Androgen Receptors in Breast Cancer

ROBERT M. BRYAN, FRCS,*† RONALD J. MERCER, FRACS,*‡ RICHARD C. BENNETT, MS,*§
GEORGE C. RENNIE, MA,*‖ TAT H. LIE, BAppSc,*¶ FRANCIS J. MORGAN, MD#**

Androgen receptor assays have been performed on 1371 specimens of histologically confirmed primary and recurrent breast cancer. Forty-two patients who had received tamoxifen as treatment for advanced disease were assessed for objective response. Another 42 patients who had received chemotherapy were similarly studied. Patients with androgen receptor-negative tumors had a significantly poorer response rate to hormone therapy than those with receptor-positive tumors ($P < 0.05$). This clinical correlation is supported by survival data of 1181 patients with primary breast cancer which showed that patients with androgen receptor-negative tumors had a highly significant trend toward shorter overall survival than those with receptor-positive tumors ($P < 0.001$). Androgen receptor data added significantly to the information provided by estrogen receptor data both in terms of response to hormone treatment and survival.

Cancer 54:2436–2440, 1984.

ESTROGEN RECEPTOR ASSAY is well established in the management of breast cancer, particularly as a guide to likely response to hormone therapy, and also as a prognostic indicator.[1–4]

The role of progesterone receptor is less well defined. Some studies have shown it to have predictive value[2,5] whereas others have not.[6]

Androgen receptor (AR) assay has been performed for some years but no place as yet has been defined for it in the management of breast cancer.[7] Recently we have reported the results of 1878 estrogen receptor (ER) assays, 1556 progesterone, and 957 AR assays showing their range and distribution and changes occurring with time, method, age, histology, and menopausal status.[8] This report correlates AR assays with both response to treatment and prognosis.

Materials and Methods

Specimens of breast cancer were received by this laboratory from Victorian city and country hospitals from 1979 to 1982, inclusive. During this period, 1371 specimens of histologically confirmed primary and recurrent breast cancer were assayed for androgen receptors.

The details of the method employed for AR assay have been described previously.[8] Briefly, the level of AR was measured by the binding of ^{3}H-methyltrienolone (R1881, New England Nuclear, Boston) to the receptor protein. As this ligand may bind nonspecifically to progesterone receptor as well as AR, the assay was carried out in the presence of 12.5×10^{-9} M triamcinolone acetonide in order to abolish this effect.[9] Gel filtration was used to separate the bound steroid from free steroid, and receptor concentration was calculated by modified Scatchard analysis.[9a] This method has remained constant throughout the period of investigation. Results are expressed in femtomoles/mg cytosol protein. A level of 0 to 5 has been regarded as androgen receptor negative (AR−), 5 to 10 as androgen receptor equivocal (AR±), and more than 10 as androgen receptor positive (AR+).

Of the 1371 patients, a retrospective study was made of 84 patients who had been treated for advanced disease at a number of centers by different clinicians. Patients who had received systemic therapy before development of recurrence were excluded. Forty-two patients with either clearly positive or negative receptor status who had received tamoxifen as hormone therapy for advanced disease were assessed for objective response to treatment by two of us (RMB and RJM). Another 42 patients who had received chemotherapy alone were similarly studied. Because these patients were entered into various clinical trials, they had not received uniformly comparable therapy.

From the *University of Melbourne Department of Surgery, St. Vincent's Hospital, and #St. Vincent's School of Medical Research, Melbourne, Australia.
† Research Fellow.
‡ Associate Surgeon.
§ Professor of Surgery.
‖ Consultant Biometrician.
¶ Senior Technical Officer.
** Director of Research.
Address for reprints: Professor R. C. Bennett, MS, Department of Surgery, St. Vincent's Hospital, Fitzroy, Victoria 3065, Australia.

The authors thank the many treating doctors throughout Victoria and also the staff of the Oncology Department, St. Vincent's Hospital, Melbourne, the Peter MacCallum Hospital, Melbourne and the Anti-Cancer Council of Victoria for their generous assistance.
Accepted for publication November 18, 1983.

The patients were assessed for objective response using International Union Against Cancer criteria.[10] Furthermore, patients were not considered to have responded unless objective response was sustained for at least 6 months, as recommended by the British Breast Group.[11] The following are categories of objective response. (1) Complete response (CR): disappearance of all known disease. This includes lytic bone metastases, which must be shown radiologically to have calcified. (2) Partial response (PR): A 50% or more decrease of the sum of the products of the largest diameters of measurable lesions and objective improvement in evaluable but nonmeasurable lesions without the development of new lesions.

For the purposes of this study CR and PR were judged as "responders" and all other patients as "nonresponders."

One thousand one hundred eighty-one patients who had AR assays carried out on histologically confirmed primary breast cancer specimens were studied to determine overall survival rates. Survival was calculated from the date of biopsy of the primary tumor using information gathered from a number of sources including the Victorian Anti-Cancer Council Registry and information supplied by the patients' doctors.

Results

Eleven of 42 patients (26%) assessed for response to tamoxifen therapy responded to treatment. Of the 42 patients, 29 (69%) were AR− and 13 (31%) were AR+. Patients with AR− cancers had a significantly lower rate of response (14%) than those with receptor-positive tumors (54%) (Table 1).

Menopausal status was known in 39 patients. Of 33 postmenopausal patients, 11 had AR+ tumors, of which six (55%) responded to endocrine treatment. Only four of 22 AR− tumors (18%) responded. There were only six premenopausal patients in this series. Two had AR+ tumors and one of these responded, whereas none of the four AR− patients responded.

In 26 patients, AR assay was performed on the primary tumor, and in 14 on metastatic tumor obtained immediately before therapy. In two patients assays were performed on both primary and metastatic tumor (receptor status was unchanged). These have been included with the first group. Table 2 shows the response rates within these two groups. The difference in response rates between AR− and AR+ tumors was not significant where assay had been performed on the primary tumor (Yates corrected $\chi^2 = 0.69$). However, where assay was performed on recurrent tumor, patients with AR− tumors had a significantly lower rate of response than those with AR+ tumors (Yates corrected $\chi^2 = 5.58$; $P < 0.05$).

TABLE 1. Response of Metastases to Tamoxifen

Receptor status of tumor	No. treated	No. responding
AR+	13	7 (54%)
AR−	29	4 (14%)
Total	42	11 (26%)

$\chi^2 = 5.54$ (Yates correction); $P < 0.05$.
AR+: androgen receptor positive; AR−: androgen receptor negative.

TABLE 2. Response to Tamoxifen Therapy

	AR assay on primary tumor		AR assay on recurrent tumor	
Receptor status of tumor	No. treated	No. responding	No. treated	No. responding
AR+	9	4 (44%)	4	3 (75%)
AR−	19	4 (21%)	10	0 (0%)
Total	28	8 (29%)	14	3 (21%)

AR+: androgen receptor positive; AR−: androgen receptor negative.

Estrogen receptor status was known in all patients. Table 3 shows the response to tamoxifen therapy by combined ER and AR status. None of the five estrogen receptor-negative (ER−) patients responded. When the estrogen receptor-positive (ER+) patients were considered, those who were both ER+ and AR+ had a significantly higher response rate to treatment than those who were ER+ AR− (Yates corrected $\chi^2 = 3.96$; $P < 0.05$).

Forty-two patients were assessed for response to chemotherapy. Of these, 27 (64%) were AR− and 15 (36%) were AR+. Androgen receptor assay was carried out on the primary tumor in all cases. Twelve patients (29%) responded to chemotherapy. There was no difference between response rates of patients with AR− or AR+ primary tumors (Table 4).

Of the 1181 patients with histologically confirmed primary breast cancer, follow-up data were available in 696. Five hundred eighty-five patients were followed for

TABLE 3. Response to Tamoxifen by ER and AR Status

Receptor status of tumor	No. treated	No. responding
ER+AR+	13	7 (54%)
ER+AR−	24	4 (17%)
ER−AR+	0	0
ER−AR−	5	0
Total	42	11 (26%)

ER+: estrogen receptor positive; ER−: estrogen receptor negative; AR+: androgen receptor positive; AR−: androgen receptor negative.

TABLE 4. Response of Metastases to Chemotherapy

Receptor status of tumor	No. treated	No. responding
AR+	15	5 (33%)
AR−	27	7 (26%)
Total	42	12 (29%)

AR+: androgen receptor positive; AR−: androgen receptor negative.

TABLE 5. Androgen Receptor in Primary Breast Cancer

Menopausal status of patients	AR+/AR±	AR−	Total
Premenopausal	102 (53.4%)	89 (46.6%)	191
Postmenopausal	296 (45.6%)	353 (54.4%)	649

χ^2 = 3.60 (not significant).
AR+: androgen receptor positive; AR−: androgen receptor negative; AR±: androgen receptor equivocal.

at least 1 year, 378 for at least 2 years, and 141 for at least 3 years.

Survival curves were constructed using these data. Figure 1 shows the overall survival rate according to receptor status. There was a highly significant trend toward a shorter survival in AR− patients than in AR+ patients (χ^2_1 for trend = 14.39; $P < 0.001$). It is interesting to note that the survival rate in AR± patients was similar to that of AR+ patients. Further separation of AR+ patients by quantitative levels did not improve prediction of survival.

Data on menopausal status were available in 840 patients (Table 5). In premenopausal women (191 patients), the difference in survival rates between AR− and AR+ patients was not significant, whereas in postmenopausal women (649 patients), AR− patients had a shorter survival than AR+ patients χ^2_1 for trend = 4.11; $P < 0.05$). These results are shown in Figures 2 and 3.

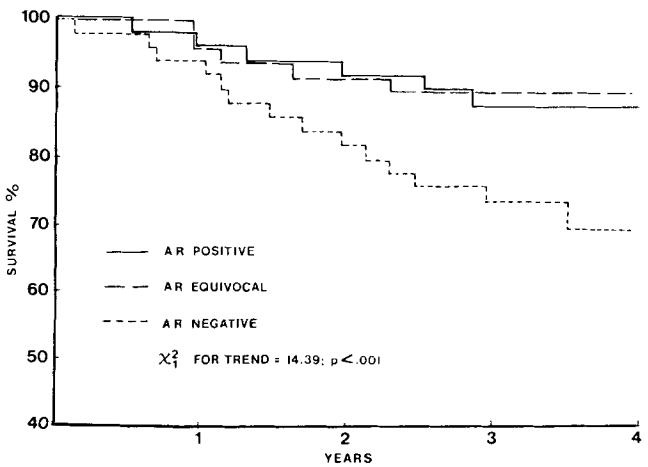

FIG. 1. Percentage survival of 1181 women with primary breast cancer according to androgen receptor status.

Previously we have reported in a study of 2006 patients that there is a trend toward improved survival with increasing levels of ER.[12] The 1181 patients described above comprise a subset of these 2006 patients for whom AR status was available. In order to determine whether the addition of AR data to ER data significantly improved prediction of survival, a further survival analysis was carried out of AR stratified by five levels of ER (0–4.9 fmol, 5–9.9 fmol, 10–19.9 fmol, 20–79.9 fmol, and ≥80 fmol). After ER was taken into account, the additional contribution of AR to prediction of survival was highly significant (χ^2_1 for trend = 7.68; $P < 0.01$).

Discussion

In this study, 26% of patients who had received no adjuvant therapy before development of recurrence responded to endocrine therapy. This response rate is lower than that reported by most other authors,[1] however this may be related to the use of different criteria for assessment of response. Workers using identical criteria have obtained similar overall response rates.[13]

It is well established that the absence of ER decreases the likelihood of a response to endocrine manipulation. Although the numbers in this study are small, the same seems to hold true for AR with receptor-negative tumors having a significantly poorer response to endocrine therapy than receptor-positive tumors. We further reviewed the results to ascertain whether assay performed upon metastatic tumor obtained immediately before therapy provided a more accurate index of response than assay performed on the primary lesion. The overall response rates to hormonal therapy were similar in both groups. Although there was discrimination in both groups, it was significant only in the metastatic group, suggesting that assay on recurrent tumor just before therapy may provide the most accurate index of response to hormonal treatment. Unfortunately, numbers in this study were relatively small and additional patients need to be assessed to fully evaluate these findings.

We have reported recently that ER assays from this laboratory provide significant discrimination in terms of response to hormone therapy.[14] In a series of 81 patients assessed by identical criteria, 34% of (ER+) patients responded, whereas only 6% of (ER−) patients responded. In this group of 42 patients, the response rates according to ER were similar: 30% and 0%, respectively. The addition of AR significantly improved the prediction of response in the ER-positive group.

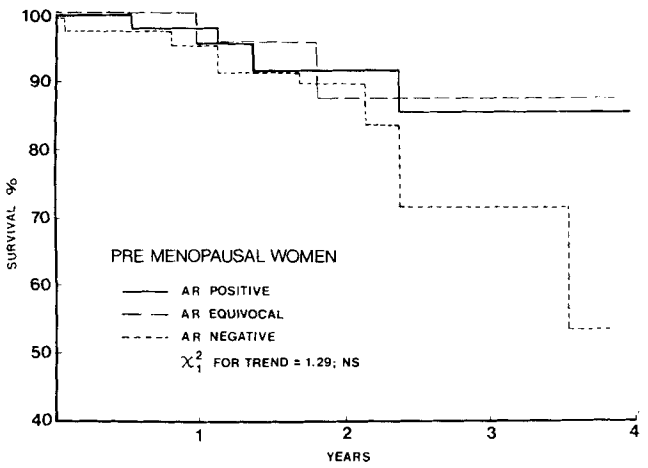

FIG. 2. Percentage survival of 191 premenopausal women with primary breast cancer according to androgen receptor status.

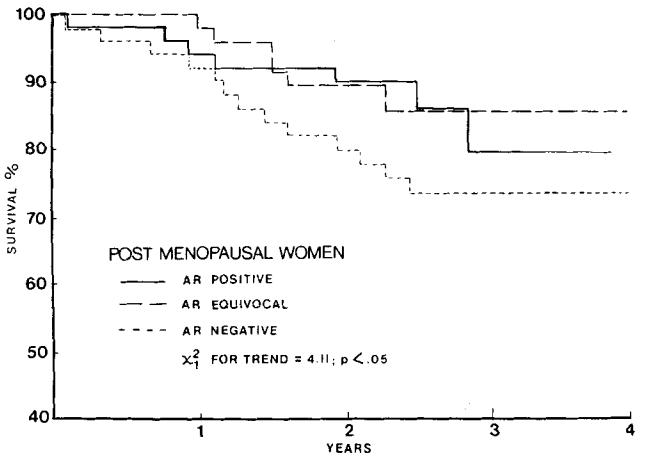

FIG. 3. Percentage survival of 649 postmenopausal women with primary breast cancer according to androgen receptor status.

The overall response rate of patients to chemotherapy in our series (29%) again is lower than that reported by most workers.[15,16] This tends to support our view that the low overall response rates obtained to hormone and chemotherapy in this study are due to the strict objective response criteria which were applied to all patients. In our series, there was no difference in response rates between AR− and AR+ tumors. This finding must be viewed with caution, however, as the chemotherapy treatment used was not uniform. Additional work is necessary to investigate the relationship between AR and specific chemotherapy regimens.

Clinical correlation between AR status and response to hormone therapy also has been found by other workers studying small numbers of patients.[17,18] Even though the number of patients in this series is also small, the relationship between receptor status and response to treatment is supported by the results of the much larger overall survival study. The lower response rate of AR− patients to hormone therapy is consistent with their poorer prognosis. As with ERs, AR may reflect the natural history of the tumor as well as the likely response to treatment.

This study shows that over a 4-year period, AR was a significant predictor of survival. It is clear from the overall survival curves that patients in the "equivocal" range should be considered as AR+ patients. Additional study is necessary to see whether the current AR− range should be subdivided.

The discrimination in survival prospects offered by AR results does not appear to be correlated with menopausal status. The difference in the chi-square values between the premenopausal and postmenopausal groups can be explained entirely by the difference in the numbers of patients. The distribution of AR results by menopausal status in these patients (Table 5) is similar to that previously reported.[8] Certainly there is no tendency toward higher androgen receptor values among postmenopausal women.[8,18] This is in direct contrast to the case with ERs.

We have reported recently in a study of 2006 patients that ER is useful as predictor of survival.[12] The addition of AR data to ER data improved the prediction of survival over the time of this study. This is in accord with current belief that the best indication of prognosis may be obtained by consideration of a number of factors.

The difference between the distribution of AR and ER results, together with the improved discrimination provided by the addition of AR data to ER data, is strong evidence that the information given by AR does not merely reflect that given by ER.

REFERENCES

1. Hawkins RA, Roberts MM, Forrest APM. Oestrogen receptors and breast cancer: Current status. *Br J Surg* 1980; 67:153–169.

2. McGuire WL. Hormone receptors: Their role in predicting prognosis and response to endocrine therapy. *Semin Oncol* 1978; 5:428–433.

3. Bishop HM, Blamey RW, Elston CW, Haybittle JL, Nicholson RI, Griffiths K. Relationship of oestrogen-receptor status to survival in breast cancer. *Lancet* 1979; 2:283–284.

4. Hahnel R, Woodings T, Vivian AB. Prognostic value of estrogen receptors in primary breast cancer. *Cancer* 1979; 44:671–675.

5. Skinner LG, Barnes DM, Ribeiro GG. The clinical value of multiple steroid receptor assays in breast cancer management. *Cancer* 1980; 46:2939–2945.

6. Manni A, Arafah B, Pearson OH. Estrogen and progesterone receptors in the prediction of response of breast cancer to endocrine therapy. *Cancer* 1980; 46:2838–2841.

7. Seibert K, Lippman M. Hormone receptors in breast cancer. In: Baum M, ed. Clinics in Oncology. London: WB Saunders, 1982; 1:735–794.

8. Mercer RJ, Lie TH, Rennie GC, Bennett RC, Morgan FJ. Hormone receptor assays in breast cancer. A 5-year experience. *Med J Aust* 1983; 1:365–369.

9. Zava DT, Landrum B, Horwitz KB, McGuire WL. Androgen receptor assay with ^{3}H-methyltrienolone (R1881) in the presence of progesterone receptors. *Endocrinology* 1979; 104:1007–1012.

9a. Scratchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949; 51:660.

10. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. *Cancer* 1977; 39:1289–1294.

11. British Breast Group. Assessment of response to treatment in advanced breast cancer. *Lancet* 1974; 2:38–39.

12. Mercer RJ, Bryan RM, Bennett RC, Rennie GC, Lie TH, Morgan FJ. The prognostic value of oestrogen receptors in breast cancer. *Aust NZ J Surg* 1984; 54:7–10.

13. Campbell FC, Blamey RW, Elston CW *et al.* Quantitative oestradiol receptor values in primary breast cancer and response of metastases to endocrine therapy. *Lancet* 1981; 2:1317–1319.

14. Bryan RM, Mercer RJ, Bennett RC, Rennie GC, Lie TH, Morgan FJ. Oestradiol receptor values in breast cancer and response of metastases to therapy. *Aust NZ J Surg* 1984; 54:3–6.

15. Hilf R, Feldstein ML, Savlov ED, Gibson SL, Seneca B. The lack of relationship between estrogen receptor status and response to chemotherapy. *Cancer* 1980; 46:2797–2800.

16. Rubens RD, Hayward JL. Estrogen receptors and response to endocrine therapy and cytotoxic chemotherapy in advanced breast cancer. *Cancer* 1980; 46:2922–2924.

17. Persijn JP, Korsten CB, Engelsman E. Oestrogen and androgen receptors in breast cancer and response to endocrine therapy. *Br Med J* 1975; 4:503.

18. Trams G, Maass H. Specific binding of estradiol and dihydrotestosterone in human mammary cancers. *Cancer Res* 1977; 37:258–261.