Circulating secretory component in breast neoplasms

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SUMMARY The serum concentrations of IgA_p and IgM_r associated secretory component (SIgA and SIgM) of 98 patients with neoplasms of the breast were measured. Of the 56 patients with carcinomas, 11 had increased concentrations of circulating SIgM, which was almost twice as sensitive as SIgA as a marker for carcinoma. Concentrations of circulating SIgA and SIgM were independent of expression of secretory component, IgA, and carcinoembryonic antigen (CEA); histological tumour grade; and tumour cell DNA ploidy, whereas a weak correlation between SIgA and SIgM and circulating CEA was seen. The three patients who had liver metastases indicated had particularly high concentrations of circulating SIgA and SIgM, whereas no difference was generally seen between patients with malignancy and those with benign tumours.

Secretory component is derived from a trans-membrane glycoprotein,¹ expressed by secretory epithelial cells. Secretory component functions as a receptor facilitating the transport of polymeric immunoglobulins (pIgA and pIgM) containing J chain into exocrine fluids², where it partly remains bound to the secretory immunoglobulins (SIgA and SIgM) and partly appears as excess free secretory component.³ Human lactating mammary glands contain an active secretory immune system and considerable amounts of secretory component and SIgA are stored in the duct system.⁴

Neoplastic secretory epithelium may synthesise secretory component, both *in vitro*³ and *in vivo*,⁵⁻¹¹ especially in adenocarcinomas of the breast,^{6,11} colon, or lungs.¹¹ Metastases from mammary^{6,9} and other carcinomas^{5,12} may also express secretory component. Synthesis of secretory component seems to be independent of tumour differentiation,^{6,11} except in large bowel carcinomas.^{7,8,11} Secretory component has thus been regarded as a potentially important tumour marker of breast carcinomas.⁶ Furthermore, it has been claimed that secretory component or SIgA in serum reflects the clinical course of breast cancer.¹³ Other disorders, however, may also produce increased serum concentrations of secretory component, particularly liver disease.¹⁴⁻¹⁶ Secretory component in serum will always be complexed with pIgA and pIgM to form circulating SIgA and SIgM.³

As far as we know this is the first study in which serum concentrations of SIgA and SIgM have been determined in relation to other biological variables of breast carcinomas—tumour expression of secretory component, IgA, and carcinoembryonic antigen (CEA); histological grading; tumour cell DNA ploidy; and circulating CEA. Furthermore, our assay reflects the actual molecular state of circulating secretory component and takes into account the different antigenicity of SIgA and SIgM.¹⁷ To the best of our knowledge previous reports on secretory component in relation to cancer have not considered the clinical relevance of circulating SIgM nor its possible influence on quantitation of SIgA—or secretory component—in serum.

Material and methods

The 93 women studied were aged between 20-81 years and had been admitted for breast tumours. Surgery for breast carcinoma had previously been performed on 15 patients, seven of whom had no recurrence but eight of whom had advanced malignant disease. Of the remaining 78 patients, 48 had carcinomas and 30 benign tumours. Immunohistochemistry, DNA flow cytometry, and serum CEA measurements were performed in 29 of the patients with malignant tumours.

IMMUNOASSAYS FOR CIRCULATING SIgA, SIgM, AND CEA

Serum samples were obtained before surgery and stored at -70°C. Differential quantitation of SIgA and SIgM was performed by an enzyme linked

immunosorbent assay (ELISA), as detailed elsewhere.¹⁷ Briefly, the assay was based on non-competitive binding of SIgA and SIgM to microplates coated with an excess of sheep IgG antibodies to human secretory component. Serum concentrations were determined in relation to appropriate SIgA or SIgM standards. Mutual competition between SIgA and SIgM in serum was avoided by testing the samples at sufficiently high dilutions. The concentration of total secretory component in serum was calculated from the SIgA and SIgM values.¹⁷ Sera from 49 age matched healthy women (aged 20–91 years) were included as controls.

The concentration of CEA in serum was measured by radioimmunoassay.¹⁸

HISTOLOGICAL GRADING AND IMMUNOHISTOCHEMISTRY

Sections routinely stained with haematoxylin and eosin were used for histological grading according to the World Health Organisation classification.¹⁹ The immunohistochemical procedure, which has been described previously,²⁰ was based on serial sections cut at 6 μ m from two ethanol fixed paraffin embedded tissue blocks, including representative neoplastic epithelium from each case. Paired immunofluorescence staining performed for secretory component, IgA, and CEA was evaluated semiquantitatively; a score of 3 represented bright overall staining of the tumour epithelium and a score of 0 virtually no staining. The degree of heterogeneous staining pattern was not evaluated in this study.

DNA FLOW CYTOMETRY

Fresh tumour tissues were studied by quantitation of DNA flow cytometry. The tumours were thus classified as nearly diploid or distinctly aneuploid. The procedure has been described and discussed in detail elsewhere.¹² Briefly, tumour cell suspensions were stained with ethidium bromide. Flow cytometrical histograms of emission measurements were analysed by planimetry. Lymphocytes from mouse spleen were used as a diploid reference.

STATISTICAL METHODS

Comparisons between groups were based on the Mann-Whitney U test. Correlations were expressed by Kendall's τ (τ_{au}). All tests were two tailed. Upper reference values for serum SIgA, SIgM, and total secretory component were taken from those corresponding to the 97.5 percentile (non-parametric) in the control group.²¹

Results

SERUM CONCENTRATIONS OF SECRETORY IMMUNOGLOBULINS

Twenty per cent of the patients with either benign or malignant tumours had circulating SIgM concentrations above the upper reference values (fig 1). Raised SIgA concentrations were found in 13% of the benign and 11% of the malignant tumours, and nearly all of these serum samples also had raised SIgM concentrations (fig 1). The two patient groups showed significantly (benign tumours, $p = 0.02$) or probably significantly (malignant tumours, $p = 0.07$) higher concentrations of total circulating secretory component than the controls. There were no differences in total serum secretory component between those with benign or malignant tumours.

Three of the four patients with carcinoma with the highest total serum concentrations of secretory component were the only ones in this study who had liver metastases indicated in their records: one had liver metastases verified by computed tomography; one developed clinical overt metastases of liver, mediastinum, and spine shortly after surgery (no advanced diagnostic test was performed before surgery to detect

Fig 1 Serum concentration of SIgA and SIgM in patients with benign (a) or malignant (b) breast tumours. Dotted lines represent upper normal reference ranges. Circles indicate patients with liver metastases (one questionable).

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liver metastases); and one died three days after admission with disseminated cancer and probable liver disease as judged by increased serum liver enzyme activities (necropsy and other tests were not performed).

The fourth patient with particularly high serum secretory component had localised cancer but was treated for rheumatoid arthritis with gold and naproxene. In contrast, seven patients with carcinoma who had moderately increased serum concentrations of SIgA and SIgM all had localised tumours, and they were otherwise healthy. Among the 45 patients with carcinoma and normal concentrations of circulating secretory component, six had disseminated disease with axillary, pleural, bone, or brain metastases.

The women with benign tumours were generally healthy except for one who was treated for heavy alcohol abuse. She had raised serum concentrations of SIgM (136 mg/l). Of the 17 patients with fibrocystic disease, four had sclerosing adenosis as the dominating histological pattern. These four women had significantly ($p < 0.03$) higher concentrations of circulating secretory component than all the others with benign breast tumours.

IMMUNOHISTOCHEMISTRY FOR SECRETORY COMPONENT, IgA, AND CEA

Of the carcinomas that were examined immunohistochemically, 76% ($n = 22$) were positive for secretory component (fig 2) with predominantly faint staining. Expression of secretory component correlated well with IgA positivity, but was not related to histopathological grade. Moreover, serum SIgA and SIgM concentrations did not correlate with the expression of secretory component in the tumours; two of five patients with raised serum SIgA or SIgM

Fig 2 Immunohistochemical staining for secretory component from distinct positive breast carcinoma.

Fig 3 Immunohistochemical staining for CEA from positive breast carcinoma.

concentrations showed no expression of secretory component in the primary tumour.

Most of the carcinomas (55%) were positive for CEA (fig 3), and 18% of these patients had raised serum concentrations of CEA. Circulating CEA concentrations showed no significant correlation with tumour CEA expression. Carcinomas with the strongest CEA staining, however, had by far the highest serum CEA concentrations. There was a weak but significant correlation ($\tau = 0.34$, $p < 0.02$) between concentrations of plasma CEA and circulating SIgA and SIgM.

DNA PLOIDY

When carcinomas were divided according to their DNA ploidy pattern into nearly diploid (31%) and aneuploid (69%), no significant differences were seen between the two groups in the concentrations of circulating secretory component and CEA, or in tumour expression of secretory component and CEA.

Discussion

Previous reports have dealt with secretory component as a marker of human breast carcinomas in terms of its expression by primary tumours,^{6 10 11} or metastases,^{6 9} and its appearance in peripheral blood.^{13 15 22} We found that 76% of primary breast carcinoma tumours were positive for secretory component, which was well within the range of observations reported by others.^{6 10}

Release of free secretory component (and preformed SIgA) from a tumour could lead to increased circulating SIgA and SIgM, as free secretory component shows high affinity not only for pIgA but par-

ticularly for pIgM.²³ Thus free secretory component is always complexed with pIg in serum, either non-covalently (mainly to pIgM) or covalently (mainly to pIgA).³ Release of free secretory component alone might be expected to cause preferential increases in SIgM concentration because pIgM is the major circulating pIg. We found that the sensitivity of SIgM as a tumour marker was almost twice that of SIgA.

Twenty per cent of the patients with carcinomas had increased preoperative serum concentrations of SIgM. Other workers have reported 13% increased SIgA concentrations,¹⁵ although patients with more advanced disease have shown a higher frequency.^{13 22} One of the patients with carcinoma had rheumatoid arthritis, a condition that may cause an increase in serum secretory component.¹⁴ Interestingly, all three patients with liver disease had particularly high concentrations of circulating secretory component. One had verified liver metastasis and another most likely also had liver disease but died before any specific diagnostic tests could be performed. The third patient developed liver metastases clinically within a short time. Conversely, six additional patients who had disseminated cancer without liver metastases had normal serum values of secretory component.

The possible association between liver metastases and high concentrations of serum secretory component agrees with the findings of previous studies in our laboratory of patients with large bowel carcinomas.²⁴ In addition, follow up studies of these patients have shown that our test can detect most patients with liver metastases up to 12 months before the liver metastases become clinically overt (Kvale D, Rognum TO, Brandtzaeg P, unpublished observations). Puleo *et al*¹³ claimed that total concentrations of secretory component in plasma might reflect the clinical course in metastatic breast cancer, but the greatest increase in secretory component in their study also occurred in patients who developed liver metastases. Other studies^{14 15} have reported an association between raised SIgA in serum and metastatic carcinomas with liver disease, although a closer evaluation of tumour properties such as expression of secretory component was not performed. It is impossible, however, to be conclusive on this matter as only three patients with liver metastases were included in our study.

The generally low concentrations of serum SIgA and SIgM in patients with breast carcinoma without liver disease, and the lack of correlation between serum component and tumour expression of secretory component, suggested that the tumours contributed very little of the circulating secretory component. Notably, Stern *et al*⁹ found lower concentrations of secretory component in cytosol from metastatic mammary carcinomas than from primary tumours.

Metastatic carcinomas may therefore produce less secretory component than that indicated by immunohistochemical observations on tumours positive for secretory component.^{6 10 11} Furthermore, increases in serum secretory component in patients with liver metastases should mainly be ascribed to secondary liver derangements and not to tumour production.²⁴

Both from a biological and clinical point of view it seemed justified to include benign breast tumours in a study of breast cancer. We found particularly high concentrations of serum SIgM in patients with sclerosing adenosis in fibrocystic disease. This might reflect the histological and immunohistochemical⁶ features of this condition. The raised SIgM value noted in one alcohol abuser could be due to alcoholic liver disease known to increase the concentrations of circulating SIgA^{7 16} and SIgM.¹⁶

In conclusion, we measured the concentrations of circulating SIgM and SIgA in patients with benign and malignant breast tumours and found a higher diagnostic sensitivity for SIgM. We did not find any significant correlations between tumour secretory component or CEA expression and the corresponding serum concentrations of secretory component and CEA. Moreover, preoperative concentrations of serum SIgA and SIgM could not indicate the type of lesion. Nevertheless, an association was indicated between high concentrations of circulating secretory component and liver metastases. Liver metastases as an isolated complication in breast cancer, however, is rare. The clinical value of quantitating serum secretory component in relation to this malignancy is therefore questionable.

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The Design and Analysis of Long-Term Animal Experiments. Statistical Methods in Cancer Research. Vol. III. IARC Scientific Publications No. 79. JJ Gart, D Krewski, PN Lee, RE Tarone, J Wahrendorf. (Pp. 219; £28.) Oxford University Press. 1987. ISBN 92 832 1179 8

Most attempts to understand and control industrial or domestic exposure to chemicals and radiation as the causes of cancer, and equally, much research into the stages of carcinogenesis are based on long term experiments in animals. The design and analysis of such studies is far more complex than is often realised, but if the work is to be worthwhile, extraneous factors must be rigorously controlled and the results must be evaluated by appropriate statistical procedures.

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AD DAYAN

Notices

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Corrections

An error occurred in the summary of *J Clin Pathol* 1987;40:879-84. (Barbatis *et al.*) The last paragraph should read: Bile duct epithelium expresses HLA-DR in primary biliary cirrhosis, large duct obstruction and drug induced cholestasis indicating that HLA-DR expression in bile duct epithelium is not exclusive to primary biliary cirrhosis.

(See also correspondence from Professor Wright)

In the paper by Kvale D *et al.* (*J Clin Pathol* 1987;40:621-5), the first line of the summary should read: The serum concentrations of IgA and IgM associated secretory component ... and not serum concentrations of IgAp and IgMr ...