, etc.) with polyamine levels and have not obtained serial samples from individual patients, we initiated a new study to screen a variety of tumor types for the appropriateness of the urinary polyamine assay as a monitor of tumor activity. All patients included in the study were treated and evaluated by only 2 physicians according to established, uniform protocols, and all results were evaluated using a statistical method that accounts for serial samples in the same patient.

Our initial report of this study (192 patients, 503 determinations) indicated statistically significant differences in polyamine levels between patients with "active" or "nonactive" disease for tumors of the breast, stomach, prostate, and the female genital tract, and (with some reservation) a variety of metastatic carcinomas of unknown origin (1). Therefore, we have subsequently concentrated our efforts on patients with just these tumor types, focusing on multiple determinations in individual patients. This paper describes our results from the analysis of 938 polyamine determinations obtained from 192 patients with these tumor types (34% of these determinations and 55% of these patients were also included in the initial report). Differences in polyamine levels between patient groups are reexamined. In contrast to the initial report, the possible effects of patient age and tumor volume on these differences are taken into account in this report. In addition, predictive values of polyamine assay for change in disease activity and stability in disease nonactivity are presented for tumors of the breast, prostate, and female genital tract.

MATERIALS AND METHODS

Since 1981, 192 patients with tumors of the breast, stomach, prostate, female genital tract, and a variety of metastatic carcinomas of unknown origin, all with histologically confirmed diagnosis, were treated and followed at the Department of Oncology at Assaf Harofeh Hospital. Patients were evaluated initially with a physical examination, blood tests (complete blood count, blood urea nitrogen, electrolytes, liver function tests), relevant X-rays, and radionuclide scans; a 24-hr urine output was collected for polyamine analysis. Chemotherapy, radiation therapy, or follow-up without therapy proceeded according to established protocols for the treatment of each disease. Every patient was evaluated periodically by the same medical team; these evaluations included routine tests and repeat urine collection for polyamine analysis. The evaluations were typically spaced 1 to 3 months apart, but sometimes they were spaced further apart. At each evaluation, based on all clinical and laboratory findings, except for the polyamine assay, patients were judged to have either active or nonactive disease. Active disease included increased tumor size and/or metastatic activity and was based on an overall clinical rather than laboratory impression, even though laboratory data were utilized in the decision-making process. In addition, in most patients with active disease and in some patients with nonactive disease, the tumor volume was estimated as being either "small" (limited local involvement) or "large" (tumor with massive local involvement or with metastases). Although we clearly understood that tumor volume and tumor proliferation do not necessarily correlate, tumor volume was a potential discriminator available for our use. Some patients with nonactive disease were placed on adjuvant therapy according to established protocols, while other nonactive patients were followed without therapy.

Urine Collection. The volume of urine collected in 24 hr was acidified and measured, and an aliquot of each was centrifuged at 900 × *g* for 20 min. One ml of each supernatant was frozen, lyophilized, and sent to San Francisco, CA, for polyamine analysis. An aliquot of the lyophilized urine was assayed for creatinine in the clinical chemistry laboratory at Assaf Harofeh.

Polyamine Assay. Each lyophilized sample was reconstituted in 1 ml of deionized water. Two hundred μl of the reconstituted samples were mixed with an equal volume of concentrated HCl and hydrolyzed at 100° for 14 to 16 hr. The hydrolysates were then lyophilized, reconstituted in 200 μl of 4% 5-sulfosalicylic acid, and centrifuged at 8000 × *g* for 10 min, and the supernatants were used for chromatographic analysis as described previously (2).

Statistical Analysis. The basic statistical analysis has been described

[1] This research was supported in part by NIH Program Project Grant CA-13525 and by a Hadassah-Wizo Canada grant.

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Received December 5, 1983; accepted June 26, 1984.

previously (1). We have expanded it, however, to account for possible tumor volume and patient age effects. Consider patients with a particular tumor type whose urine is measured, *i.e.*, assayed, serially for levels of a particular polyamine. The *j*th serial measurement from the *i*th patient, y_{ij}, is modeled by

$$\log y_{ij} = \mu + \beta x_i + \theta_1 z_{ij} + \theta_2 w_{ij} + \eta_i + \epsilon_{ij}$$

where x_i is the age of the patient, z_{ij} is 0 or 1 according to whether at the time y_{ij} is taken, the patient's disease is classified as nonactive or active, respectively, and where w_{ij} is 0 or 1 according to whether at the time y_{ij} is taken, the patient's tumor volume is classified as small or large, respectively (if $z_{ij} = 0$, then w_{ij} is taken to be 0). Moreover, η_i is a random interpatient effect, and ϵ_{ij} is random residual error. Consequently, each log measurement from a patient with age x is regarded as arising from one of 3 populations of log measurements, one population corresponding to nonactive disease, one population corresponding to active disease with small tumor volume, and one population corresponding to active disease with large tumor volume. The means of the log measurements from each of these 3 populations are $\mu + \beta x$, $\mu + \beta x + \theta_1$, $\mu + \beta x + \theta_1 + \theta_2$, respectively; $\mu + \beta x$ is a base-line mean, and θ_1, θ_2, and $\theta_1 + \theta_2$ are group mean differences, all of which are to be estimated. The use of the logarithmic transformation mitigates the skewness effect of the data (see Charts 1 and 2) on the statistical analysis. The method of maximum likelihood is used to estimate the base-line mean and mean differences and to establish confidence intervals about them. The estimates and confidence limits presented in the tables (see below) are obtained by taking the antilogs of the numbers obtained with the maximum likelihood method. Therefore, tabled means are estimates of geometric means and are in the units of the polyamine assay, while the confidence intervals given are for proportional differences and are dimensionless. The results of our earlier study (1) can be compared to the results of this study by taking the antilogs of the numbers given in Tables 2 and 3 therein.

Predictive values for change in disease activity and stability in disease nonactivity are simply estimated as observed proportions. Consider, again, patients with a particular tumor type whose urine is measured serially for levels of a particular polyamine, and consider all pairs of consecutive measurements from these patients spaced within *l* months apart, and such that the second measurement of the pair exceeds the first measurement of the pair by Δ% of the first measurement. The ratio of (*a*) the number of such pairs which are associated with a change in disease activity from nonactive to active between the 2 measurement times to (*b*) the number of all such pairs is an estimate of the predictive value of the polyamine assay for an (adverse) change in disease activity. On the other hand, consider all pairs of consecutive measurements from these patients spaced within *l* months apart and such that the second measurement of the pair does not exceed the first measurement by Δ% of the first measurement. The ratio of (*c*) the number of these pairs which are associated with nonactive disease activity at both measurement times to (*d*) the number of all such pairs is an estimate of the predictive value of the polyamine assay for stability in disease nonactivity. Both estimates were computed for $l = 3$, 4, and 6 and $\Delta = 30$, 50, 80, and 110.

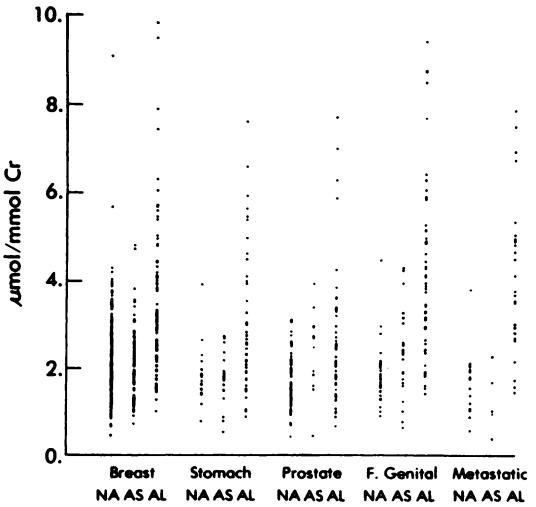

Chart 1. Putrescine levels grouped by tumor type and subgrouped by nonactive (*NA*), active small tumor (*AS*), and active large tumor (*AL*) disease. *Cr*, creatinine.

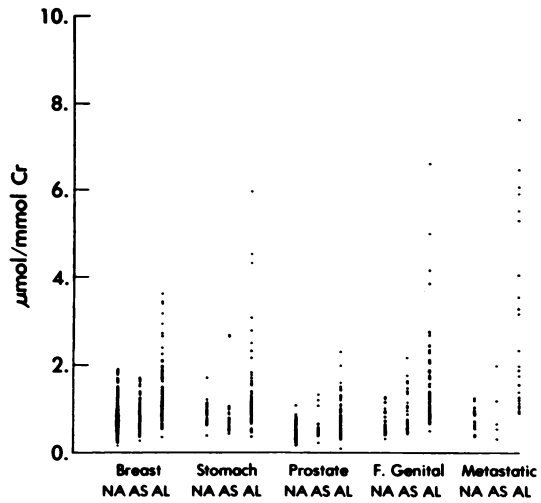

Chart 2. Spermidine levels grouped by tumor type and subgrouped by nonactive (*NA*), active small tumor (*AS*), and active large tumor (*AL*) disease. *Cr*, creatinine.

RESULTS

Table 1 in conjunction with Charts 1 and 2 shows the distribution of the 938 polyamine determinations from the 192 patients in terms of tumor type, disease activity, and tumor volume. There is no statistical evidence whatsoever that age affects polyamine measurements; *i.e.*, β is effectively zero, and graphical display of the data confirms this result. Therefore, for all analyses presented, β is constrained to be zero. Tables 2 and 3 show the results of the statistical analysis for differences in putrescine and spermidine, respectively, between nonactive tumors and active tumors. When at the 5% statistical significance level there is a significant (proportional) difference, a 95% confidence interval for this difference is given (of which the lower limit exceeds one). For example, the geometric mean of the putrescine levels with nonactive stomach tumors, λ, is estimated to be 1.39, and with 95% confidence the geometric mean of the putrescine levels with active stomach tumors is no less than 114% of (the true value of) λ, but no greater than 170% of this value. In this expanded study, significant differences were observed only with tumors of the stomach, prostate, and female genital tract for putrescine, and with breast, female genital tract, and metastatic carcinoma of unknown origin for spermidine. Note though, that for each tumor type, a significant difference in either putrescine or spermidine occurs. (In this analysis, θ_2 is constrained to be zero.)

Tables 4 and 5 are similar to Tables 2 and 3 but further subdivide the population of measurements into groups corresponding to nonactive, active small, and active large tumors for putrescine and spermidine, respectively. Metastatic tumors of unknown origin are not represented in these tables, since all such tumors were of large volume. In these comparisons, all

Table 1

Number of patients and polyamine samples

Tumor type	No. of patients	Age range (yr)	No. of polyamine determinations					
			Disease activity			Tumor volume		
			Active	Nonactive	Total	Small	Large	Total
Breast	104	29–78	190	356	546	96	99	195
Stomach	21	37–90	69	22	91	38	53	91
Prostate	30	56–85	72	66	138	78	60	138
Female genital	25	32–79	84	32	116	49	57	106
Metastatic carcinoma of unknown origin	12	58–75	30	17	47	22	25	47
Total	192		445	493	938	283	294	577

Table 2

Statistical analysis for putrescine, nonactive versus active

	Geometric mean nonactive tumors (μmol/mmol Cr)[b]	Confidence interval for proportional difference in level between nonactive and active tumors
Breast	2.03	NS[a]
Stomach	1.39	1.14, 1.70
Prostate	1.60	1.06, 1.63
Female genital	1.68	1.40, 2.01
Metastatic carcinoma	1.99	NS

[a] NS, not significant.
[b] *Cr*, creatinine; also used in Tables 3, 4, and 5.

Table 3

Statistical analysis for spermidine, nonactive versus active

	Geometric mean nonactive tumors (μmol/mmol Cr)	Confidence interval for proportional difference in level between nonactive and active tumors
Breast	0.74	1.11, 1.43
Stomach	0.86	NS[a]
Prostate	0.44	NS
Female genital	0.68	1.39, 2.20
Metastatic carcinoma of unknown origin	0.80	1.95, 2.56

[a] NS, not significant.

Table 4

Statistical analysis for putrescine accounting for tumor volume

For all tumor types, the difference in level between nonactive and active small tumors is not significant.

	Geometric mean nonactive tumors (μmol/mmol Cr)	Confidence interval for proportional difference in level between nonactive and active large tumors	Geometric mean active small tumors (μmol/mmol Cr)	Confidence interval for proportional difference in level between active small and active large tumors
Breast	2.01	1.27, 1.75	1.76	1.42, 2.01
Stomach	1.71	1.28, 1.88	1.50	1.29, 2.44
Prostate	1.58	1.06, 1.77	1.84	NS[a]
Female genital	1.69	1.73, 2.44	2.28	1.13, 2.08

[a] NS, not significant.

tumors except prostate showed significant differences between nonactive and active large tumors for both polyamines. Prostate tumors showed a positive result for the difference in level between nonactive and active large tumors for putrescine. Unfortunately, for both putrescine and spermidine, comparisons in level between nonactive and active small tumors showed no significant differences. (Note that the numbers in Column 1 differ slightly between Tables 2 and 4 and between Tables 3 and 5, since in this analysis, θ_2 is not constrained to be zero.)

Table 5

Statistical analysis for spermidine accounting for tumor volume

For all tumor types, the difference in level between nonactive and active small tumors is not significant.

	Geometric mean nonactive tumors (μmol/mmol Cr)	Confidence interval for proportional difference in level between nonactive and active large tumors	Geometric mean active small tumors (μmol/mmol Cr)	Confidence interval for proportional difference in level between active small and active large tumors
Breast	0.73	1.40, 1.88	0.73	1.28, 2.08
Stomach	0.86	1.07, 1.79	0.65	1.34, 2.48
Prostate	0.44	NS[a]	0.50	NS
Female genital	0.63	1.77, 2.92	0.82	1.25, 2.44

[a] NS, not significant.

Table 6

Predictive values of polyamine assay for patients with breast tumors

Predictive value for change in disease activity	Predictive value for stability in disease nonactivity	*n*	Required difference in 2 consecutive levels (%)	Interval between levels (mo)
Putrescine				
9		32	50	3
	96	171	50	3
11		18	80	3
	89	185	80	3
7		59	30	4
	97	184	30	4
8		38	50	4
	97	205	50	4
6		65	30	6
	97	210	30	6
7		42	50	6
	97	232	50	6
Spermidine				
7		59	30	3
	97	150	30	3
12		17	80	3
	99	187	80	3
7		71	30	4
	97	181	30	4
15		20	80	4
	97	232	80	4
7		76	30	6
	97	208	30	6
9		21	80	6
	97	263	80	6

Tables 6 to 8 present the predictive values of the putrescine and spermidine assay for change in disease activity and stability in disease nonactivity for tumors of the breast, prostate, and female genital tract. Inadequate sample sizes account for the nonavailability of results for the other tumor types. For the results

Table 7
Predictive values of polyamine assay for patients with prostate tumors

Predictive value for change in disease activity	Predictive value for stability in disease nonactivity	*n*	Required difference in 2 consecutive levels (%)	Interval between levels (mo)
Putrescine				
29		7	50	3
	100	34	50	3
50		2	80	3
	97	39	80	3
30		10	50	4
	97	38	50	4
50		4	80	4
	95	44	80	4
50		2	110	4
	91	46	110	4
Spermidine				
0		9	30	3
	94	33	30	3
8		12	30	4
	92	38	30	4

Table 8
Predictive values of polyamine assay for patients with female genital tumors

Predictive value for change in disease activity	Predictive value for stability in disease nonactivity	*n*	Required difference in 2 consecutive levels (%)	Interval between levels (mo)
Putrescine				
50		2	110	3
	100	15	110	3
50		4	50	4
	100	17	50	4
50		2	110	4
	95	19	110	4
Spermidine				
50		2	80	3
	100	15	80	3
66		3	80	4
	100	18	80	4
33		3	110	4
	95	19	110	4

presented, the numbers given in the column labeled *n* are the denominators of the predictive values. The results for all tumor types show excellent prediction of stability in disease nonactivity, based on adequately large *n*. However, the prediction of change in disease activity was considerably poorer, especially for tumors of the breast. For this prediction, however, *n* was not adequate, except for tumors of the breast. Where predictive values for the 6-month interval are not tabled, it is because the first predictive value is not greater than the first predictive value for the 4-month interval. Where predictive values for a given Δ are not tabled, it is either for a similar reason or because *n* is zero.

DISCUSSION

This expanded study revealed a decrease in the number of significant differences in urinary polyamine levels between nonactive *versus* active tumors from the number of such differences reported in our initial study (1). Nevertheless, when both putrescine and spermidine levels are considered, a significant difference was noted for each of the 5 tumor types studied, for at least one of the 2 polyamines.

However, the significant additional information offered by this study is the results obtained by separating active tumors into small and large categories and the results of predicting tumor stability and tumor progression. It is particularly disappointing to note that, for all tumor types successfully separated into small and large, there was no statistically significant difference between nonactive and active small tumors. Obviously, the urinary polyamine assay would be of greatest value if it could distinguish these 2 states. The differences between nonactive tumor and active large tumor states, and between active small tumor and active large tumor states are certainly of interest, but they can translate into clinical utility only if the involved disease states could not otherwise be distinguished.

Our study of predictive values for tumors of the breast, prostate, and female genital tract was revealing, but only partially encouraging. For all tumor types and for both polyamines, the prediction of stability in disease nonactivity was excellent. However, this information is of value only if the predictive value of the progression of tumor growth is sufficiently large. In the case of breast tumors, where the number of determinations was quite large, the predictive value of tumor growth progression was poor. Therefore, although stable urinary polyamine levels in the nonactive state of this disease might be reassuring, elevated levels would add little to our knowledge of the status of the patient's tumor, since in most instances it does not reflect tumor growth progression. For patients with tumors of the prostate or female genital tract, the results are more promising, stable levels being indicative of stability of disease nonactivity, and elevated levels indicating tumor growth progression 30 to 50% of the time. Although there are many false-positive results, following a patient with a positive result more closely for other signs of tumor growth progression might have a substantial payoff. Unfortunately, our sample sizes of patients with these tumors are too small. Continued study of these patients will be necessary to see if the promise of this assay will become a reality.

Finally, there are 2 other points to be emphasized. (*a*) The time intervals between assays within a given patient were often as large as 3 months, and sometimes even larger. Additional and more frequent assays from a patient might provide more suitable data for early detection of disease progression. (*b*) Although a number of data analysis approaches were applied to our data, only some of which are reported in this paper, these do not necessarily exhaust all of the possible data analysis approaches. It therefore is conceivable, although we feel not likely, that yet another data analysis approach could yield a more positive picture of the urinary polyamines as indicators of tumor growth than we have reported here.

ACKNOWLEDGMENTS

We thank Jeanne Elliott for typing the manuscript and Meir Cohen of the Veterinary Institute, Beit Dagon, Israel, for lyophilizing the urine specimens.

REFERENCES

1. Horn, Y., Beal, S. L., Walach, N., Lubich, W. P. Spigel, L., and Marton, L. J. Further evidence for the use of polyamines as biochemical markers for malignant tumors. Cancer Res., *42:* 3248–3251, 1982.
2. Marton, L. J., Edwards, M. S., Levin, V. A., Lubich, W. P., and Wilson, C. B. Predictive value of cerebrospinal fluid polyamines in medulloblastoma. Cancer Res., *39:* 993–997, 1979.
3. Oredsson, S. M., and Marton, L. J. Polyamines: the elusive cancer markers. Clin. Lab. Med., *2:* 507–518, 1982.