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Urinary Androgen Metabolites and Recurrence Rates in Early Breast Cancer

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Abstract—*Androsterone and aetiocholanolone were measured in urine specimens obtained from 218 women with early breast cancer. Patients who excreted less than the median amount of these steroids had a significantly higher recurrence rate ($P < 0.005$) than patients excreting more than the median value. The relationship between androgen excretion and recurrence is stronger in pre-menopausal women than in the post-menopausal group. The androgen assays are still associated with recurrence rates when patients are stratified by pathological stage, but no clear relationship was found with histological grade.*

INTRODUCTION

IT IS generally accepted that the prognosis of women with advanced breast cancer who excrete sub-normal amounts of urinary androgen metabolites is poor[1]. In the early disease, by which is meant operable Stages 1 and 2, the relation between androgen metabolite excretion and the clinical course of the disease is not clear. Several workers have found that such patients have a sub-normal excretion of these compounds, associated with rapid recurrence after mastectomy[2–5]; others have not[6, 7], while a recent publication[8] showed a correlation with post-menopausal women but not with pre-menopausal patients. Advances in gas–liquid chromatographic methods have now made it possible to analyse urinary steroid profiles with accuracy[9]. At the same time, rigorous procedures have been laid down for the organisation of clinical trials and the evaluation of recurrence rates[10,11]. Accordingly, the relationship between the excretion of two of the androgen metabolites (androsterone and aetiocholanolone) and recurrence rates after mastectomy has been reinvestigated.

METHODS

Patients

Urine specimens (24 hr) were collected from 700 patients with breast cancer, 10 days after they had been treated by mastectomy. The urine was stored at −20°C until analysed. All patients were in pathological Stages 1 and 2. Histological grading of the tumours was by the method of Bloom and Richardson[12]. Steroid metabolites were assayed in the urine of 218 women selected from the first 450 of these patients. The patients were followed-up at 3-monthly intervals for the first year and, if still free from the disease, were then followed-up at 6-monthly intervals. In the recurrent cases, time of recurrence varied from 1 to 58 months; in those without recurrence, follow-up examination varied from 4 to 62 months. In the latter group, deaths from causes other than cancer occurred in 4 women at 4, 8, 32 and 40 months. For statistical analysis, these patients were classified as non-recurrent up to the time of death.

There were 22 recurrences in the 90 patients with Stage 1 disease and 61 in 128 patients with Stage 2 disease. Note that these recurrent rates are not representative of the total 700 patients: the patients chosen for investigation were deliberately weighted to include all known recurrences, with the non-recurrent controls chosen by an independent statistician. When all the steroid assays were completed, the median values for androsterone (A) and aetiocholonolone (Ae) were calculated for the 218 sets of results. Patients with values above the median were designated as 'high' (above 390 μg/24 hr for A and above 590 μg/24 hr for Ae); those with values below the median were classed as 'low' excretors. Recurrence rates were then calculated for patients above or below the median values and compared by the log-rank test[11].

Analytical methods

Urinary steroid profiles were measured by

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capillary gas–liquid chromatography[9] using a Pye–Unicam GCV model, with flame-ionisation detector, connected to a Kemtronix Supergrator-3 integrator for identification of the steroids and computation of their concentration. Only two compounds in the steroid profile are considered here: androsterone (3α-hydroxy-5α-androstan-17-one) and aetiocholanolone (3α-hydroxy-5β-androstan-17-one).

RESULTS

Patients who excrete amounts of A below the median value for the 218 cases have a much more rapid recurrence rate after mastectomy than women excreting amounts above the medium value (Fig. 1). The difference is highly significant ($\chi^2 = 8.54$; $P < 0.005$).

Of the 83 recurrences, 53 occurred in the 'low' excretors of A, compared with 30 in the 'high' excretion group. Similar results were found for Ae ($\chi^2 = 7.69$; $P < 0.01$).

Menopausal status

A similar relationship between A excretion and recurrence rates was found when pre-menopausal women were considered. Patients excreting less than the median value (Fig. 2) had significantly faster recurrence rates than those excreting larger amounts ($\chi^2 = 6.66$; $P < 0.01$). A similar result was found for Ae ($\chi^2 = 5.61$; $P < 0.025$). In the post-menopausal group, the same trends were observed and although the differences did not reach formal significance, it was considered justified to pool both pre- and post-menopausal women for further statistical analyse.

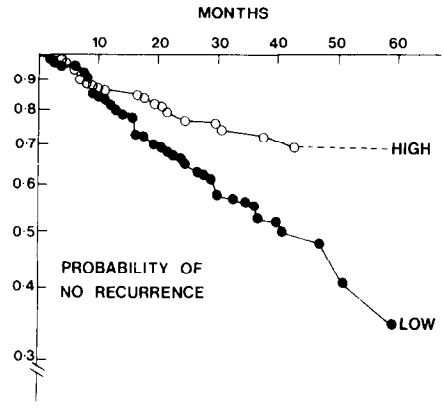

Fig. 1. Androsterone excretion and recurrence rates in pre-menopausal, menopausal and post-menopausal women after mastectomy. 'High' designates values of androsterone above the median value (380 μg/24 hr); 'low' refers to values below this figure. There were 83 recurrences in 218 patients. Differences in recurrence rates: $\chi^2 = 8.54$ ($P < 0.005$).

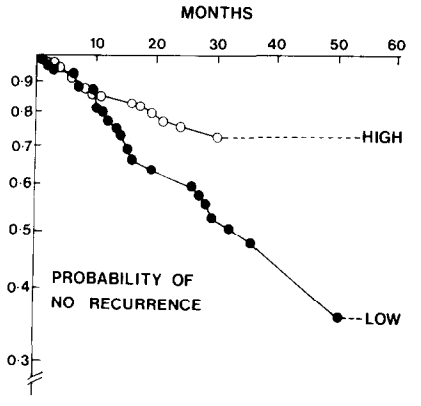

Fig. 2. Androsterone excretion and recurrence rates in pre-menopausal and menopausal women after mastectomy. There were 45 recurrences in 122 patients. Differences in recurrence rates: $\chi^2 = 6.66$ ($P < 0.01$). Other details as for Fig. 1.

Stage

Recurrence rates after mastectomy are related to the pathological stage of the disease, and the rates increase with increasing number of nodes[14]. Within the three usual nodal categories (Stage 1; Stage 2, 1–3 nodes; Stage 2, 4 or more nodes), urinary A levels remain of prognostic significance. Patients in the first two nodal groups who excrete low amounts of this steroid have faster recurrence rates than those who excrete high amounts (Fig. 3).

Similar results were found for Ae only in the Stage 2, ≥4 nodes group ($\chi^2 = 4.33$; $P < 0.05$).

Grade

Histological gradings were obtained for 184 patients. In the 21 cases with Grade I tumors there was only one recurrence, which made it impossible to determine whether androgen metabolite levels were related to recurrence in this group. In the 95 Grade II cases, the 33 recurrences were independent of androgen status. Only in the 68 patients with Grade III tumours (31 recurrences) was the recurrence rate faster in women with a low A excretion than in those with high levels of this compound (Fig. 4). This result was not found with Ae.

DISCUSSION

The urinary androgen metabolites, androsterone and aetiocholanolone, are significantly related to recurrence rates after mastectomy. This relationship is still found after stratifying the patients by pathological stage, which indicates that androgen metabolite excretion may be a useful additional variable for prediction of recurrence rates. As far as histological grade is concerned, a consistent relationship with androgen excretion was found only in Grade III, which may imply that these steroids are

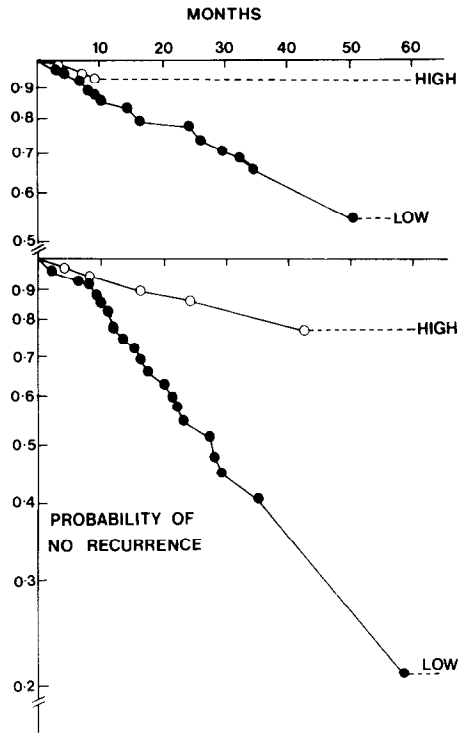

Fig. 3. Androsterone excretion and recurrence rates in Stage 1 cases after mastectomy. There were 22 recurrences in 90 patients. Differences in recurrence rates: $\chi^2 = 7.46$ ($P < 0.01$). *Other details as for Fig. 1.*

Androsterone excretion and recurrence rates in Stage 2 cases (1–3 nodes) after mastectomy. There were 26 recurrences from 67 patients. Differences in recurrence rates: $\chi^2 = 11.2$ ($P < 0.005$). *Other details as in Fig. 1.*

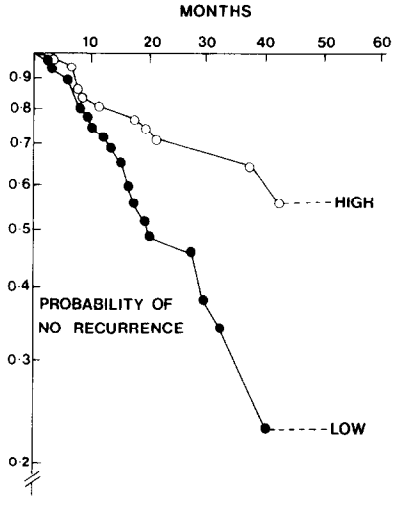

Fig. 4. Androsterone excretion and recurrence rates in Grade III cases after mastectomy. There were 31 recurrences from 68 patients. Differences in recurrence rates: $\chi^2 = 5.87$ ($P < 0.025$).

involved in tumour differentiation, but the data are sparse.

The practical clinical value of androgen metabolite assays for prediction of recurrence will be difficult to determine, as very large numbers of patients would be required for a reasonable estimate of the accuracy of prediction of response. This problem has already been encountered in studies of advanced breast cancer[12]. While sophisticated statistical analyses may help, there is no way of avoiding the necessity for accumulating large numbers of patients.

The patients studied were not strictly serial: there was a deliberate selection of a disproportionate number of recurrent cases. This bias is almost universal in the literature dealing with prediction of recurrence rates and it means that predictive tests based on retrospective data will rarely be as efficient when they are used prospectively. Another source of bias is the use of arbitrary cut-off points. In much of the work on prediction of response, the definition of positivity or negativity appears to be intuitive and may be varied to suit the investigator. We have used the median value of the urinary androsterone and aetiocholanolone levels for the whole group of patients as an objective cut-off point, although this is not necessarily the most sensitive way of discriminating between patients with slow or fast recurrence rates. We examined the possibility that there is a biological gradient, with the lowest androgen values being associated with the worst prognosis. When the patients were stratified into 3 equal groups (on the basis of the amounts of androgen excreted), those excreting the largest amounts of steroids had the best prognosis and those excreting the smallest amounts had the worst prognosis, but the numbers of cases in each group were small and the biological gradient was only marginally significant. More cases are needed to clarify this point.

In the long term, the only logical method of testing the usefulness of androgen metabolite measurements for predicting recurrence rates after mastectomy is by carrying out a prospective study, and this is in hand. Such a study is a formidable undertaking since it involves assays on all patients and should run for the same length of time as the original retrospective study (i.e. approximately 5 years).

As far as the biology of breast cancer is concerned, our current findings indicate an involvement of the androgens in the clinical course of the disease. This is in line with previous results which show that a sub-normal excretion of androgen metabolites is a determinant of risk of breast cancer,[15, 16] that androgen metabolite excretion is correlated with response to endocrine ablation[17, 18] and that androgen receptor levels in the tumors are related to response[1]. Recently, Abul-Hajj[19]

has shown that there is a correlation between urinary 11-deoxy-17-oxosteroid levels and oestrogen receptor sites. The latter are clearly related to recurrence rates after mastectomy[20] and to response to endocrine ablation[21]. A model study was carried out by Segaloff and his colleagues[8] in which they found a significant correlation between a subnormal excretion of 17-ketosteroids and recurrence at 2 years only in their post-menopausal patients. This study is not strictly comparable with ours, since urine specimens were collected shortly after operation and there are considerable changes in steroid excretion brought about by mastectomy[22]. Futhermore, recurrence was assessed as two-year recurrence-free rates. Our own results analysed in a similar fashion would also show no significant differences, whereas the life-table method we used, with follow-up running to 5 years, shows a clearly significant result.

We thus have a degree of harmony in that results from widely diverse experiments all indicate an association between androgen status and clinical course. Urinary androsterone and aetiocholanolone are mainly derived from adrenal dehydroepiandrosterone (5-androsten-3β-hydroxy-17-one) and dehydroepiandrosterone sulphate circulating in the blood, and are excreted in the urine mainly as glucuronidate conjugates following conversion by the liver[23]. Whether there is a low conversion of the precursor steroids to the urinary metabolites due to a metabolic deficiency at either the liver of peripheral level is still to be established. Until more is known of the biological mechanisms by which endocrine manipulations bring about regressions of breast tumours, there must always be the suspicion that the association between clinical result and androgen status is an indirect one.

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