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Nuclear DNA content of fine needle aspirates of invasive ductal carcinomas of the breast

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Abstract. Patients with aggressive breast cancers benefit from chemotherapy prior to surgery. If the biology of the breast cancers were better characterised pre-operatively, more patients at risk could be offered chemotherapy. We have assessed nuclear DNA content of fine needle aspirates (FNA) of 103 invasive ductal breast cancers and compared this to tumour size, node status and histological grade. Median follow-up was 18 months so no prognostic studies were made. Diploid and non-diploid tumours were distributed equally in node negative and positive patients. However non-diploidy status increased in line with known prognostic markers of tumour size and histological grade. This suggests that ploidy might contribute to the pre-operative assessment of prognosis. We conclude that nuclear DNA of breast cancer FNAs may be of value in the pre-operative biological assessment of breast cancer patients.

Keywords: Breast, carcinoma, cytology, DNA

1. Introduction

The treatment of breast cancers has changed in the last few years, from radical surgery to breast conserving surgery with or without adjuvant therapy [25,34,38]. Patients can now have pre-operative neo-adjuvant therapy and/or anti-oestrogen therapy prior to any surgery with the aim of shrinking the tumour and prolonging survival. Knowledge of biological status pre-operatively of the tumour would help in the selection of therapy as the response is dependent on the histological grade of the cancer. High grade tumours respond better to chemotherapy than low grade tumours which in turn respond better to anti-oestrogen therapy. Acceptance of prognostic grading of histological specimens of invasive ductal cancers has been used for many years [4], however, this can only be confirmed retrospectively, after the tumour has been excised. This does not permit the breast cancers to be prognostically graded prior to any surgery or neo-adjuvant therapy. There has been one study which

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compared histological grading to ploidy on FNA of breast cancers, but the study evaluated only a small sample of patients [10].

We have therefore elected to do ploidy studies on fine needle aspirates (FNA) to determine if this improves evaluation of ploidy status which may be an independent prognostic factor in breast cancers when tissue sections [5,7–9,13,14,19,24,32,33,36] or cytological specimens are used [1,3,30]. In present study we have compared the ploidy status of FNAs to the TNM staging system and to histological grade.

2. Materials and methods

2.1. Diagnostic cytological smears

FNA samples were obtained from women attending the Breast Unit at St Mary's Hospital, London with invasive ductal breast cancers. They were obtained using a 20 G needle and a 10 ml syringe. Smears were prepared from the aspirates, air dried and stained with modified Giemsa. They were analysed under light microscopy and reported according to the Royal College of Pathologists' guidelines for malignant lesions [27]. Available for nuclear DNA studies were consecutive 103 invasive ductal breast carcinomas.

2.2. Nuclear DNA cytological smears

After reporting the pre-operative diagnostic smear slides were placed in xylene for 72 hrs to remove the coverslip and synthetic mounting resin. The slides were then destained in 100% methanol for 15 min and washed in distilled water for 10 min. The slides were then fixed in 10% neutral buffered formalin for 30 min and then in 5 N HCl for 1 h which hydrolyses the ribose-purine bonds in the DNA to give sugar aldehyde residues [11]. The slides were then Feulgen stained using a CAS™ DNA ploidy staining kit following the manufacturers staining protocol which uses the thionine reaction (Beckton/Dickinson, Belgium). The Feulgen dye couples stoichiometrically via the Schiff reaction to the sugar aldehyde to give a blue colour [2]. The stained slides were then placed sequentially in acid alcohol, 100% ethanol and xylene. The slides were then mounted with coverslips using synthetic resin.

2.3. DNA rat hepatocytes controls

Rat hepatocytes embedded on a slide (Beckton/Dickinson) were Feulgen stained in the same manner of the previous specimens. The cells were used to calibrate the human DNA content and also acted as a control for the intensity of the Feulgen staining, and were placed in each batch of slides. Two rat hepatocyte control slides were placed in each batch of 12 specimens. The calibration slides were part of the multicentre European Quality Control Program run by Beckton/Dickinson. The coefficient of variation was 3.2% for the rat hepatocytes.

2.4. DNA tonsillar controls

Aspirate smears of normal tonsillar tissue were also used as controls and were Feulgen stained in the same manner as previously described. Two such controls were placed in each batch of specimens, they acted as a control for the staining procedure as well as a normal control the DNA index. Their coefficient of variation was 2.9%.

2.5. Image analysis

The Feulgen stained cytological slides were analysed on a CAS™ 200 image analysis system (Beckton/Dickinson). This is a video-based interactive image cytometer that uses the sum of the optical density of each Feulgen stained nucleus to calculate the amount of DNA present [21,28]. The ploidy status of a cytological sample was based on the analysis of at least 100 cells (range 100–220). The DNA indices of the cytological specimens were recorded. DNA indices of cytological specimens of breast cancers < 1.20 were classified as diploid with a coefficient of variation of 2.4% and > 1.20 as non-diploid with a coefficient of variation of 8.1%.

2.6. Histological specimens

Tissue sections of 2 μm were taken from the post-operative surgical specimens and routinely processed for light microscopy from all those patients included in the study to confirm the histological diagnosis of breast cancer. They were fixed in 10% neutral-buffered formalin and embedded in paraffin and stained with Haemotoxylin and Eosin for histological studies. Histological specimens were reported in accordance with national guidelines [27]. Each patient had their TNM stage measured using clinical pathological findings.

3. Results

There were a total of 103 women with a mean age of 52 yrs (range 31–82). Of the patients 59% were node negative and 41% were node positive. Comparing tumour size, 29% were less than 2 cm, 46% between 2–5 cm and 25% greater than 5 cm. Of the invasive ductal tumours 15% were grade 1, 45% were grade 2 and 40% were grade 3. The cancers non-diploid were 70%, and 30% were diploid. The women were 25% premenopausal and 75% postmenopausal. The results are summarised in Tables 1 and 2. Comparison of ploidy status and tumour size and histological grade is graphically displayed in Figs 1 and 2. The median follow-up time was 18 months thus no prognostic studies were made because they would not be of any clinical value.

Table 1
Patient characteristics

Character	Number of patients	Percentage of total
Node positive	42	41%
Node negative	61	59%
Tumour size < 2 cm	30	29%
2–5 cm	47	46%
> 5 cm	26	25%
Histological Grade I	15	15%
II	46	45%
III	42	40%
Premenopausal	26	25%
Postmenopausal	77	75%
Diploid	31	30%
Non-diploid	72	70%

Table 2
Ploidy status, node involvement, tumour size and histological grade

Variables	Diploid	Non-diploid
Node negative	18 (30%)	43 (70%)
Node positive	14 (33%)	28 (67%)
Tumour size $<$ 2 cm	15 (50%)	15 (50%)
2–5 cm	14 (30%)	33 (70%)
$>$ 5 cm	3 (12%)	23 (88%)
Histological Grade I	9 (60%)	6 (40%)
II	15 (33%)	31 (67%)
III	14 (34%)	27 (66%)

Values in parentheses are percentages of ploidy status in each variable.

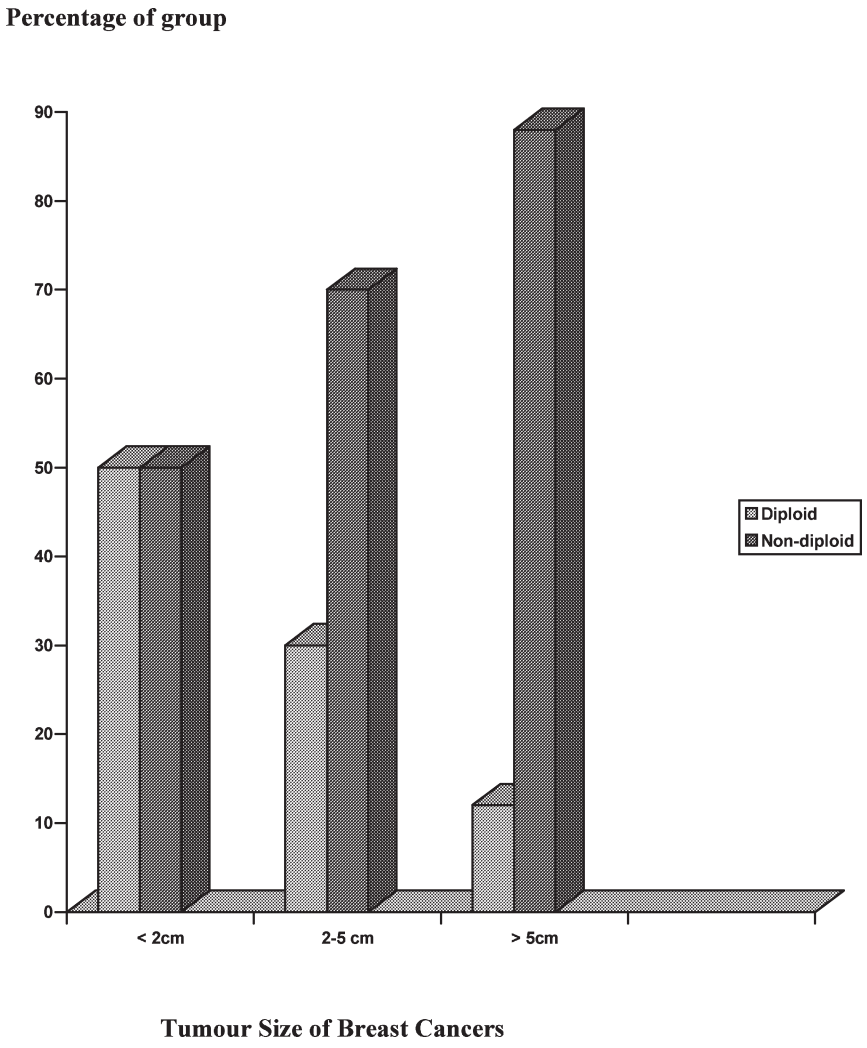

Fig. 1. Distribution of diploid and non-diploid in fine needle aspirates of breast cancers compared to tumour size.

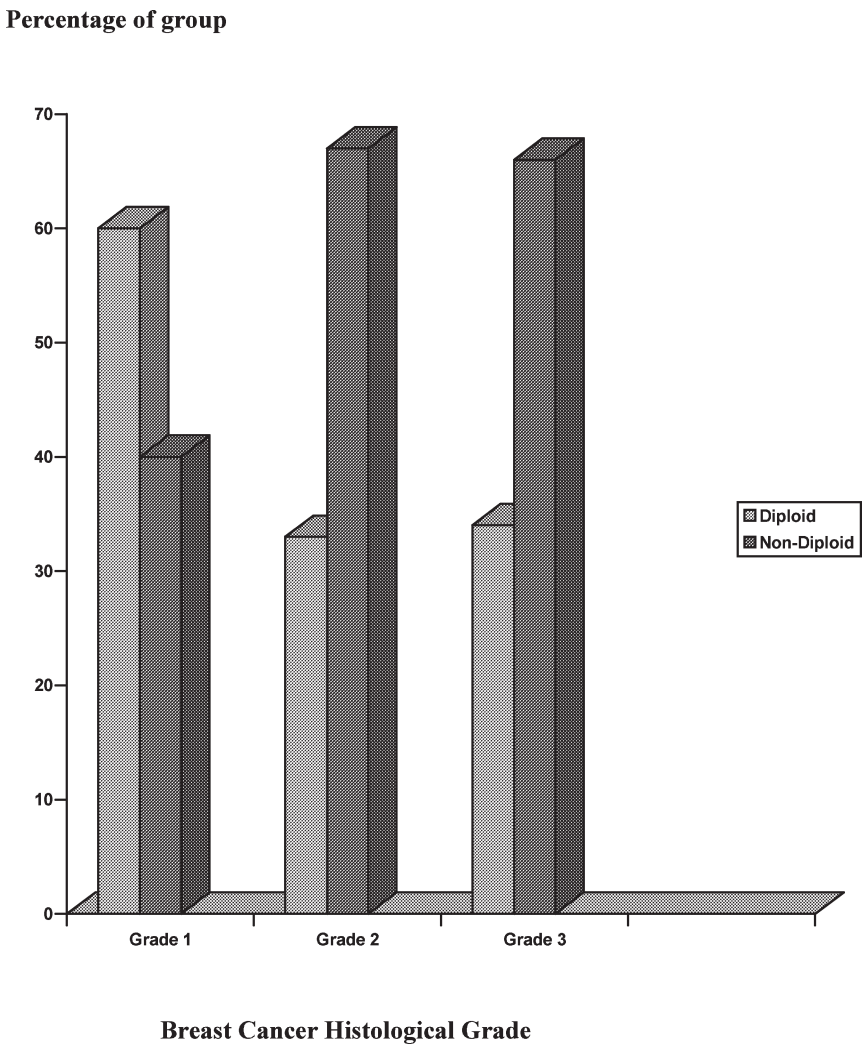

Fig. 2. Distribution of diploid and non-diploid of breast cancer cytology compared to histological grade.

4. Discussion

Nuclear DNA content is a reflection of the amount of DNA present in the chromosomes of the cell. It is usually defined as ploidy status. The issue of whether the DNA ploidy classification (diploid, tetraploid or aneuploid) is an independent prognostic factor is less clear, and not all studies are in agreement [5,7–9,13,14,19,24,32,33,36]. These studies were made on tissue sections of breast cancers, and flow cytometry was used to determine ploidy status. There have only been a few prognostic studies of nuclear DNA which have used cytological specimens of breast cancers [1,3,30].

It has been shown that ploidy status of tissue sections and cytological specimens of breast cancers using flow cytometry are comparable [20]. Also comparable are the measurements of nuclear DNA in breast cancer cytology using flow and image cytometry [16]. The latter method being more advantageous as cells are sparse in cytological specimens and debris and benign cells are eliminated from the analysis [15]. Although nuclear DNA content of breast lesions are comparable with image

cytometry on tissue sections and FNAs [29], cytological specimens are preferable since tissue sections are liable to contain sliced tumour nuclei [15]. We have shown that ploidy of FNAs of breast cancers correlate with histological grading and with tumour size and therefore may have a prognostic role in the pre-operative treatment of these patients.

More patients are being given pre-operative treatment in the form of neo-adjuvant therapy and anti-oestrogen therapy. It would be of clinical value if the tumour biology could be measured prior to any treatment. Studies prognostically grading breast cancer cytology have been carried out with differing results [6,12,13,17,22,23,26,28,31,35,37]. Our results show that determination of ploidy status of invasive ductal breast cancers can be easily achieved on FNAs using image cytometry. We have found there is an equal distribution of ploidy status in node positive and negative patients which is unlike some previous studies [12,18]. Our distribution of ploidy status, 30% diploid and 70% non-diploid is similiar to previous reports [9,12,18]. When comparing the ploidy status to tumour size and to histological grade, it is evident that non-diploid cells are present in a higher frequency as tumour size and histological grade increases. The latter relationship has been previously observed [18,24], but the relationship between tumour size and ploidy status differs in these studies. Because tumour size and histological grade has long been established as having prognostic value, DNA ploidy status of FNAs of invasive ductal cancers may also have a possible prognostic role.

We therefore aim to follow up this group of patients in order to evaluate the prognostic role of nuclear DNA content in FNAs of breast cancers. This may lead to a better choice of treatment regimes available to breast cancer patients and to a better assessment of these patients prior to any treatment.

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