Polyamine Concentrations in Bone Marrow Aspirates of Children With Leukemia and Other Malignancies

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High-pressure liquid chromatography analysis of polyamines in bone marrow from leukemic and nonleukemic subjects demonstrated increased concentrations of putrescine, spermidine, and spermine associated with increased cellularity. The most striking abnormality was the marked elevation of putrescine. Bone marrow polyamine analysis may be an adjunct for evaluation of leukemia patients.

THE NATURALLY OCCURRING POLYAMINES putrescine, spermidine, and spermine function in physiologic processes such as tRNA methylation, amino acylation of tRNA, RNA synthesis, bacterial cell division, and the growth of animal cells in tissue culture systems.[1] In vitro cell culture studies have demonstrated an early rise and subsequent fall in polyamine levels in rapidly growing, normal tissue as opposed to their accumulation in malignant cells.[2] Striking alterations in the biosynthesis of polyamines accompanies various phases of growth and differentiation in many animal tissues.[3]

The potential that polyamine quantitation might be a clinical, biochemical marker for malignancy was an outgrowth of the work of Russell and co-workers, which indicated increased amounts of these aliphatic amines in the urine of cancer patients.[4] Subsequent investigations have demonstrated that fluctuations in serum or urinary polyamines parallels the clinical status of patients.[4,5] Elevated concentrations are often found in association with localized or metastatic tumors, in contrast to lower values associated with remission of neoplastic disease.

The basic abnormality responsible for symptoms and signs in the acute leukemias is the proliferation and infiltration of abnormal leukocytes in the marrow or other organs. Initial therapy in the acute leukemias of childhood is designed to eliminate the life-threatening manifestations of the disease by restoring normal bone marrow function. A complete remission is defined as the absence of histologic evidence of leukemia, i.e., less than 5% blast cells in the marrow. The total number of malignant or leukemic cells in the body when the diagnosis of leukemia is established is of the order of 10^{12} cells. Cytologic or symptomatic remission is associated with a reduction of the total number of neoplastic cells to approximately 10^9 or 10^{10} cells per individual. At this stage, the bone marrow or peripheral blood diagnosis of leukemia is not possible, even though a significant number of residual malignant cells may be present.

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Table 1. Free Polyamine Concentration (nmole/ml) in Bone Marrow in Nonleukemic Diseases

					Bone Marrow Data											
	Putrescine	Spermidine	Spermine	Spd/Spm	Cellularity	Blasts	Promyelocyte	Myelocyte	Mature Neutrophil	Eosinophil	M/E	Miscellaneous	Erythrocyte	Lymphocyte	Megakaryocyte	M/L
(1) Nonmalignant																
Infectious mononucleosis	12.8	157.5	239.5	0.66	I	8	5	17	20	—	1.7	—	25	24	N	1.7
Dysgammaglobulinemia	Trace	55.3	57.0	0.97	N	0	1	28	44	—	3.3	—	22	5	N	14.6
Idiopathic thrombocytopenia	—	43.5	63.6	0.68	N	0	1	9	21	—	0.6	—	51	18	I	1.7
Sickle cell disease	Trace	20.1	22.8	0.88	D	1	2	7	31	—	1.0	—	41	18	D	2.2
Average	—	69.1	95.7	0.80												
(2) Malignant																
Rhabdomyosarcoma	Trace	53.6	85.4	0.63	N	2	1	20	44	—	2.5	—	26	7	N	9.3
Neuroblastoma	Trace	70.3	90.4	0.78	N	1	2	14	56	—	4.5	—	16	11	N	6.5
Retinoblastoma	5.5	58.0	62.0	0.94	N	2	3	13	32	—	1.4	—	33	17	N	2.8
Average	—	60.6	79.3	0.78												
(3) Histiocytosis-X																
Case 1	1.10	126.0	196.5	0.64	I	5	4	24	35	—	2.5	—	25	7	N	9.0
Case 2	0.27	3.1	5.1	0.61	D	0	0	13	60	—	4.3	—	17	10	N	7.3
Case 3	—	5.2	6.4	0.81	N	1	1	13	51	—	2.6	—	25	9	N	7.2
Average	—	44.8	69.3	0.69												
Total average	1.97	53.7	82.9	0.76												
Range	0–12.8	3.1–157.5	5.1–239.5	0.61–0.97												

N, normal; D, decreased; I, increased; M/E, myeloid/erythroid; M/L, myeloid/lymphoid.

To increase the clinician's capability to diagnose the presence of neoplastic cells below this limit, quantitative studies of polyamine concentrations in the bone marrow of children with leukemia and other malignancies have been undertaken. A relationship between the degree of leukemic infiltration and the bone marrow concentration of polyamines appeared likely. Since the quantitation of these aliphatic amines is reproducible in nanomoles, it should be a more sensitive indicator than present histologic and histochemical methods.

MATERIALS AND METHODS

Thirty-five bone marrow specimens were obtained from children with leukemia, and ten additional specimens were obtained from other children with benign disorders for diagnostic evaluation. Bone marrow, 0.1-1.0 ml, was obtained from the posterior ileum in heparinized, plastic syringes. The specimens were extracted with an equal volume of 10% sulfosalicylic acid. The mixture was agitated and subsequently centrifuged at 10,000 *g* for 20 min. The supernatant was extracted, lyophilized, and resuspended in one-fifth the original volume. The suspension solvent was 0.2 *N* sodium citrate buffer, pH 2.2. Prior to analysis the sample was filtered through a Millipore.

Polyamines were determined on the Durrum D-500 amino acid analyzer. The analyzer contained a standard 5-mm path-length flow cell, and all machine functions were accomplished by means of PDP8/M computer. The analytic column was 8.9 cm long with an inner diameter of 0.175 cm. The column was packed with Durrum DC46-96 cation exchange resin.

The elution system consisted of three sodium citrate buffers: buffer 1: 0.2 *M* sodium citrate, pH 6.6; buffer 2: 2.4 sodium citrate, pH 4.68; and buffer 3: 3.05 *M* sodium citrate, pH 4.68.[6]

The method used measured the free polyamine concentration, i.e., acid soluble polyamines. An additional study[7] has contrasted the free, protein bound and acetylated polyamine concentrations in biologic fluids. No increased sensitivity nor qualitative difference was evident with regard to bone marrow polyamine profiles in neoplastic states. The recovery rate of polyamines was greater than 92%. Studies evaluating polyamine concentrations in serum or urine utilizing acid hydrolysis[4-7] have recovery rates of 50%-70% and offer no increase in sensitivity.

Histologic examination of peripheral blood and bone marrow was performed by 500 cell differential counts on each patient.

RESULTS

The free polyamine concentration in the bone marrow of several nonleukemic diseases is presented in Table 1. The cumulative data indicate a spermidine/spermine ratio in bone marrow of 0.76 nmole/ml, with a range of 0.61-0.97. The very low levels of putrescine found in the various conditions are significant (Table 1). Detectable or quantifiable levels of putrescine were found in only two situations: infectious mononucleosis and histiocytosis-X. Elevations of both spermidine and spermine are found in these two situations. Additionally, in both of these conditions the spermidine/spermine ratio is at the lower end of the range. The bone marrow data presented in Table 1 indicate: (1) increased polyamine concentration parallels increased bone marrow cellularity as exemplified by infectious mononucleosis and histiocytosis-X, and (2) both of these diseases were associated with an increased number of blast cells in the marrow.

Bone marrow specimens from patients with leukemia who are in relapse or in whom the diagnosis has just been established demonstrate a significant change in the polyamine concentration. There is a marked elevation of the putrescine concentration with a mean average of 7.93 nmole/ml for all the leukemic conditions (Table 2). Similarly, there is a 20%-50% increase in the mean spermidine and spermine concentration in the marrow (Table 2). Finally, there appears to

Table 2. Free Polyamine Concentration (nmole/ml) in Bone Marrow in Leukemia in Relapse

					Bone Marrow Data											
	Putrescine	Spermidine	Spermine	Spd/Spm	Cellularity	Blasts	Promyelocyte	Myelocyte	Mature Neutrophil	Eosinophil	M/E	Miscellaneous	Erythrocyte	Lymphocyte	Megakaryocyte	M/L
(1) Acute lymphocytic																
Case A (1)	17.2	136.7	128.0	1.06	I	84	0	0	6	—	1.0	—	6	4	D	1.5
(2)	19.7	136.9	66.6	2.06	I	95	0	0	0	—	0	—	2	3	D	0
Case B	8.7	107.0	143.4	0.75	I	97	—	—	—	—	0	—	3	0	D	0
Case C	2.7	142.3	196.1	0.73	I	79	—	4	0	—	1.0	—	4	13	D	0.3
Case D (1)	1.4	41.6	41.3	1.01	D	23	0	8	23	—	1.0	—	32	14	D	2.2
(2)	0.3	50.0	45.5	1.10	D	25	1	4	13	—	0.4	—	47	10	D	1.8
Case E	4.5	63.8	302.5	0.21	I	84	0	0	1	—	0.3	—	4	11	D	0.1
Case F	—	36.0	75.0	0.48	I	97	0	0	0	—	0	—	3	0	D	0
Average	6.81	89.3	124.8	0.93												
(2) Acute myelocytic leukemia	—	32.8	51.8	0.63	I	80	2	6	8	—	4.0	—	4	0	D	16.0
(3) Acute myelocytic leukemia	24.8	52.9	46.3	1.14	I	35	25	10	6	—	2.9	—	14	10	D	4.1
Total average	7.93	80.0	109.7	0.92												
Range	0–24.8	32.8–142.3	41.3–302.5	0.21–2.06												

Abbreviations are defined in footnote to Table 1.

Table 3. Free Polyamine Concentration (nmole/ml) in Bone Marrow in Leukemia in Remission

					Bone Marrow Data											
	Putrescine	Spermidine	Spermine	Spd/Spm	Cellularity	Blasts	Promyelocyte	Myelocyte	Mature Neutrophil	Eosinophil	M/E	Miscellaneous	Erythrocyte	Lymphocyte	Megakaryocyte	M/L
(1) Acute lymphocytic																
1	0.99	44.5	56.5	0.79	N	4	3	23	44	—	3.3	—	21	5	N	14.0
2	5.25	123.4	133.5	0.93	N	0	1	8	25	—	0.5	—	63	3	N	11.3
3	0.70	49.5	64.0	0.77	N	1	2	28	39	—	2.5	—	28	2	N	35.0
4	1.75	56.5	57.0	0.99	N	1	1	13	55	—	2.6	—	26	4	N	17.5
5	0.25	9.8	24.1	0.41	D	3	1	6	59	—	2.5	—	26	5	D	13.8
6	4.60	63.8	76.9	0.83	N	3	1	14	45	—	1.9	—	32	5	N	12.6
7	9.65	117.5	158.8	0.74	N	7	6	15	18	—	0.7	—	51	3	N	15.3
8	1.85	10.3	5.9	1.75	D	2	—	5	30	—	0.8	—	41	22	D	1.7
9	1.55	89.0	113.3	0.79	N	1	1	22	46	—	2.7	—	25	5	N	14.0
10	1.19	54.5	82.8	0.66	N	3	2	9	51	—	2.0	—	31	4	N	16.2
11	7.05	88.5	144.5	0.61	N	0	0	12	16	—	0.4	—	68	4	N	7.0
12	1.15	37.5	51.5	0.66	N	2	1	15	42	—	1.6	—	36	4	N	15.0
13	1.25	42.7	27.0	1.58	N	1	0	6	60	—	2.7	—	24	9	N	7.4
14	0.50	42.4	54.0	0.79	N	0	1	15	45	—	2.0	—	30	9	N	6.8
15	1.15	53.5	57.0	0.93	N	2	1	16	40	—	1.6	—	35	6	N	9.8
16	—	25.5	31.3	0.81	D	5	1	10	45	—	2.1	—	26	13	D	4.3
17	2.40	59.0	65.5	0.90	N	0	0	15	41	—	1.4	—	41	3	N	18.7
18	4.00	62.0	91.0	0.68	N	2	1	12	32	—	0.9	—	50	3	N	15.6
19	4.80	76.5	159.5	0.48	N	3	3	15	39	—	1.4	—	40	0	N	60.0
20 (a)	—	3.6	4.4	0.82	D	2	0	4	36	—	0.9	—	46	12	D	3.3
(b)	—	9.1	12.9	0.71	D	5	0	12	47	—	1.9	—	30	6	D	9.8
21	1.35	25.5	30.5	0.84	N	3	1	10	57	—	3.4	—	20	9	N	7.8
Average	2.34	52.03	70.0	0.87												
(2) Acute myelocytic leukemia																
1	—	8.0	5.6	1.43	N	4	12	32	36	—	7.3	—	11	5	D	16.0
2	—	—	trace	±	D	4	0	8	10	—	0.7	—	26	52	D	0.35
3	trace	75.2	131.3	0.57	N	1	2	16	60	—	4.1	—	19	4	N	19.5

Abbreviations are defined in footnote to Table 1.

be a shift in the spermidine/spermine ratio. However, no precise association with either the bone marrow differential or the percentage of blast cells is apparent. The increased polyamine concentrations may presumably be ascribed to the marked increase in bone marrow blast cells in leukemia and/or the increased cellularity.

The difference between leukemias in remission and in relapse is highly significant. Most noticeable is a threefold decrease in the mean putrescine concentration of leukemic marrows in remission (Table 3). The average concentration for remission in acute lymphocytic leukemia is 2.34 nmole/ml and trace levels of putrescine in myelocytic leukemias (Table 3). Additionally, decreased concentrations of spermidine and spermine and a depression of the spermidine/spermine ratio is evident (Table 3). The acute lymphocytic leukemia samples 2, 6, 7, 9, 11, and 19 (Table 3) show relatively high putrescine and/or spermidine and spermine values as contrasted to the remainder of the specimens. In these situations there is a marked decrease in the myeloid to erythroid ratio in the bone marrow. Whether these individuals will demonstrate longer remission duration or if some other feature characterizes the relationship between polyamine concentration, the cell types and total cellularity of the bone marrow is unclear at the present time. The data in Table 3 suggest a potential relationship between polyamine concentration and the myeloid to lymphocyte ratio of these marrows. Longitudinal follow-up and increased numbers of patients will be required to determine whether elevated polyamines simply relate to increased cellularity and blast cells or if they have some more specific connotation.

Markedly increased levels of polyamines were detected in bone marrow specimens from acute leukemia patients in relapse and remission, in childhood solid tumor patients, and also in such unrelated hyperproliferative disorders as infectious mononucleosis, sickle cell disease, and histiocytosis-X. However, putrescine accumulation seemed to correlate specifically with onset, intensity, and duration of chemotherapy, and in certain acute leukemia patients in relapse (Table 2; samples A1, A2, B, C, E, and 3) and in remission (Table 3; samples 2, 6, 7, 9, 11, and 19). For example, putrescine accumulations and increased spermidine/spermine ratios were more often seen when therapy with antimetabolites (6-mercaptopurine and cytosine arabinoside) were being given to sustain clinical remissions. In contrast, disproportionate elevations of putrescine were not observed in solid tumors or in other myeloproliferative disorders untreated with antimetabolites. Putrescine accumulation as a side-effect of cancer chemotherapy is highly significant in view of the facilitating effect of this polyamine on the in vitro growth of lymphoid cell lines.[8]

DISCUSSION

The relationship of increased polyamine synthesis of hyperproliferative states is well established. Recently, the potential applicability of this observation has been suggested by the demonstration of increased polyamine excretion associated with neoplasms. It has been proposed that measurement of polyamine excretion be used for diagnostic monitoring of patients with neoplasms, both solid tumors and leukemia. Russell and several other groups[4,5] have indicated that monitoring of urinary polyamine concentrations in cancer patients shows a

correlation with the presence of the tumor. We have explored the usefulness of a quantitative polyamine determination in blood[9] and bone marrow of children with acute leukemias. Our data indicate significant elevations of all polyamines in acute leukemias of childhood. The elevations noted in the bone marrow are associated with increased cellularity of the marrow in nonneoplastic conditions such as infectious mononucleosis or with a shift and increase in the number of lymphoid and myeloid progenitors. The quantitation of these compounds may be a more sensitive index than the histologic evaluation of bone marrow for establishing remission or recrudescence of neoplasm. The elevation in the putrescine concentration, noted in all the hyperproliferative states in this report, is striking. The potential usefulness of this approach for monitoring leukemia therapy is related to the application of automated analysis, which allows the reproducible detection of less than nanomole quantities of each of the polyamines.

The quantitation of polyamines for clinical diagnostic purposes in the bone marrow has greater sensitivity than the previously reported studies looking at urinary polyamine concentrations.

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