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Detection of Mammary Micrometastases by Pregnancy-associated α_2-Glycoprotein (PAG, α_2-PAG or PAM) and Carcinoembryonic Antigen (CEA)*

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Abstract—*Eleven of* 30, *stages* 1 *or* 2 *mammary cancer bearers developed conventionally detectable metastases within* 8–37 (*median*, 21) months. *Serum PAM rose by* $>75\%$ *above baselines before detection of metastases in all but one of these patients and in* 5 *of the* 19 *remaining well. The means of the maximum percentage rises in PAM were* 421.9 *for the metastatic and* 59.9 *for the well patients* ($P<0.01$ *in Mann–Whitney U-test*). *In contrast, there was a similar sustained rise in serum CEA in only* 2 *of* 10 *patients with metastases* (*one other rose* $>75\%$ *above baseline but fell before metastases appeared*), *and* 10 *of the* 19 *clinically well patients had rises exceeding* 75%. *This was not bettered by taking a* 45% *rise in CEA as the discriminant or by using a function of the combined rises in PAM and CEA. Also there were no significant correlations between the absolute values of serum PAM and CEA nor between their incremental changes. Thus PAM rises often detect micrometastases with growth potential in mammary cancer bearers. Whilst CEA rises are only occasionally helpful.*

INTRODUCTION

THE PLACE of pregnancy-associated α_2-glycoprotein α-macroglobulin (PAM) as a detector (a more accurate term than "marker" in this context) of expanding subclinical mammary micrometastases [1] is widely debated at present. Previously, enthusiasms for biochemical aspects of cancers have often dissolved in the light of subsequent extended investigations [2–4] and it is clear that the following two principles must be established from the outset in assessments of biochemical and clinical inter-relationships. Firstly the study of diagnostic tests requires a different investigational design from that of follow-up tests. Diagnosis needs reliable comparison of cancer bearers with non-cancer bearers presenting similar diagnostic problems to the clinician, and follow up needs assessment of changes from reliable initial baseline values in individuals. Secondly, a judicious combination of sound biochemistry, careful statistical control and detailed clinical perception is essential. The difficulties lie in predicting which basic research is relevant, in creating the appropriate clinical perception and in fostering fruitful communication amongst disciplines.

PAM (other abbreviations are PAG, α_2-PAG, PAAG, PZP and SP3) is not a diagnostic substance for mammary cancers. Women presenting with cancers have similar blood levels to those with benign diseases of the breasts or those with no mastopathy. Its blood levels appear to rise in women who will soon develop clinically obvious metastases from mammary cancers [1]. One aspect of the worth of this proposition has been tested in

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accordance with the foregoing principles by comparing the changes in PAM with those of CEA in the same patients, since CEA has been proposed [5, 6] as a biochemical detector in this context.

MATERIALS AND METHODS

Thirty women with resectable carcinomas of the breast and axilla (stages 1 or 2) were admitted to the study without special selection apart from receiving no regular medication. They were aged 34–78 yr when initially treated by mastectomy alone (11), by mastectomy plus radiotherapy (16) or by mastectomy and radiotherapy plus specific stimulatory immunotherapy (3) using autografts of irradiated cancers [7, 8] Blood samples were obtained and sent to the laboratories in coded batches as previously described [1]. The codes remained unbroken until the measurements were finally correlated with clinical status at the time of assessment for this report.

Parameters of assay

PAM was measured by an enzyme linked immunoassay [9]. The intra-assay coefficient of variation for PAM was 7.1% ($n=500$) and the inter-assay coefficient of variation was 10.5% at 1 μg/ml ($n=500$).

CEA was measured by an established radioimmunoassay [10] used in our laboratory service.

Baseline values for both CEA and PAM were calculated for each patient, excepting one for CEA, as the average of 3 or 4 weekly measurements made immediately after the patient entered the study. Subsequent measurements were made generally at intervals no greater than 3 months for between 8 and 37 months.

RESULTS

Nineteen of the 30 patients remained well and apparently free from cancer upon the grounds of routine clinical examination and general biochemical and radiographic investigations. The remaining 11 patients developed macrometastases in the mastectomy scars or the axillary/supraclavicular lymph nodes (4 cases) in bones alone or with other sites (7 cases) in the liver (3 cases), in the lungs (4 cases) and in the brain (1 case).

A clear difference emerged between the PAM profiles of the 11 patients who developed metastases [Fig. 1(a)] and of the 19 who remain clinically well [Fig. 1(b)]. All in the metastatic group had elevations of serum PAM in the range 57–1800% (mean=421.4) above baselines (Table 1) before metastases were detected by conventional clinical examination, liver function tests or radiography of chest or skeleton; only one patient at 57% had a maximum PAM rise of less than 75%. In the clinically well patients PAM rises of 1–316% (mean=59.9) occurred, in 5 subjects

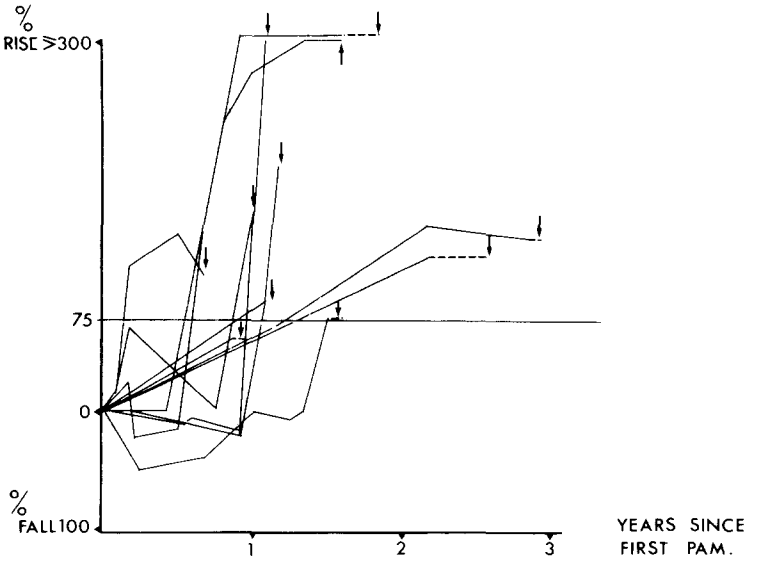

Fig. 1(a). Changes of serum PAM in the metastatic group. Arrows indicate the times of clinical detection of metastases.

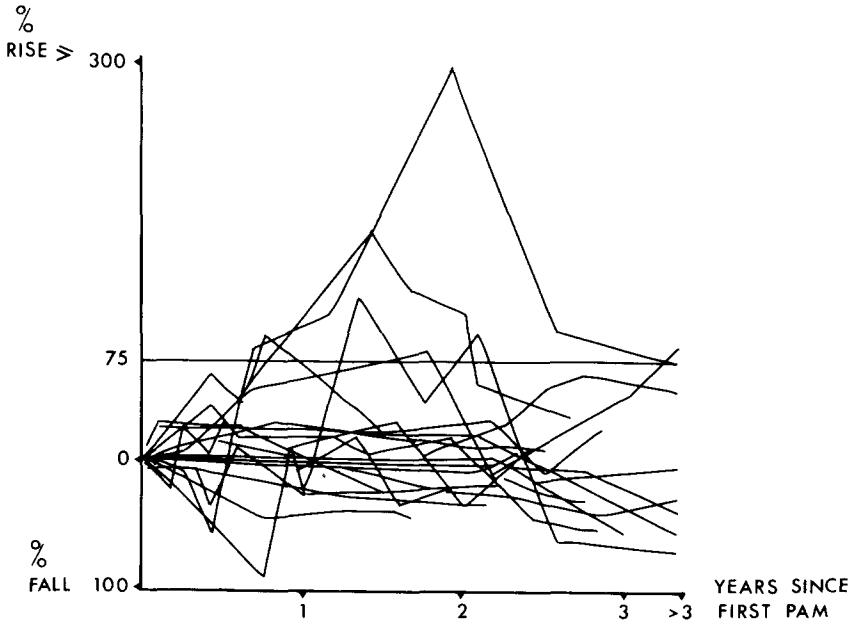

Fig. 1(b). Changes of serum PAM in the clinically well group.

the rises exceeded 75% and subsequently fell towards baselines. The difference between individual maximum PAM rises in the two groups was statistically significant with $P < 0.01$ in a Mann–Whitney U-test. This is in keeping with the result of a previous preliminary assessment [1] on the same patients when the whole profiles of PAM changes in the two groups were compared by attempting to develop a method of constructing zero-constrained regression lines and carrying out an analysis of variance.

The percentage changes of CEA from baselines have been assessed in the same way. The profiles for the metastatic group [Fig. 2(a)] show rises exceeding 75% in 4 of 10 patients and in 2 of these there were 2 later observations below the 75% level before metastases were detected. Ten of the clinically well group [Fig. 2(b)] had single rises exceeding 75%, 7 fell to baselines later, 1 remained close to the 75% level and 1 maintained a rise at 230%. The difference between the individual maximum percentage CEA rises in the two groups is not significant in a Mann–Whitney U-test (Table 1). When a rise in CEA of >45% was examined for discrimination between metastatic and clinically well patients, 8 of 10 patients with metastases were correctly detected but 12 of 19 patients remaining well were incorrectly suspected of harbouring active metastases. A discriminant analysis to obtain a weighted combination of the individual maximum percentage PAM and CEA rises which might separate the well and the metastatic patients more clearly, did not reduce the misclassifications obtained with PAM alone. The optimal discriminant function was:

$$7.07e^{-x_1/170} - 1.16e^{-x_2/170},$$

where x_1 is the maximum percentage rise in PAM and x_2 is the maximum percentage rise in CEA. The exponential transformation was used to preserve the difference in means between the well and the metastatic groups and to give equality of covariances. This gave 1 out of 10 misclassifications in the metastatic group and 5 out of 19 in the well group. Thus as shown in Table 2, using a discriminant of a 75% rise from baselines the test sensitivity* is 91% for PAM and 40% for CEA while the test specificity† is 74% for PAM and 47% for CEA. With a 45% rise as the discriminant for CEA the test sensitivity becomes 80% and the specificity falls to 37%. The combined discriminant for PAM and CEA has a test sensitivity of 91% and a specificity of 74%.

No significant correlations were found between the absolute values of PAM and CEA, or between their incremental changes.

*The percentage of correct classifications in the metastatic group.

†The percentage of correct classifications in the well group.

Table 1. Individual maximum percentage increases for PAM and CEA in the metastatic and clinically well patients*

Detector	Metastatic				Clinically well				*P* in Mann–Whitney U test
PAM	125	90	57	144	777	40	27	1	26	8	<0.01
	76	1056	198	150	162	16	0	28	5	0	
	1800					0	29	92	15	63	
						316	122	182	169		
	$\bar{x} = 421.4$					$\bar{x} = 59.9$					
CEA	60	39	83	1184	50	85	371	15	26	48	NS
	47	107	0	445	—	86	79	0	730	0	
	73					57	44	26	243	26	
						114	81	88	229		
	$\bar{x} = 208.8$					$\bar{x} = 123.6$					

*Lists of the absolute values are available upon request.

DISCUSSION

The interpretation of variations in putative biochemical detectors of microcancers is more difficult than it appears at first. Variations in a detector characteristic of established macrometastases do not necessarily mean they will be found at a micrometastatic stage and conversely, variations noted with micrometastases may be absent at a macrometastatic stage. Also, normal ranges of values for most potential detectors are imperfectly established and statements based upon the proportions of "abnormal" values obtained should be viewed with caution. These difficulties can be managed by frequent sequential measurement of the detectors, by the use of index numerals for changes from baseline values in individuals acting as their own controls and by retrospective comparison of results in patients remaining clinically well with those developing metastases. This approach was put to the test in this study by using the mean values of several measurements at the start of surveillance. The approach to the problems of the reliability of the assays at low values of the putative detectors is an integral feature of the experimental design since the detective significance of variations from such levels, particularly for CEA is unknown. The lowest level of sensitivity of the PAM assay is 10 ng/ml, which is well below the microgram levels found in the blood of most females.

The difference in PAM profiles between the metastatic and the clinically well groups allows the definitive conclusion that PAM often detects growing metastases from mammary cancers as previously suggested [1, 11] However, the odds that a rise of 75% or more in serum PAM might be followed by a fall with maintenance of the clinically well status, are about 1:3 in this series since this course was followed by 6 of the 17 patients with such a change in PAM. However, the number of patients is small in relation to the protean natural behaviour of mammary cancers and studies of larger numbers are required. Further clarification of the time sequences of PAM changes may help to identify those with potentially lethal metastases. Comparison of PAM measurements with those of CEA disclosed a much wider range of accurate detection by PAM, which is not approached by reducing the discriminant level for CEA to a 45% rise from baselines or by the optimal combination of the PAM and the CEA data. The possibility that CEA might detect the

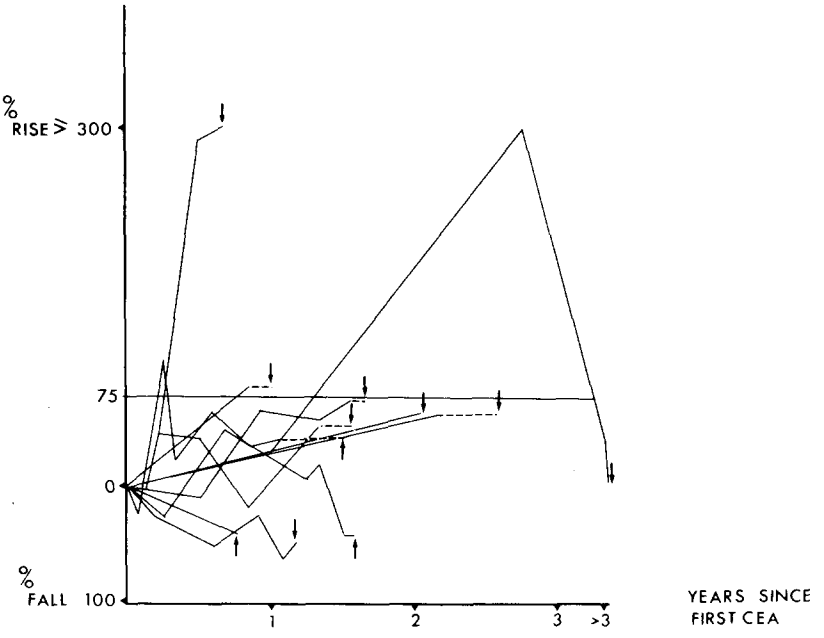

Fig. 2(a). Changes of serum CEA in the metastatic group. Arrows indicate the times of clinical detection of metastases.

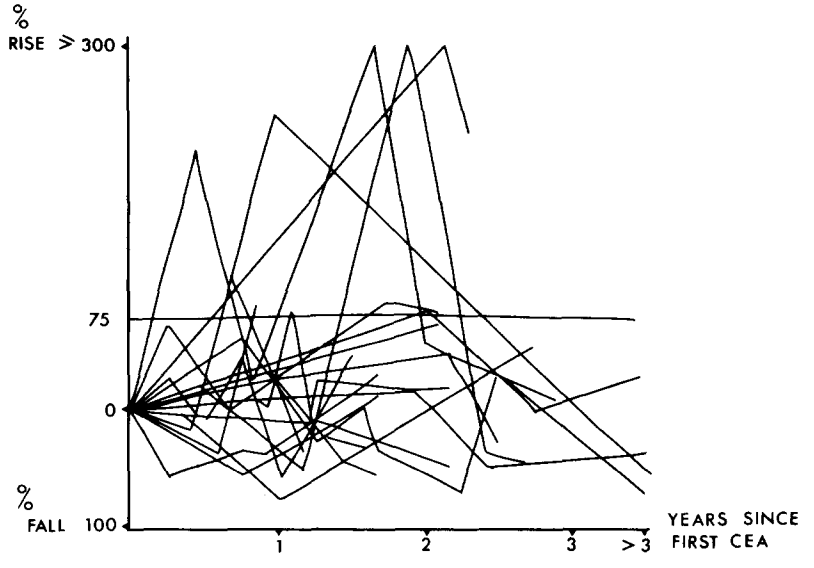

Fig. 2(b). Changes of serum CEA in the clinically well group.

Table 2. Performance of tests

Discriminant	Sensitivity %	Specificity %
PAM > 75%	91	74
CEA > 75%	40	47
CEA > 45%	80	37
PAM + CEA	91	74

growth of hepatic metastases has not been investigated.

Further refinement in the detection of expanding micrometastases from mammary and possibly other cancers [12] may emerge from combining other parameters with PAM. The simple comparison of levels of possible detectors in the presence of localised cancers with those in the presence of disseminated cancers is not an adequate assessment. This is clearly illustrated in the case of PAM where the wide range of values in different individuals precludes its use in diagnosis, although rises from well established baselines in individuals alert us to the presence of otherwise undetected micrometastases that will soon expand to clinical awareness and that may be more sensitive to treatments.

Values of putative detectors should be measured repeatedly and sequentially in carefully controlled clincal circumstances where unwanted variables have been eliminated. Statistical research into the comparison of multiparametric profiles of data obtained in this way has much to contribute to the multidisciplinary problems this work imposes.

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