Breast carcinoma cellularity and its relation to oestrogen receptor content

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SUMMARY The cellularity of 104 primary breast carcinomata was determined by semiautomated image analysis to allow the relation between cellularity and oestrogen receptor content values to be assessed. The oestrogen receptor content of tumours detected by a steroid binding assay showed no correlation with cellularity, although a possible weak negative correlation was observed between tumour cellularity and oestrogen receptor content detected by enzyme immunoassay. It is concluded that there is no single direct correlation between tumour cellularity and oestrogen receptor content.

It is widely accepted that assessment of the presence of oestrogen receptors (ER) in breast carcinomata is of value in predicting prognosis and the response to hormone therapy.¹ The most widely used method determines receptor content on tissue homogenates. The results, therefore, do not directly take into account what proportion of the homogenised tissue comprises malignant cells rather than collagenous stroma or inflammatory cells, and the cellularity of individual breast carcinomas may vary 10-fold or more.^{2,3} Several studies have tried to show the effect of tumour cellularity of ER content but the methods used assessed cellularity subjectively and produced contradictory findings.⁴⁻¹¹ In this study we assessed cellularity objectively using semiautomated image analysis. The study was designed to allow the relation between cellularity and ER values to be assessed at two different times in an attempt to evaluate any influence of changes in ER assay "performance" and to determine if any difference in cellularity occurred. The data also allowed the two methods of quantitation of ER and their relation to cellularity to be compared.

Material and methods

Patients with a histological diagnosis of primary infiltrating breast carcinoma, in whom tumour ER assays had been performed and for which satisfactory tissue blocks were still available, were selected retrospectively from the Ninewells Breast Survey database. Sufficient histological material was needed so that the cellularity of a tissue section, which was subjectively

representative of the tumour as a whole, could be assessed. Fifty consecutive cases which satisfied these criteria were selected from 1983 and 54 from 1986. The latter group had ER data determined by both assay methods (see below). Patients ranged from 28 to 84 years of age (median 58 years): 32 were premenopausal and 74 postmenopausal at diagnosis.

MICROSCOPIC EXAMINATION

The cellularity of a neoplasm was assessed by image analysis, which was used to determine the proportion of a section of tumour occupied by malignant cells. The method, using the Quantimet 970, has been described in detail elsewhere.¹² Sections stained with haematoxylin and eosin considered to be representative of the tumour were used. Due to the non-uniform distribution of malignant cells in a neoplasm consecutive non-overlapping fields (total magnification $\times 100$) across a section from periphery to centre and back were assessed. This was repeated until a total of 20 fields had been analysed. The average result was calculated and expressed as a percentage of the neoplasm (malignant cells plus stroma) occupied by malignant cells.

OESTROGEN RECEPTORS

The storage of tumour tissue, homogenisation and preparation of cytosol, and determination of oestrogen receptor content by radioligand steroid binding assay (ER-SBA) were as previously described for this laboratory.¹³ A Scatchard plot which did not yield a significant linear correlation was considered to indicate that the tumour had no receptor content.¹³ In 1986 ER was also quantitated in the same cytosol

Table Correlation coefficients (Kendall's τ) for cellularity and ER (fmol/mg protein)

Groups compared			(n =)	τ	z	p value
Cellularity	ER-SBA	(1983)	50	-0.138	-1.412	> 0.16
Cellularity	ER-SBA	(1986)	54	-0.156	-1.66	> 0.1
Cellularity	ER-EIA	(1986)	54	-0.237	-2.53	< 0.02
Cellularity	ER-SBA	(1983 + 86)	104	-0.136	-2.05	< 0.05
*ER-SBA	ER-EIA	(1986)	54	-0.568	6.06	< 0.00001

*Pearson product-moment correlation coefficient, $r = 0.784$, ER-EIA = 1.05, ER-SBA = 15.69.

preparations by an enzyme immunoassay (ER-EIA; trogen receptor content by radioligand steroid binding assay (ER-SBA) were as previously described for this laboratory.¹³ A Scatchard plot which did not yield a significant linear correlation was considered to indicate that the tumour had no receptor content.¹³ In 1986 ER was also quantitated in the same cytosol preparations by an enzyme immunoassay (ER-EIA; Abbott Laboratories) according to the assay procedure provided with the EIA kits. The results obtained were expressed as fmol oestrogen receptor/mg cytosol protein. The steroid receptor laboratory was an active member of the UK Steroid Receptor Quality Assessment Group during the period of this study and routinely assayed EORTC quality control material.

The significance of any correlation between cellularity and ER content was tested by determining the correlation coefficient using a non-parametric ranking (Kendall's τ method). As multiple correlations were being performed a result was considered to be significant at a probability of 0.01 or less. Comparison of cellularity and ER-SBA between 1983 and 1986 was carried out by the non-parametric Mann Whitney

U test, for which a p value of ≤ 0.05 was considered to be significant.

Results

As expected, no difference in the range of cellularity was observed in the carcinomas diagnosed in 1983 and 1986 (1983 median = 25%, 1986 median = 29%, $p > 0.7$) or for ER content (1983 median = 16 fmol/mg cytosol protein, 1986 median = 0 fmol/mg cytosol protein $p > 0.3$). Equally, no significant difference was observed in the ER content (ER positive ≥ 20 fmol/mg) obtained with the SBA method in the two years examined (1983 ER positive = 23, ER negative = 27; 1986 ER positive = 22, ER negative = 32) (χ^2 test = 0.28, 1 df, $0.75 > p > 0.5$).

A good correlation was observed between the ER content in breast tumours detected by the different methods of analysis. Surprisingly, the non-parametric analyses suggest an inverse correlation between cellularity and ER content, although only for the enzyme immunoassay did the results approach significance. A summary of the findings is presented in the table. The menopausal status of the women did not affect the findings.

The figure shows the correlation between tumour cytosol protein expressed as mg/g tissue wet weight (data available for 96 cases) and cellularity. Analysis of the data with ER expressed as fmol/mg wet weight of tissue resulted in similar findings to those in the table.

Discussion

No significant difference was observed in the cellularity or ER content between the two groups of tumours taken from 1983 and 1986. A good correlation was also shown to exist between the ER results obtained by the two assay methods used.

The detection of ER content in breast cancer by biochemical methods relies on the use of tissue homogenates and ER content has traditionally been expressed as fmol/mg tissue cytosol protein. Previous workers have found no correlation between ER-SBA content expressed as fmol/mg tumour cytosol protein or fmol/mg weight wet and tumour cellularity when

Figure Relation between tumour cytosol protein (mg/g tissue wet weight) and cellularity.

assessed subjectively.^{4-6,10} Other authors, however, have claimed that cellularity still influences ER-SBA content but only for ER positive tumours,^{7,11} and Mumford, using an objective point counting technique, found a significant positive correlation between cellularity and all ER values, but only for postmenopausal women.⁸

In contrast to the findings made by these workers, we observed a possible, albeit weak, negative correlation between cellularity and ER detected by the newer enzyme immunoassay. This assumes that the tissue submitted for biochemical analysis was of a similar cellularity to that used to assess cellularity. In a prospective study it may be possible to obtain a better correlation if contiguous samples are taken. The negative correlation between measurement of cellularity and ER-EIA raises the interesting question as to the possibility that the stroma may be producing a local paracrine effect which stimulates the malignant epithelial cells to produce oestrogen receptor.

The lack of a significant correlation between ER content and cellularity could be attributed to the method of expressing oestrogen receptors (fmol/mg tumour cytosol protein). In this paper we have shown that there may be a seven- to 10-fold difference in tumour cytosol protein concentration at any given cellularity. In an attempt to establish if there is a more direct correlation between cellularity and oestrogen receptor content, oestrogen receptors were expressed as a function of wet weight (fmol/mg wet weight homogenised tissue). The results did not change the findings. Presumably, ER content is a function of both cellularity and the amount of receptor expressed by individual malignant cells, and both parameters may vary from tumour to tumour. Another variable—namely, a stromal paracrine effect—could also influence the amount of ER harvested from tissue homogenates.

Individual tumour cell oestrogen receptor content may also be important in determining overall ER content in breast carcinomata, and there is evidence, albeit from tissue homogenates, to suggest that the relative amount of ER in tumour cells may be of prognostic value.^{14,15} It is therefore important that a technique is developed which can determine the ER content of a tumour by taking into account both the cellularity and receptor content in individual cells, particularly as a positive correlation has been shown to exist between tumour cellularity and survival.³ An assessment of ER, which takes into account both of these factors, may produce a more meaningful result of greater prognostic value. Image analysis using the Quantimet 970 has recently been shown to be accurate in quantifying differentiation products detected by

Parham, Baker, Robertson, Vasishta, Baker, Smith monoclonal antibodies in breast carcinomata and the results can be expressed as a function of tumour cellularity.¹² By using this method, the true relation between oestrogen receptors and cellularity may be determined. A monoclonal antibody directed against oestrogen receptors, which can be reliably used in paraffin wax sections, would be of considerable benefit.

We thank Professor JS Beck for his encouragement and support in this work.

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by pathologists for pathologists", and volume 1 compares favourably with the *Recent Advances in Pathology* series. The articles cover aspects of gynaecological, urological, and nephrological pathology.

Much of this volume describes cancers with low malignant potential, and the term "borderline" in ovarian tumours is used not only for serous and mucinous tumours, but also endometrioid, clear cell, Brenner and mixed Mullerian tumours. The diagnosis of persistent and proliferative gestational trophoblastic disease is clearly described.

There are chapters on early prostatic malignancy, and the lack of editorial censorship is shown by one author warning of the risks of treating incidental prostatic carcinoma, while the subsequent chapter advocates radical prostatectomy for similar lesions. The book also describes papillary prostatic urethral lesions and malignant testicular stromal tumours, and there are chapters on the multiple causes of crescentic glomerulonephritis and fibrillary glomerulonephritis.

This is a very valuable collection of articles, and reasonably priced. If subsequent volumes are of a similar standard *Progress in Reproductive and Urinary Tract Pathology* will be a very welcome series of publications.

KM GRIGOR

Cyclosporin. Mode of Action and Clinical Application. Ed AW Thomson. (Pp 372; £50.) Kluwer Academic Publishers. 1990. ISBN 0 7462-0124-9.

It is trite to note that the use of cyclosporin has greatly enlarged the clinical possibilities of transplantation, and it is a truism to state that trying to understand the mechanism of its actions has pained immunologists by showing up a huge area of their ignorance. The understanding of molecular and cellular signal transduction and effector activation are being applied to cyclosporin and its new rival FK506, but there is still much to learn—and much to be learnt from studies of the pharmacology of cyclosporin.

Dr Thomson, whose own work has combined functional and morphological investigations in this field, has edited an attractive and useful synoptic account of our general understanding up to early 1989. There are 15 chapters by active scientists and clinicians from Australia, Britain, France, Switzerland and the USA. They review in varying detail the effects of cyclosporin A on mechanisms of cellular and humoral immune response initiation and amplification (20% of the book), and its therapeutic potential or proven value in human diseases of the bone marrow, eye, diabetes, skin and autoimmune disorders (40%). The remaining third covers pharmacokinetics, drug metabolism—harmful effects in clinical practice and pathological changes in experimental models.

Each chapter contains a tidy statement of current knowledge, plentiful illustrations, and a good supply of references. Most can only report phenomena, as our understanding is deficient, but others do discuss likely mechanisms involving binding to a specific cytoplasmic protein and downstream consequences on interleukin 2-mediated cell activation and proliferation.

To the basic scientist the book offers a useful but inevitably slightly dated review. For the applied researcher and clinician it provides valuable guidance on the possibilities and problems of treatment. For a compound with such a narrow therapeutic range it is disappointing that almost no author

describes the detail of any dosing regimen used—the drug is just administered! Apart from that lapse, Dr Thomson has provided a book of great use to immunologists and pathologists.

AD DAYAN

Management of Orbital and Ocular Adnexal Tumours and Inflammations. Ed JA Mauriello, JC Flanagan. (Pp 285; Hardcover DM 280.00.) Springer. 1990. ISBN 3 540 51155 5.

This is a profoundly disappointing book, which is a great pity because it contains some very good things. These include excellent photomicrographs, clinical illustrations, and gross pathological photographs, many of rare entities, culled from AFIP alumni and from Dr Flanagan's extensive practice. There are also a large number of well organised tables throughout the book. These good points only serve to highlight the deficiencies and chaos of the text. Although designed to be dipped into, rather than read as a whole, the organisation of the text constantly flits from one topic to another and frequently places a whole team of horses before the cart. I asked two "busy ophthalmologists", at whom the book is aimed, to look at it; both found the format and indexing irritating and elusive. From the pathologist's standpoint it is unsatisfactory to have paragraph headings of conditions that are not synonymous and to describe only the first, an example being: angiolymphoid hyperplasia (Kimura's disease, eosinophilic granuloma, eosinophilic folliculosis) when only Kimura's disease is described. Pick out the plums and you will enjoy it. Read it all and you risk indigestion.

ACE MCCARTNEY

Pharmacology—Drug Actions and Reactions. Ruth R Levine. (Pp 576; soft cover £24.95) Churchill Livingstone. 1990. ISBN 0-316-52217-1

This book is a useful introduction to the concepts of pharmacology and toxicology. Many medical graduates will have covered most of the ideas presented in their undergraduate years. The clinical scientist or MLSO rotating through his or her department's drug analysis section for the first time, however, will find it invaluable. Indeed, the hardest part of writing this review has been prising the book loose from the toxicology section, where it has rapidly become a fixture on the bookshelf alongside Clark, Goodman, and Gilman, the *British National Formulary*, and the *Data Sheet Compendium*. Strongly recommended: a definite "best buy".

ARW FORREST

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Corrections

An error appeared in the bottom line of the first column of the table in the letter, "Are calculated globulin measurements useful in screening for paraproteinaemia?" (*J Clin Pathol* 1990;43:694). The correct line should have read:

Specificity = $\frac{TN}{FP + TN} \times 100 = 96.7\%$.

Two authors names were omitted from a letter to the Editor, "Breast carcinoma cellularity and its relation to oestrogen receptor content." (*J Clin Pathol* 1989;42:1166–8). The names of P Coy of the Victoria Cancer Clinic, The Cancer Control Agency of British Columbia, and C Fletcher of the Special Development Laboratory, Greater Victoria Hospital Society, should have been included.

NOTICES

ACP Locum Bureau

The Association of Clinical Pathologists runs a locum bureau for consultant pathologists.

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