Pyridinium crosslinks as markers of bone resorption in patients with breast cancer

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Summary  Collagen breakdown, and thus bone resorption, can now be assessed by measuring the urinary excretion (Pyd) and deoxypyridinoline (Dpd) metabolites. High values of the crosslinks have been found in patients with malignant disease, and have been shown to correlate with the severity of bone destruction. The crosslinks have been measured in 20 patients with breast cancer, ten with known bone metastases and ten with no evidence of metastatic disease. In all the patients the crosslink excretion values were higher than the reference interval, but the crosslinks were measured in patients with breast cancer and compared with those from a group of patients with no known metastases and who had been followed subsequently for not less than 1 year.

Patients and methods

All the patients were surveyed at the time the samples were obtained with a limited skeletal survey, including a chest X-ray, and with 99mTc-polyporphosphate scintigraphy. The ten patients with known metastatic disease in bone included two with metastatic disease also in the lungs and one who had a lesion thought to be metastatic in the contralateral breast. At the end of the 1 year follow-up period, the patients without metastases were examined clinically and biochemically but the isotope scintigraphy was not repeated at this stage.

Urine samples were frozen within 6 h of collection and stored at −20°C. In an earlier study we had demonstrated that repeat assays on samples stored at −20°C showed no deterioration of the crosslinks (Robins et al., 1991). The samples from the patients with and without metastatic disease were treated identically and the assays were carried out without knowledge of the diagnosis.

Analyses of the crosslinks were performed by HPLC (Black et al., 1988; Seibel et al., 1989). In all cases the urine samples (250 µl) were hydrolysed with an equal volume of concentrated HCI to release bound forms of the crosslinks. The results were expressed relative to the urinary creatinine concentration. Creatinine, calcium and alkaline phosphatase assays were done with standard automated procedures.

Statistical analyses were carried out using Student's t-test and linear regression with Pearson's correlation coefficients.

Results

Figure 1 shows the urinary concentrations of Pyd and Dpd relative to creatinine in the two groups of patients. Values for a group of 118 healthy volunteers aged 21 to 74 (Seibel et al., 1989) are shown for comparison. There were clear differences between the two groups of patients; for Pyd/Cre the mean value (± s.d.) for the group with metastases (102.3 ± 67.1) was significantly higher (P < 0.025) than that for the group without metastases (44.5 ± 17.2). The corresponding values for Dpd (with metastases, 24.2 ± 17.4; without metastases, 12.3 ± 7.4) were statistically not significantly different. The values for the patients without known metastases were, however, significantly higher than those for the controls, with...
ever, demonstrated that phosphatase activities were raised in many patients who had metastases. For the whole group the correlation coefficient was 0.86 (P<0.001) for Pyd and 0.77 (P<0.001) for Dpd. For the patients without metastases the correlation coefficients were 0.72 (P<0.05) for Pyd and 0.78 (P<0.02) for Dpd. The adult reference range for serum alkaline phosphatase is 20–120 IU.

Discussion

The assays in our small study were carried out blind but demonstrated that many of the patients known to have bone metastases had raised values for urinary Pyd and Dpd. Rather surprisingly Pyd assays appeared to discriminate better than Dpd assays between the two groups of patients although the greater range of values for Dpd in the group without metastases must have contributed to this finding. It was, however, noted that some patients not thought to have metastatic disease at the time also had raised values of Pyd and Dpd excretion relative to our control group. Further long-term follow up is needed to determine whether these represent 'false positive' results or signify occult bony metastases. One possible explanation would be that this finding indicates generalised bone resorption in both patient groups, perhaps caused by a tumour-derived cytokine or prostaglandin component; tumour necrosis factors and PG-E2 have been shown to increase bone resorption in vitro (Bertolini et al., 1986; Garrett et al., 1987). The excretion of Pyd and Dpd was correlated with the serum alkaline phosphatase activity suggesting that osteoblastic activity and osteoclastic activity

![Figure 1](image1.png)  
**Figure 1** Urinary excretion of Pyd and Dpd (relative to creatinine) in patients with and without known metastatic disease in bone.

![Figure 2](image2.png)  
**Figure 2** Urinary excretion of Pyd and Dpd (relative to creatinine) compared with serum alkaline phosphatase activities. For the whole group the correlation coefficient was 0.86 (P<0.001) for Pyd and 0.77 (P<0.001) for Dpd. For the patients without metastases the correlation coefficients were 0.72 (P<0.05) for Pyd and 0.78 (P<0.02) for Dpd. The adult reference range for serum alkaline phosphatase is 20–120 IU.
increased together. However the serum assays carried out at the time did not include γ-glutamyl transferase or 5'-nucleotidase so that we cannot be confident that all the alkaline phosphatase was derived from bone. Again, further work with larger numbers would be worthwhile.

We conclude that assays of the urinary excretion of Pyd and Dpd could provide a valuable additional indicator of metastatic spread to bone. We feel that further studies with larger numbers and additional information on follow up are needed.

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