Pure and mixed mucinous breast carcinomas: DNA stemline and prognosis

S TOIKKANEN, E EEROLA,* T OEKFORSS

From the Departments of Pathology and *Medical Microbiology, University of Turku, Finland

SUMMARY The DNA stemline of 45 mucinous breast carcinomas was determined by flow cytometry using paraffin embedded archival tissue sections. The material consisted of 26 pure mucinous and 19 mixed mucinous carcinomas. The patients were followed up for at least 15 years or until death. Nearly all pure mucinous carcinomas had a normal DNA stemline (25 of 26) with only one aneuploid tumour. Mixed mucinous carcinomas had a DNA content resembling that of common ductal carcinoma with 11 aneuploid tumours. Aneuploid tumours tended to be of higher grade and stage than diploid tumours. The survival of patients with pure mucinous carcinoma was better than that of patients with mixed mucinous carcinoma. Mucinous carcinoma should be classified as such only if it is a pure mucinous carcinoma.

DNA flow cytometry is a rapid and efficient method for evaluating the DNA content of tumour cells.¹ Although many mammary carcinomas have already been tested with this method,²⁻⁸ surprisingly little attention has been paid to different histological types. This is probably due to the fact that the most common type, infiltrating duct carcinoma, comprises most mammary cancers, and therefore material for study from other types is limited. We were able to find only eight reported cases of mucinous carcinoma of the breast measured by DNA flow cytometry.^{5,6,9}

The method described by Hedley *et al*¹⁰ allows paraffin embedded archival material to be used in DNA flow cytometry, which helps considerably in prognostic studies. We report the results of the DNA flow cytometric analysis of 62 cases of pure or mixed mucinous mammary carcinomas followed up long term.

Material and methods

The cases were retrieved from the files of the department of pathology, University of Turku, Finland, and covered the period from 1945 to 1969. Slides stained with van Gieson, haematoxylin and eosin, periodic acid Schiff with and without diastase treatment, high iron diamine-alcian blue and the Grimelius stain were reviewed. The tumours were divided into pure mucin-

ous carcinomas and mixed mucinous carcinomas according to the following principles.

Pure mucinous carcinomas contained abundant extracellular mucin, which surrounded all the invasive cell groups at the tumour margins. Mixed mucinous carcinomas were similar in all other respects, but with a minor component of invasive cell strands without extracellular mucin—that is, they showed features of ductogenic carcinoma (figs 1 and 2).

As histological grading according to Bloom and Richardson¹¹ was difficult (because it emphasises the value of tubule formations) nuclear grade was determined according to Black and Speer instead.¹² The clinical staging was performed according to the TNM system.¹³ The patients were followed up for at least 15 years or until death. The cause of death was confirmed from various sources: necropsy; hospital files; the Finnish Cancer Registry; and the Central Statistical Office of Finland (cause of death statistics).

Single cell suspensions from paraffin blocks were prepared using the method described by Hedley *et al*.¹⁰ In addition to the thick sections for DNA flow cytometry, a 5 µm section was cut as a histological control. The detached cells were treated with RNase (100 µg/ml, Sigma Chemical Company, St Louis, Missouri, USA) for 15 minutes at room temperature and stained thereafter with propidium iodide (416 µg/ml, Sigma). The cells were then filtered through a 50 µm nylon mesh to remove aggregates. The cells were analysed using an Epics-C clinical flow cytometer (Coulter Electronics, Hialeah, Florida, USA). The excitation wavelength was 488 µm at a power setting

Fig 1 Pure mucinous carcinoma of the breast. All invasive cell clusters contain extracellular mucin.

of 400 mW. Fluorescence was detected at a range of more than 570 μm . The minimum accepted cell number for each analysis was 5000. The occurrence of cell aggregates was further elucidated by the use of an electronic gate in the channel detecting cell size (forward angle light scatter). The coefficient of variation averaged 6.1 for G_0/G_1 peak. Clear histograms were obtained from 45 tumours. In 17 tumours (13 pure mucinous carcinomas and 4 mixed mucinous carcinomas) the amount of intercellular mucin was so great that the cell number did not exceed the required 5000 cells, or the cell debris disturbed the histogram, and these tumours were therefore not regarded as eligible for the study. The first peak in the histogram (the lowest fluorescence intensity) was taken as the normal DNA stemline (diploid) and other peaks as the abnormal DNA stemline (aneuploid) except the peak at the tetraploid region if the cell number was less than 10%. Because no control cells were used, hypodiploidy could not be detected.

The Fischer-Irwin test was used for statistical analysis.¹⁴

Results

Only one of the 26 pure mucinous carcinomas was aneuploid; 11 of 19 mixed mucinous carcinomas showed a cell line with abnormal DNA content. The difference was highly significant ($p = 0.00007$).

The clinical stage at the time of diagnosis was known in 44 cases. Table 1 shows the prevalence of diploid and aneuploid tumours at each stage. Aneuploid tumours tended to be of a higher stage. Mixed tumours were of a higher stage than pure tumours (table 1). Table 2 shows the correlation between nuclear grade and DNA stemline. The tumours with small and regular nuclei (grade III) were diploid in 90% of the cases, whereas only 58% of tumours with grades II and I nuclei (combined) had a normal DNA stemline.

One patient with a diploid tumour was lost to follow up and one with an aneuploid tumour died post-operatively. These patients were not included in the follow up data. In the diploid group there were six deaths from cancer; 15 patients died of other causes. Eleven patients were alive after 15 years. In the aneuploid group eight patients died of their cancer,

Fig 2 Mixed mucinous carcinoma of the breast. Infiltrating duct carcinoma comprises a minor component of tumour.

Table 1 Distribution of tumours according to clinical stage at time of diagnosis

Stage	Diploid	Aneuploid	PMC	MMC
I	5		4	1
II	16	3	13	6
III	8	7	6	9
IV	3	2	2	3

PMC, Pure mucinous carcinoma; MMC, mixed mucinous carcinoma.

two patients succumbed to other diseases, and only one was alive at the end of the follow up. Three patients with pure mucinous carcinoma and 11 patients with mixed mucinous carcinoma died of their cancer. This difference was significant ($p = 0.001$). Twenty two patients with pure mucinous carcinoma and seven with mixed mucinous carcinoma had no evidence of recurrent disease, were alive or had died of other causes.

Discussion

Our material comprised relatively advanced cancers, because many cases dated from the 50's and the 60's when patients sought medical treatment later than is usual today.

The DNA stemline of only a few mucinous carcinomas of the breast has been determined by DNA flow cytometry. Of the eight reported determinations that we found^{5,6,9} only one tumour showed abnormal DNA content,⁹ which agrees with our findings. Erhardt *et al*¹⁵ also reported abnormal DNA index using Feulgen microspectrophotometry in four of 13 colloid carcinomas of the breast. No information, however, was given in any of these reports as to whether the tumours contained areas resembling ordinary infiltrating carcinoma. The results of the present study show clearly that even a minor component of infiltrating ductal carcinoma is enough to change the DNA stemline and afford a prognosis similar to that of common ductogenic cancer. Rasmussen *et al*¹⁶ recently reported similar findings based on histological features.

Mucinous mammary carcinoma has been regarded as one of the more benign forms of breast cancer,¹⁷⁻¹⁹ though this notion has recently been questioned.^{20,21} The reasons for the relatively benign behaviour are

obscure, but mucinous carcinoma has several histological characteristics that may explain it. The growth pattern is often expanding, cellularity and mitotic activity are low, and cell atypia is not very prominent. In the light of these facts it was interesting to find that the DNA stemline of pure mucinous carcinoma was almost exclusively normal. It is tempting to speculate that this is the factor responsible for those benign features and consequent good prognosis. This is, however, difficult to prove. The evidence collected from studies on other types of mammary cancers includes some controversial findings. For example, medullary carcinoma of the breast seems to have a prevalent abnormal DNA stemline despite relatively benign biological behaviour,^{6,9,15} whereas invasive lobular carcinoma often has normal DNA content despite the fact that it behaves like infiltrating duct carcinoma.¹⁵

Our material is too small to provide a basis for definite conclusions, but it seems to indicate that pure mucinous carcinomas usually have a normal DNA stemline, which is probably related to the slow growth and good prognosis of this rare form of mammary cancer. By contrast, the DNA profile of mixed mucinous carcinomas is similar to that of infiltrating duct carcinoma, which has been reported to be abnormal in 60–80% of cases.²⁷ Thus our study shows that mixed mucinous carcinoma should be classified according to the non-mucinous component of the tumour.

This study was financially supported by the Finnish Foundation for Cancer Research.

References

- Crissman MA, Steinkamp JA. Rapid simultaneous measurement of DNA, protein and cell volume in single cells from large mammalian cell populations. *J Cell Biol* 1973;69:766–71.
- Hedley IW, Rugg CA, Ng ABP, Taylor IW. Influence of cellular DNA content on disease-free survival of stage II breast cancer patients. *Cancer Res* 1984;44:5395–8.
- Ewers SB, Långström E, Baldetorp B, Killander D. Flow cytometric DNA analysis in primary breast carcinomas and clinicopathologic correlations. *Cytometry* 1984;5:408–19.
- Kute TE, Hyman B, Hopkins M, Marshall R, Case D, Kammire L. Relationship of flow cytometry results to clinical and steroid receptor status in human breast cancer. *Breast Cancer Treatment Res* 1985;6:113–21.
- McDivitt RW, Stone KR, Craig RB, *et al*. A proposed classification of breast cancer based on kinetic information. Derived from a comparison of risk factors in 168 primary operable breast cancers. *Cancer* 1986;57:269–76.
- Horsfall DJ, Tilley WD, Orell SR, Marshall VR, Cant EL. Relationship between ploidy and steroid hormone receptors in primary invasive breast cancer. *Br J Cancer* 1986;53:23–8.
- Cornelisse CJ, van de Velde CJH, Caspers RCJ, Moolenaar AJ, Hermans J. DNA ploidy and survival in breast cancer patients. *Cytometry* 1987;8:225–34.
- Owainati AAR, Robins RA, Hinton C, *et al*. Tumor aneuploidy, prognostic parameters and survival in primary breast cancer. *Br J Cancer* 1987;55:449–55.

Table 2 Correlation of nuclear grade, DNA stemline, and histological type

Nuclear grade	Diploid	Aneuploid	PMC	MMC
III	19	2	13	8
II	12	9	11	10
I	2	1	2	1

PMC, Pure mucinous carcinoma; MMC, mixed mucinous carcinoma.

- 9 Olszewski W, Darzynkiewicz Z, Rosen PP, Schwartz MK, Melamed MR. Flow cytometry of breast carcinoma. I. Relation of DNA ploidy level to histology and estrogen receptors. *Cancer* 1981;**48**:980-4.
- 10 Hedley DW, Taylor IW, Rugg CA, Musgrove EA. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. *J Histochem Cytochem* 1983;**31**:1333-5.
- 11 Bloom HJG, Richardson WH. Histological grading and prognosis in breast cancer. A study of 1409 cases of which 359 have been followed 15 years. *Br J Cancer* 1957;**11**:359-77.
- 12 Black MM, Speer FD. Nuclear structure in cancer tissues. *Surg Gynecol Obstet* 1957;**105**:97-102.
- 13 UICC. *TNM classification of malignant tumours*. Third ed. Union Internationale Contre le Cancer: Geneva, 1978:47-59.
- 14 Hodges IL Jr, Lehman EL. *Basic concepts of probability and statistics*. San Francisco: Holden-Day Inc, 1964:297-303.
- 15 Erhardt K, Auer G, Folin A, Silferswärd C, Skoog L. Mammary carcinoma: comparison between histologic type, estrogen receptor and nuclear DNA content. *Am J Clin Oncol* 1986;**1**:83-9.
- 16 Rasmussen BB, Rose C, Christensen I. Prognostic factors in primary mucinous breast carcinoma. *Am J Clin Pathol* 1987;**87**:155-60.
- 17 Lee YT. Surgical treatment of carcinoma of the breast: III. Pathological findings and probability of relapse. *J Surg Oncol* 1984;**25**:109-15.
- 18 Norris HJ, Taylor HB. Prognosis of mucinous (gelatinous) carcinoma of the breast. *Cancer* 1965;**18**:879-85.
- 19 Silverberg SG, Kay S, Chitale AR, Levitt SH. Colloid carcinoma of the breast. *Am J Clin Pathol* 1971;**55**:355-63.
- 20 Clayton FC. Pure mucinous carcinomas of breast: Morphologic features and prognostic correlates. *Hum Pathol* 1986;**17**:34-8.
- 21 Rosen PP, Wang TY. Colloid carcinoma of the breast: analysis of 64 patients with long-term follow-up. *Am J Clin Pathol* 1980;**73**:304.

Requests for reprints to: Dr S Toikkanen, Department of Pathology, University of Turku, Klimamyllynk 10, SF-20520 Turku 52, Finland.