Clinical Relevance of Polyamines as Biochemical Markers of Tumor Kinetics

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The polyamines, spermidine and spermine, and their di-amine precursor, putrescine, constitute a unidirectional biosynthetic pathway whose biosynthetic enzymes and accumulation patterns appear to play important roles in the regulation of growth processes. Concentrations of these compounds in physiological fluids are low or undetectable under normal conditions, are elevated in patients with metastatic cancer, and are thought to reflect growth (putrescine concentrations) and cell turnover (spermidine concentrations) of the organism. Cancers are a broad spectrum of diseases in which there are altered growth fractions and cell-turnover fractions, and therefore cancer chemotherapeutic agents have been developed to take tumor kinetics into account. Because changes in polyamines in physiological fluids reflect cell kinetics, this review compiles evidence of their efficacy as biochemical markers of cancer and suggests their possible usefulness to clinicians in rapidly assessing tumor response to chemotherapy or to multimodality therapy.

Additional Keyphrases: cancer • monitoring therapy • column chromatography • diagnostic aids • screening • spermidine, spermine, putrescine

As early as 1889, studies suggested that more polyamines appear in urine in certain pathological states. Using simple chromatographic procedures, Udránszky and Baumann (1) detected putrescine and cadaverine in the urine of patients with cystinuria (1, 2). The presence of cadaverine suggested bacterial contamination of the urine, because it is not found in mammals to any substantial extent (3). The excretion of spermidine by patients with cystinuria and other conditions was not reported until almost a century later, when more sophisticated ion-exchange techniques were used to separate and estimate diamines and polyamines in biological material (4, 5).

Increased Polyamines in Physiological Fluids of Cancer Patients

Increased amounts of polyamines in the urine of human cancer patients were reported in 1971 (6). In the case of one patient with a large ovarian teratoma, the bulk of the tumor was surgically removed and polyamine excretion decreased toward a normal value. Patients with acute myelocytic leukemia, lymphomas, carcinomas, and sarcomas exhibited some pattern of enhanced polyamine concentrations in their urine. Further, this report indicated that leukemic patients in remission had only slightly increased concentrations of spermidine and spermine but no detectable putrescine in their urine. It was suggested that the increased concentrations of polyamines in the urine might provide a diagnostic tool to evaluate tumor activity (7), and many studies since this time have corroborated this original idea.

The ability to detect increased polyamines in the urine of cancer patients at this time was based on acid hydrolysis of the specimen at high temperatures, followed by paper electrophoresis (8). It was not possible to detect polyamines in the urine before such hydrolysis, and it was postulated that the polyamines in the urine were mainly in a conjugated form (9). Recent studies of [14C]polyamine metabolism in response to tracer injections into rats and humans have indicated that putrescine and spermidine are rapidly conjugated in both rats and humans and that most of the putrescine and spermidine excreted is conjugated (10). Spermine is not conjugated to any degree but is the polyamine that usually is not increased in the urine of cancer patients (10), with certain exceptions: patients with breast carcinomas and colon carcinomas usually excrete above-normal amounts of spermine (11, 12).

The early studies were extended by the same investigators to evaluate changes in polyamine excretion in response to chemotherapy (9, 13, 14). For patients with
acute myelocytic leukemia and Hodgkin's disease, excretion during chemotherapy was markedly increased (9). In those cases in which the patients entered either a partial or complete remission, polyamine excretion decreased as the patients entered remission.

In these early studies high-voltage electrophoresis was used for separation and ninhydrin staining for quantitation of the amines. Although this method is adequate for tissues, with physiological fluids such as urine there was an unknown compound that migrated identically with spermine and was ninhydrin positive (15, 16). Whatever this unknown was—and it has still not been identified—it was not excreted in detectable amounts by normal persons, and it appeared to be characteristic of neoplasia. Further, mice with L1210 leukemia also excrete this unknown compound in high concentrations whereas normal mice excrete considerably less (15).

Thus, the potential usefulness of polyamines as cancer markers was realized, but it also was evident that a more sensitive, convenient, and rapid assay was necessary to adequately evaluate their concentrations in physiological fluids. A rapid, automated amino acid analyzer technique was adapted for the separation and quantitation of polyamines in physiological fluids (16). This was originally adapted to a Beckman Model 121 analyzer but most investigators today who routinely measure putrescine, spermidine, and spermine in physiological fluids use a Durrum D-500 high-pressure amino acid analyzer (17, 18). This method is sensitive to the picomole range and 15 to 20 samples can be run per day with excellent reproducibility. Studies of polyamine concentrations in physiological fluids with an amino acid analyzer corroborated the earlier findings that polyamines are increased in urine and serum of the great majority of cancer patients. Further, the excretion appeared to correlate with the clinical status of the patient (19). Since that time, there have been reports from other laboratories of increased polyamines in the urine and blood of patients with cancer (20–25), as well as reports of decreased polyamine values after successful treatment of various types of malignancy (12, 21–27).

Intracellular and Extracellular Polyamines in Animal Models of Tumor Regression

To better understand why these increases occurred in cancer patients, investigators studied two animal model systems: (a) the MTW9 mammary carcinoma of the rat, a hormone-dependent tumor that spontaneously regressed when the source of hormone was extinguished; and (b) the 3924A hepatoma of the rat, a rapidly growing hepatoma, and its response to 5-flurouracil and to local irradiation. Polyamines were measured in the tumor, liver, and serum of rats with either spontaneously regressing MTW9 carcinoma or radiation- or chemical-induced regression of the 3924A hepatoma (28–30). A decreased amount of spermidine in both the liver and tumor of the rats with MTW9 mammary carcinomas paralleled an increase in spermidine in the serum (28). The period of maximal tumor regression, i.e., within 48 h of removal of hormonal support, corresponded with the time of the highest values for spermidine in the serum or in the tumor interstitial fluid (28). This animal model suggested that intracellular spermidine, which increased during tumor growth, was diminished by excretion during regression, and, further, that spermidine concentrations in the serum or urine reflected tumor cell death. Rats with 3924A hepatomas that received 5-flurouracil had detectable spermidine in their serum, more than double the control value within 36 h of drug administration (29). During this same period, the concentration of spermidine in the tumor dropped to two-thirds of the pre-treatment value. To assess the increment in polyamines in body fluids attributable to tumor cell death vs. increments attributable to toxic effects on host tissues, changes in polyamine concentrations in tumors, livers, and in body fluids of rats with 3924A hepatomas in response to local irradiation were assessed (30). Putrescine and spermidine in serum increased rapidly, similar to the pattern after treatment with 5-flurouracil (30). These data support the hypothesis that the increased polyamines in extracellular fluids are derived mainly from tumor cells. In a preliminary study of a group of cancer patients who had polyamines determined before, during, and after chemotherapy (19), those who were responding to chemotherapy exhibited rapid increases in urinary putrescine and spermidine. Figure 1 illustrates a model of polyamines as biochemical markers of tumor growth and tumor cell death.

Pretreatment Excretion of Polyamines in the Urine

Table 1 quantitates the excretion of putrescine, spermidine, and spermine by patients with hematologic tumors, solid tumors, and by normal persons. The normals consisted of men and women, 18 to 60 years of age, ambulatory, and in apparent good health. This age range closely paralleled the ages of the patients in the tumor categories. The ranges were wider in all tumor categories and the mean and median were elevated. Putrescine, spermidine, and spermine values were highest in patients with hematologic tumors, were above normal in patients with solid tumors, and were signifi-
Table 1. Polyamines in Urine of Cancer Patients before Treatment

<table>
<thead>
<tr>
<th></th>
<th>Putrescine Mean ± SE</th>
<th>Median</th>
<th>Range</th>
<th>Spermidine Mean ± SE</th>
<th>Median</th>
<th>Range</th>
<th>Spermine Mean ± SE</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic tumors</td>
<td>4.4 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2 (n = 59)</td>
<td>0.45–38</td>
<td>3.7 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 (n = 54)</td>
<td>0.58–25</td>
<td>0.8 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 (n = 53)</td>
<td>0.25–8.0</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>3.7 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8 (n = 29)</td>
<td>0.29–10</td>
<td>2.7 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 (n = 30)</td>
<td>1.09–8.1</td>
<td>0.6 ± 0.28</td>
<td>0.55 (n = 30)</td>
<td>0.12–3.7</td>
</tr>
<tr>
<td>Normal persons</td>
<td>2.1 ± 0.62 (n = 16)</td>
<td>1.0</td>
<td>0.40–5.5</td>
<td>1.2 ± 0.18</td>
<td>0.8 (n = 16)</td>
<td>0.40 = 2.1</td>
<td>0.04 ± 0.04</td>
<td>0.02–0.3</td>
<td>0.007 (n = 16)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Amounts in 24-h collections, assayed with an amino acid analyzer.

<sup>b</sup> Data differ from normals (P < 0.001).

significantly lower in the normal persons. In general, the patients studied had advanced disease with a considerable tumor cell burden and had been referred for palliative chemotherapy.

Because urine is the body fluid from which much of the data concerning polyamines as markers of tumor kinetics have been compiled, it is important for those workers in the field to consider relating the excretion of the major constituent to that of a reference compound in urine. As stated by Dr. David Seligman, Professor of Laboratory Medicine at Yale, in a report to the American Cancer Society, “Creatinine, poor as it is, is the best we have. We ought to report polyamines per mg creatinine (or an equivalent).” In our laboratory, we have found that serial 24-h urines from the same patient show remarkably consistent values for polyamine concentration when the excretion of the polyamine is expressed per milligram of creatinine. When this normalizing technique is not used, values can be two- to threefold different from day to day. We must assume that these fluctuations, when no reference is used, are due to improper collections or changes in kidney function, or both.

Polyamines as Markers of Tumor Kinetics

Normal and neoplastic tissues with the highest growth rates accumulate the highest concentrations of putrescine and spermidine (15, 27, 31). The differences in extracellular polyamines appear to be related to the different cell-loss factors of normal tissues vs. neoplastic tissues. Steel (32) and others have published extensive studies indicating that certain cancers have high cell-loss factors. For instance, carcinomas may have cell-loss factors as high as 70%. That means that most of the tumor cells generated rapidly die; perhaps this explains the increased polyamines in the body fluids of cancer patients. Other data support the hypothesis that those tumors with the highest growth rates also excrete the most polyamines. For instance, patients with Burkitt’s lymphomas excrete the highest amounts of polyamines reported to date (27). Burkitt’s lymphoma is a rapidly proliferating tumor with a high growth fraction (33).

Those patients with tumors with small growth fractions and slow growth rates, such as colon carcinoma and breast carcinoma, excrete much lower amounts of urinary polyamines (11, 12). Interestingly, however, colon-carcinoma patients had increased spermine excretion in 66% of those patients studied (12).

Polyamine Excretion as a Function of Tumor Kinetics

It has already been indicated that urinary polyamine values are increased in response to chemotherapy. A greater than twofold increase in spermidine within 24 to 48 h predicted partial or complete response of the tumor to treatment with a high degree of accuracy (19, 34) (Table 2). This finding could have significant clinical implications because a lack of response within two days would indicate to the clinician that the chemotherapeutic regime should be changed, to facilitate a response. At the present time, responses are based on routine clinical tests that are performed after the chemotherapeutic regime ends.

Careful studies of polyamine excretory patterns in response to chemotherapy indicate that increased putrescine excretion tended to appear slightly later than increased spermidine excretion, and that these increases were greatest in those patients who failed to respond to chemotherapy (19, 34). This suggests that the appearance of putrescine in the serum or urine of a patient may reflect recruitment of cells into the growth fraction, a concept tested in patients with multiple myeloma by evaluating putrescine pre- and post-treatment in relation to the thymidine labeling index of the tumor cells. A very close correlation was found between labeling index and quantitative putrescine excretion (r = 0.837) (19). Since then, many other labeling indices and putrescine values have been obtained, and this concept appears to be upheld (34).

Figure 2 illustrates three chemotherapeutic regimes for the same patient with multiple myeloma. In the first graph, the patient had no response to chemotherapy and a low labeling index; there were no increases in either putrescine or spermidine excretion in response to che-
motherapy. With a minor response (the middle graph), there was a slight increase in both putrescine and spermidine excretion; however, a partial response, at a time when the labeling index had increased to 11%, consisted of a high concentration of putrescine initially and a late response to multiple courses of chemotherapy. The progressive increase in putrescine excretion by this patient is compatible with the hypothesis that urinary putrescine reflects the growth fraction of a tumor.

Effects of Duration and Intensity of Chemotherapy on Polyamine Excretory Patterns

Figure 3 illustrates the response to chemotherapy of a patient with advanced breast carcinoma. This patient received a single dose of doxorubicin (50 mg/m²) with cyclophosphamide (200 mg/m², four times daily), which resulted in rapid, marked excretion of putrescine and spermidine. In those cases in which chemotherapy is prolonged, there is usually a less dramatic early rise, with concentrations being increased all during chemotherapy and decreasing after chemotherapy is stopped.

Relative Usefulness of Measuring Polyamines in Serum, Plasma, and Urine

Although several investigators have published that putrescine and spermidine are increased in the sera of cancer patients (16, 35), only one published report compares serial samples of plasma and serum to 24-h urine specimens for the same patient. Values for serum and plasma collected at the same time from the same patient differ only slightly, suggesting that either is acceptable for polyamine analyses (18). Further, increases in the concentrations of putrescine and spermidine in the serum and plasma correlate well with increases in corresponding 24-h urine samples. Spermidine concentrations in the sera were consistently about 10-fold lower than corresponding urine values; putrescine concentrations were 10- to 100-fold different. It was postulated that the difference in the putrescine values might be due to its more active metabolism by diamine oxidase (EC 1.4.3.6), an enzyme known to be present in the bloodstream. Therefore, a greater assay sensitivity is necessary to accurately measure putrescine and spermidine in serum or plasma. The recent development of a fluorescent detector, based on o-phenaldehyde as the fluorescent tag, for the Durrum D-500 amino acid analyzer makes possible more accurate determinations of the polyamines in plasma and serum, because this detection method is more sensitive than are ninhydrin staining procedures. However, there are certain precautions in the collection of the serum or plasma sample that must be observed. For instance, the serum and plasma must be rapidly processed so that there is not significant lysis of leukocytes or erythrocytes. Because intracellular polyamine concentrations are much greater than extracellular, a little cell lysis can alter markedly the values obtained. Also, if any of the buffy coat is removed along with the plasma during processing, high and erratic values for polyamines will be obtained. The time of day at which the plasma or serum was drawn did not seem to affect the value obtained before chemotherapy (19). That is, samples taken at 0730 or 1730 h the same day had similar values for the polyamines. However, after chemotherapy was begun, the values in the serum rose markedly and consistently to a peak value, usually 48 to 72 h later, and thereafter declined (19). This study suggested that

### Table 2. Effect of Response to Chemotherapy on Urinary Spermidine and Putrescine Excretion

<table>
<thead>
<tr>
<th>Response</th>
<th>No. cases</th>
<th>(Post-/pretreatment ratio)</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Putrescine</td>
<td>Spermidine</td>
</tr>
<tr>
<td>No response</td>
<td>22</td>
<td>2.7 ± 3.5</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Partial response</td>
<td>15</td>
<td>3.0 ± 3.1</td>
<td>3.7 ± 2.1</td>
</tr>
<tr>
<td>Complete response</td>
<td>23</td>
<td>2.5 ± 1.2</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>All hematologic tumors</td>
<td>33</td>
<td>2.6 ± 2.2</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>All solid tumors</td>
<td>20</td>
<td>2.8 ± 3.4</td>
<td>2.0 ± 1.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of the ratios
a: Different from no response (P < 0.00001).
b: Different from solid (P < 0.05).

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**Fig. 2.** Effect of three separate chemotherapy regimes for the same patient with multiple myeloma, illustrating the change of response as a function of the labeling index before therapy

**Fig. 3.** Rapid response of polyamine excretion in a patient with breast carcinoma to a single high dose of doxorubicin and cyclophosphamide (Adriamycin–Cytoxan, A–C)

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CLINICAL CHEMISTRY, Vol. 23, No. 1, 1977 25
polyamine measurements in serum and urine in response to chemotherapy might be useful to clinicians as a rapid assessment of the efficacy of anticancer therapy. Further studies are in progress in this laboratory to assess whether a casual plasma sample can be used to accurately assess the response of the patient to cancer chemotherapy.

Usefulness of Polyamines as Early Detectors of Cancer

Apparently, polyamines are useful indicators of the efficacy of chemotherapy, and their potential usefulness may extend to early prediction of relapse. However, the early suggestion that they might be useful in early detection of occult cancer seems highly improbable at this time. Firstly, the methodology is still time-consuming and has not been applicable to mass-screening procedures, which would be necessary to ascertain their possible usefulness in early diagnosis. Secondly, other pathological conditions in which there is high cell turnover and high growth fractions also appear to result in increased urinary polyamines, e.g., patients with psoriasis, cystic fibrosis, myocardial infarctions, and bacteremias (21, 36, 37). In bacteremias the major increase is in putrescine, and in pathological conditions other than cancer the original model appears to hold; that is, increased putrescine in body fluids reflects cell growth and increased spermidine reflects cell loss.

Further Developmental Approaches

The clinical importance of rapid assessment of tumor kinetics in relation to treatment and the fact that changes in the concentrations of putrescine and spermidine in body fluids appear to accurately reflect growth and cell loss point to the importance of developing rapid, sensitive tests for the polyamines. It should be possible to raise specific antibodies to putrescine, spermidine, and spermine, and to the naturally conjugated polyamines. For radioimmunoassays to have clinical relevance, skilled immunologists will have to establish the optimal conditions for the interaction of each antibody/antigen complex to ensure reliable radioimmunoassay procedures—specific, precise, sensitive, accurate, and reproducible. This goal will only be attained with interactions of teams of basic scientists, clinical chemists, and clinicians. These tools could be valuable to the clinician for rapidly assessing response to chemotherapy and early prediction of relapse, which would facilitate the timing of chemotherapy. Perhaps they would even afford the ease of multiple-sample analysis that will be necessary to initiate broad-spectrum screening programs to ultimately answer the question of whether polyamines are markers of early neoplastic processes. Lipton et al. (24) found increases of polyamines in body fluids to be highest in the early stages of tumor growth. Because growth and tumor cell loss are probably greatest during tumorigenesis, there is always the possibility that a rapid assay procedure could at least be used to screen those populations with known high cancer incidence.

Further studies are needed as to the efficacy of plasma and serum vs. urinary polyamines as markers of tumor kinetics. At present, it appears that plasma would be better, because there is less possibility of cellular contamination.

References