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The Diagnostic and Prognostic Value of Urinary Polyamine Measurement in Bladder Cancer

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Summary. This study concerns a group of 54 patients with bladder cancer aged from 51 to 80 years (50 males, 4 females). Polyamines (putrescine - PU, spermidine - SPD) were measured on 24 h urine collections prior to surgery by an automatic ion exchange analyzer. Both polyamines, and especially PU, correlated well with the degree of tumor infiltration (JEWETT-MARSHALL stage) and mitotic activity (BRODER'S grade). Retrospectively, 28 patients for whom follow-up after surgery was 6 months to one year were classified into two groups: R+ (21) patients with disease recurrence or progression, and R_0 (8) patients remaining tumour free. Twenty patients with high preoperative PU levels were in the R+ group. By contrast, 6 patients with normal preoperative PU levels were in the R_0 group.

Key words: Bladder cancer, Putrescine, Spermidine.

Polyamines are cations required for cellular proliferation and growth (3, 8). The extremely high polyamine levels observed during embryonic development decrease progressively with age. Only those tissues which continue protein synthesis have high concentrations of polyamines, the major ones being putrescine and spermidine. Polyamines are not markers of tumour mass, but are involved in cell growth (9). Tumour growth is associated with an increased intracellular putrescine concentration and putrescine excretion. Cellular death produces an increased extracellular spermidine concentration (6). In bladder tumours, the urologist faces the problem of identifying those tumours which, at a given stage or grading, will relapse, infiltrate and metastasise rapidly (5).

MATERIALS AND METHODS

Polyamines were measured using an automatic ion exchanger (LIQUIMAT III, KONTRON ANALYTIQUE, France) (4) on 24 h urines from 54 patients with bladder tumours. Measurements were made prior to any treatment and patients were monitored by cystoscopy every two months. The longest period of follow-up was two years; the minimum follow-up period for detection of tumour recurrence was six months.

The upper limits were determined on the basis of 22 healthy controls, as follows: putrescine (m + 2 SD) = 2.00 µg/mg creatinine; spermidine (m + 2 SD) = 1.60 µg/mg creatinine. The serum creatinine level was taken into account to correct for the possibility of incomplete 24 h urine collections and also for correction of variations in renal function. Reproducibility was evaluated with replicate analyses of a pooled urine sample; the coefficients of variation for intra-assay reproducibility were putrescine 2.8% and spermidine 0.8% (7 identical samples in the same series). The coefficients of variation for interassay reproducibility were putrescine 4.5% and spermidine 5.0% (1 identical sample in 7 different series).

RESULTS

Preoperative urinary putrescine and spermidine concentrations were related to the JEWETT-MARSHALL staging and BRODER'S grade. From a diagnostic viewpoint, the median polyamine values increased in direct proportion to the rise of the staging or grade. Stage 0 and grade 1 tumours generally had normal polyamine levels and a median value in the normal range. By contrast, stage C and grade IV tumours had higher polyamine concentrations.

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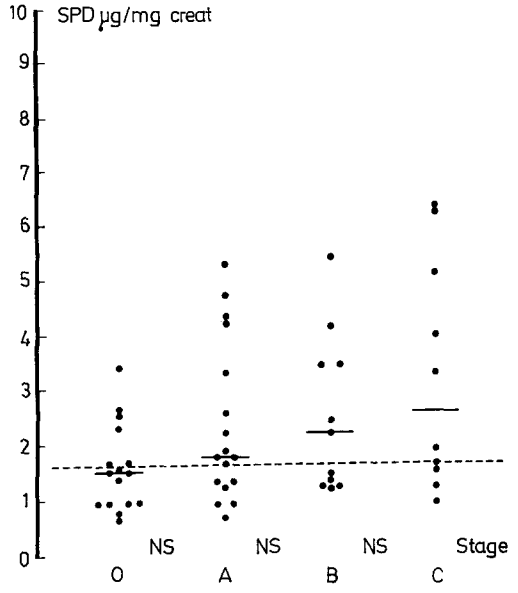

Fig. 1. Distribution of spermidine (SPD) values as a function of the JEWETT-MARSHALL stage

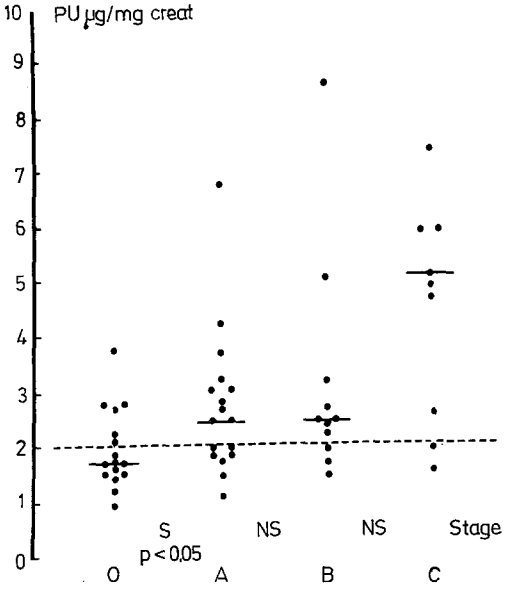

Fig. 3. Distribution of putrescine (PU) values as a function of the JEWETT-MARSHALL stage

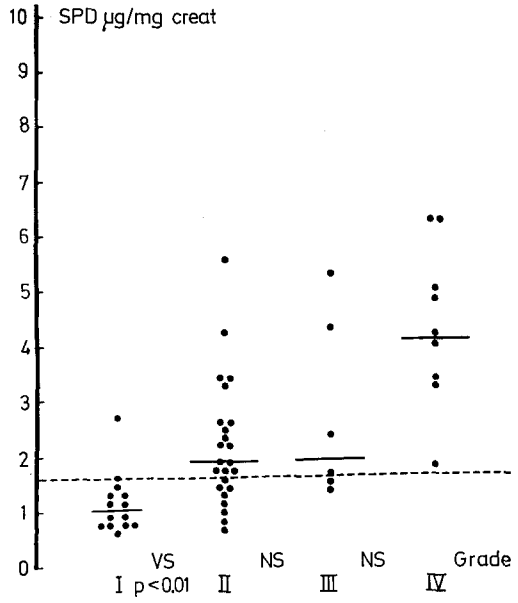

Fig. 2. Distribution of spermidine (SPD) values as a function of the BRODER'S grade

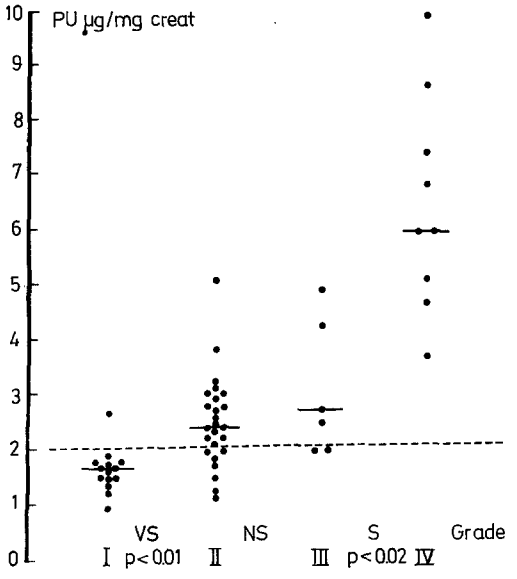

Fig. 4. Distribution of putrescine (PU) values as a function of the BRODER'S grade

Figure 1 shows the distribution of spermidine concentrations according to the stage. Although there was no significant difference in distribution between stages, the proportion of elevated spermidine levels appears to rise as the stage increases.

In Figure 2, which shows the distribution of spermidine concentrations as a function of the grade, there is a significant difference between grade I and grade II: the proportion of raised spermidine levels increased in close correlation to the severity of the grade. Figure 3 shows that the change of putrescine concentrations as a function of the stage is much more evident than for spermidine. In this case, there is a significant difference between stage 0 and stage A. The highest putrescine levels clearly occur in advanced stages.

Figure 4 illustrates the putrescine concentration as a function of the grade. Putrescine levels appear to be closely correlated with the severity of the grade.

Thus, an association generally exists between a high preoperative urinary polyamine con-

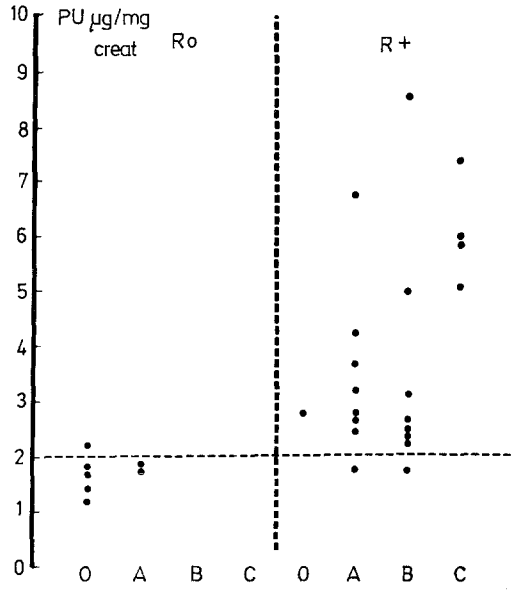

Fig. 5. Distribution of putrescine (PU) values as a function of the clinical outcome. R_0 = group free of relapses at 6 months; R+ = group having relapsed within 6 months

centration and the presence of a malignant bladder tumour. Putrescine appeared to be the more sensitive of the two polyamines studied, and correlated particularly well with the increase in the grade.

FOLLOW-UP STUDY

Only putrescine was considered in this study. Twenty-eight patients were followed-up for at least 6 months. Two groups of patients were defined: group R_0 consisting of 7 patients with no recurrence at 6 months, and group R+ consisting of 21 patients who had relapsed. Figure 5 shows the distribution of preoperative putrescine values for each group: the results are clearly different. Out of 20 patients with high preoperative putrescine values, 19 relapsed. By contrast, of the 8 patients with normal preoperative putrescine levels, only two had relapsed at 6 months.

The interest of polyamine measurements is not to determine whether infiltrating (B_2 and C) or unquestionably malignant (III and IV) tumours will recur after a treatment but to predict the behaviour of lower stage and grade tumours. While the number of cases is insufficient to allow definitive conclusions, the following preliminary data for 16 patients warrants attention.

- stage 0 and A tumors: in 7 cases with normal putrescine levels there was only 1 relapse. By contrast, in 9 cases with raised putrescine levels, 8 relapses occurred within 6 months, as follows:
  - 1 0 II tumour
  - 4 A III tumours
  - 2 A IV tumours
  - 1 III tumour

DISCUSSION

Although considerable attention is paid at present to markers in oncology, no marker appears significant at this time in bladder cancer. CEA in blood is of no value (7). Serum beta$_2$ microglobulin and acute phase reactant protein levels in bladder cancer have both recently been reported on (1, 2); the levels of these proteins rise as a function of the severity of the stage. Aside from markers, no procedure to date has allowed evolution of a urothelial tumour in a particular patient to be predicted. However, the expression of blood group antigens as a marker of differentiation in bladder tissue appears to be an area worthy of investigation. We are presently conducting a retrospective study on cell sections embedded in paraffin. Out of 8 stage A patients found to have antigens we noted 4 cases free of disease and 4 recurrences (two stage A and two stage B). Antigen disappearance always preceded recurrence by several months. Investigation of antigens thus appears extremely interesting for the prognostic study of bladder tumours. Indeed, although our figures are derived from a small number of cases, they agree with the 133 observations published in U. S. literature. The principle of measuring a product of a urothelial tumour cell is an attractive propositon. The possibility of measuring a molecule which reflects more the mitotic activity of the tumour cells rather than its mass has several theoretical advantages. Although the existence of a high urinary polyamine concentration does not constitute a specific test for bladder tumours, we believe that putrescine measurement offers a promising means of establishing the prognosis in such cases. This study included only the results of polyamine measurements made prior to any treatment. Putrescine levels are apparently directly related to the likelihood of recurrence.

We are presently enlarging this study by taking more measurements prior to each follow-up cystoscopy, and the correlation between recurrence and putrescine concentration seems to persist.

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