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Simple method for comparing reliability of two serum tumour markers in breast carcinoma

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Abstract

***Aims*—To compare the two breast tumour markers, CA15-3 and mucinous-like carcinoma associated antigen (MCA), using Receiver Operating Characteristic (ROC) curve analysis.**

***Methods*—One hundred and ninety six patients "presenting" with breast carcinoma had serum CA15–3 and MCA concentrations measured.**

***Results*—Using these markers as indicators of stage IV disease at the recommended laboratory level, true positive rates (TPR) and false positive rates (FPR) were obtained as follows: CA15–3 TPR = 75%, FPR = 7·4%, MCA TPR = 80%, FPR = 59·1%. By increasing the CA15–3 cutoff level to 45 U/ml, a TPR and FPR of 75% and 0·6%, respectively were obtained. By increasing the MCA cutoff level to 23 U/ml, a TPR and FPR of 65% and 2·3%, respectively, were obtained.**

***Conclusions*—Using ROC curve analysis shows that CA15-3 is a superior indicator of metastatic breast disease than MCA at recommended laboratory levels, and by altering the cutoff points, the specificity and sensitivity for both these markers can be improved.**

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Tumour markers are widely used in monitoring patients with cancer, and they have proved useful in those with carcinoma of the breast.[1–5] The clinical application of these markers may (i) aid in the staging of the condition, (ii) monitor response to treatment, (iii) aid in the detection of disease recurrence or progression, and (iv) aid in the diagnosis of an occult primary in metastatic disease. The use of tumour markers for screening for occult primary cancer is impractical and has yielded poor results.[6] Tumour markers are not specific for cancer tissue and none is organ specific, the notable exception being prostate specific antigen (PSA).[7] Some markers have an increased affinity for particular types of tumour (breast CA15-3, MCA, CA50; gastrointestinal CA19-9, CEA; ovary CA125; testis human chorionic gonadotrophin, α fetoprotein), but there is nevertheless some degree of overlap. Clearly, some markers are more sensitive and more specific for some tumours than for others.

The value of a diagnostic test hinges on its ability to detect those with the disease (sensitivity) and on its ability to exclude those without the disease (specificity). Most laboratory results are on a continuous scale rather than a dichotomous one (false/negative result). The determination of a "cutoff" or "threshold" point in those tests with continuous scales will affect the sensitivity and specificity of that test. Receiver Operating Characteristic (ROC) curve analysis is a simple graph plotting the true positive rates (sensitivity) against the false positive rates (100/specificity).[8] The true positive rate (TPR) is plotted on the Y axis and the false positive rate (FPR) on the X axis. ROC curve analysis can be used objectively to (i) compare and contrast two or more different tests measuring a common entity, such as two tumour markers detecting metastatic disease, and (ii) determine the optimal point or value on the decision axis at which a test becomes positive.

Methods

One hundred and ninety six patients presenting with mammary carcinoma were studied: 101 had stage I disease, 50 stage II, 25 stage III and 20 stage IV. Staging was done according to the UICC (International Union against Cancer) breast cancer classification. All patients with breast carcinoma attending our breast clinic at University College Hospital, Galway, are carefully staged and screened at presentation. This involves history taking, clinical examination, CA15–3 and MCA assays, liver function tests, chest x rays and isotope bone scans (with raised tumour marker concentrations).[9] CA15–3 was measured by radioimmunoassay (CIS biointernational, Gif-sur-Yvette, France). The recommended normal range is less than 30 U/ml. MCA was measured by an enzyme immunoassay (Roche Products Ltd., Herts, England). The recommended normal range is less than 11 U/ml. Receiver Operating Characteristic (ROC) curve analysis was used objectively to compare, contrast, and assess these two tumour markers. The TPR refers to the number of patients (per cent) with stage IV disease who have tumour marker concentrations above a preselected level. The FPR refers to the number of patients (per cent) with marker concentrations above a preselected level but without metastatic disease. The TPR and FPR values for various cutoff points were plotted using a graphics package (Cricket Graph, Apple Computers Inc., San José, California, USA).

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Table 1 Details of CA15-3 concentrations in patients with breast cancer

	CA15-3 (U/ml)				
Stage	*I*	*II*	*III*	*IV*	*Total*
Minimum	15·0	15·0	15·0	15·0	15·0
Maximum	43·1	34·3	47·4	240·0	240·0
Mean	19·7	19·6	24·1	104·7	28·9
Median	17·2	17·7	21·7	75·2	18·9
SD	5·7	5·2	9·4	86·2	37·6
Number	101	50	25	20	196

Table 2 Details of MCA concentrations in patients with breast cancer

	MCA (U/ml)				
Stage	*I*	*II*	*III*	*IV*	*Total*
Minimum	1·3	2·6	3·6	6·9	1·3
Maximum	22·4	25·0	25·0	25·0	25·0
Mean	11·7	12·0	13·7	20·8	13·0
Median	10·2	11·6	11·5	25·0	11·5
SD	4·0	4·3	6·2	6·6	5·4
Number	101	50	25	20	196

Results

Table 1 and 2 illustrate the minimum, maximum, mean, median and standard deviations for CA15–3 and MCA in all patients. Stage IV disease was associated with the highest serum concentration of both markers and the results were statistically skewed. To give the data a normal distribution without interfering with the intrinsic values,[10] the data were transformed to the logarithmic form (to the base 10). The standard error of the difference (SED) between the mean values from each stage was calculated. The actual difference between the means of both groups was then calculated and divided by the SED, which gives the number of standard errors this difference represents. Values greater than 1·96 ($p < 0·05$) were considered significant. For both CA15–3 and MCA, there was no significant difference between stages I, II, and III, but there were significant differences between stages IV and the preceding stages I, II, and III for the two markers ($p < 0·001$). There was a strong correlation between both CA15–3 and MCA ($r = +0·65$, Student's t test = 12; degrees of freedom = 194; $p < 0·001$).

Table 3 True positive rates (TPR) and false positive rates (FPR) at various "cutoff" points for CA15-3 and MCA in diagnoses of metastatic breast disease

CA15–3			MCA		
Cutoff (U/ml)	*TPR (%)*	*FPR (%)*	*Cutoff (U/ml)*	*TPR (%)*	*FPR (%)*
15·0	95·0	63·9	7·0	95·0	88·6
17·0	90·0	51·1	9·0	95·0	85·2
20·0	80·0	37·5	*11·0	80·0	59·1
22·0	80·0	25·0	13·0	80·0	29·0
25·0	75·0	13·6	15·0	75·0	20·5
27·0	75·0	10·8	19·0	70·0	11·9
*30·0	75·0	7·4	21·0	70·0	10·2
32·0	75·0	5·7	**23·0	65·0	2·3
35·0	75·0	2·6	24·0	65·0	2·3
37·0	75·0	2·3	25·0	65·0	0·0
40·0	75·0	2·3			
42·0	75·0	1·1			
**45·0	75·0	0·6			
46·0	75·0	0·6			
47·0	70·0	0·0			
50·0	70·0	0·0			

*Denotes the recommended cutoff values used in our laboratory. **Denotes "new" cutoff values with respective TPRs and FPRs.

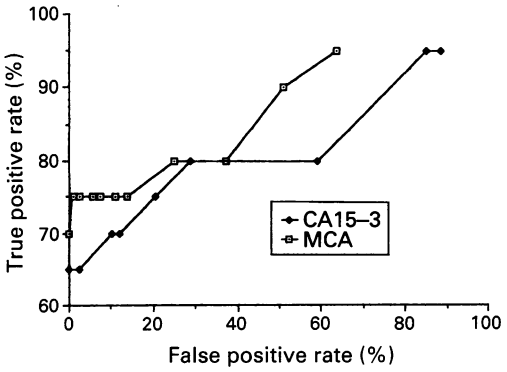
By altering the cutoff point for each tumour marker, the respective TPR (sensitivity) and FPR (100% specificity) changed (table 3, figure). For the recommended cutoff levels in our laboratory, MCA had a greater TPR (80·0%) than CA15–3 (75·0%), but CA15–3 had a lower and more favourable FPR—7·4% compared with 59·1% (figure).

We can deduce that, firstly, CA15–3 seems to be superior to MCA in detecting metastatic breast disease as it gives higher TPR than MCA for similar FPR, and, secondly, the optimal cutoff points (for the detection of stage IV disease) for both tumour markers should be reviewed. These new cutoff points are obtained by selecting the value for each marker which gives a high true positive rate before there is an increase in the false positive rate. In the case of CA15–3, an arbitrary optimal value would be 45 U/ml (new TPR = 75·0%; new FPR = 0·6%) and for MCA, an arbitrary optimal value would be 23 U/ml (new TPR = 65·0%; new FPR = 2·3%). Using both markers at their new ideal level would yield a true positive rate of 65·0% and a false positive rate of 2·3%, which is inferior to CA15–3 alone.

Discussion

Tumour markers are widely used in clinical medicine. An increasing number are available and each claim to be superior to others. With the greater number of laboratory tests available to clinicians, inevitably there will be an overlap and indeed a duplication among several assays. The most important characteristic of any assay is its "reliability" to measure what it purports to assay. In the comparison of two tests which purport to measure the same entity (two tumour markers as indicators for metastatic disease), an examination of the relative true positive rates (sensitivity) and false positive rates (100% specificity) can objectively differentiate between them and can therefore assess their credibility. The Receiver Operating Characteristic (ROC) curve permits such an investigation.[8 11–13] It can also be used to select the optimal point or value for a particular test which would yield the prime true positive rate before there is an adverse increase in the false positive rate.

Monoclonal antibodies are widely used in



ROC curve

clinical medicine, including the management of mammary carcinoma.[14] CA15–3 is a circulating marker defined and identified by two monoclonal antibodies designated DF3 (prepared against a membrane fraction of a metastatic breast carcinoma[15]) and 115D8 (produced against antigens of human milk fat globule membranes[16]). It has been extensively investigated in patients with mammary carcinoma,[2 5 9 17–21] and a good correlation demonstrated between it and the extent of the disease and high concentrations are usually associated with metastatic disease.[2 9 17–22]

Mucinous-like carcinoma associated antigen (MCA) is identified by a particular antibody (MCA-b-12) using as an immunogen a mixture of human breast cancer cell lines (MCF-7, SK-BR3, Hs-578T and ZR-75/–1).[23] MCA belongs to a group of mucin-like glycoproteins released from breast cancers and includes CA15–3. Analogous to CA15–3, high concentrations of MCA have been shown to be a reliable indicator of metastatic disease.[3 24–27]

Several studies have compared both these markers. Molina *et al*[26] showed that the sensitivity was 77·5% and 70·0% for MCA and CA15–3, respectively, in metastatic disease. Gozdz *et al*[27] showed that patients with stage IV disease had raised CA15–3 values (>40 U/ml) and raised MCA values (>17 U/ml) in 48% and 52% of cases, respectively. Miserez *et al*[28] used the 95th percentile of healthy volunteers to obtain the cutoff points for MCA (<11 U/ml) and CA15–3 (<23 U/ml). They obtained sensitivities of MCA and CA15–3 of 84% and 78%, respectively, and specificities of 81% and 78%, respectively. Silver *et al*[29] compared the three tumour markers CA15–3, MCA, and CEA in patients with breast carcinoma (n = 158) and various control groups using ROC analysis. The authors illustrate the process of cutoff point selection and also emphasise that the cutoff point selected for a particular clinical situation, such as the probability of only stage IV breast disease being present or not, may be inappropriate for another clinical situation (such as the probability of breast cancer (all stages) being present or not). In the context of determining the likelihood of the presence or absence of metastases in patients with breast carcinoma and utilising the recommended cutoff points of 30 U/ml for CA15–3 and 11 U/ml for MCA, a greater sensitivity (TPR) for MCA was obtained (CA15–3 = 75·0%; MCA = 80·0%). MCA had a greater FPR (100% specificity) than CA15–3 (59·1% and 7·4%, respectively). This finding detracts from the usefulness and reliability of MCA as an indicator of stage IV disease. By altering the cutoff value and plotting TPR against FPR on an ROC curve, the optimal value can be ascertained. This optimal cutoff point is selected on the basis of achieving the highest TPR value before there is an increase in the FPR. Such a selection is arbitrary, but nevertheless rational. From the data presented here, we recommend cutoff points of 45 U/ml for CA15–3 and 23 U/ml for MCA. These are the positions on the decision axis where values exceeding them indicate metastatic breast disease with reasonable certainty. In the instance of CA15–3 this would give a TPR of 75·0% and an FPR of 0·6%, and in the case of MCA would give a TPR of 65·0% and an FPR of 2·3%. Utilisation of both markers at the optimum level simultaneously confers no additional benefit with a TPR of 65·0% and an FPR of 2·3%.

In conclusion, our ROC curve analysis of serum CA15–3 and MCA in patients with mammary carcinoma would suggest that CA15–3 may be superior to MCA in identifying stage IV disease. Moreover, a change in the cutoff limits would decrease the FPR —increase the specificity—without adversely affecting the sensitivity, and thereby increase the reliability of the tests and greatly facilitate surveillance of stage IV disease in patients with breast carcinoma.

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