

Correlation of DNA Content and C-erb B-2 Protein (Her-2/Neu) Reactivity with Cytologic Grade in Breast Carcinoma*

*Meme Karsinomunda Sitolojik Derece ile DNA İçeriği ve C-erb-B2 Protein (Her-2/Neu) Reaktivitesinin Karşılaştırılması**

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Özet: Bu çalışmanın amacı meme kanserlerinde DNA indeksi, S-faz fraksiyonu (SPF), C-erb B-2 ekspresyonu ve sitolojik derecenin prognostik önemini değerlendirebilmek ve önceden boyanmış sitolojik materyalin DNA analizi için uygun olup olmadığını araştırmaktır. Meme karsinomalı 35 hastada daha önce boyanmış olup silinerek Feulgen boyanan ince iğne aspirasyon materyalinde DNA içeriği araştırıldı. 6 olgu suboptimal Feulgen boyaması nedeniyle değerlendirme dışı tutuldu. SPF'u 20 olguda belirlenebildi. 9 olguda piklerin üstüste gelmesi nedeniyle SPF'u belirlenemedi. Her olguda Papanicolaou boyalı yaymalara dayanarak sitolojik derece belirlendi. 22 olguda parafin kesitlere C-erb B-2 onkoprotein için immün dokükimyasal boyanma yapıldı. DNA indeksi, S-faz fraksiyonu (SPF), C-erb B-2 ekspresyonu ve sitolojik derecenin prognostik etkilerini değerlendirebilmek amacıyla bu ölçütler arasında bir korelasyon olup olmadığını anlamak ve önceden boyanmış sitolojik materyalin, DNA içeriğini saptamak amacıyla kullanılabilirliği araştırıldı. Yalnız SPF'u ve sitolojik derece birbiriyle korelasyon göstermiş olup, bu bulgu onların prognostik önemini göstermektedir. Kendi bulgularımıza dayanarak, önceden boyanmış yayma-

Summary: The aim of this study is to evaluate prognostic value of DNA index, S-phase fraction (SPF), C-erb B-2 expression and cytologic grade and to investigate if previously stained cytologic material is appropriate for DNA analysis in breast carcinomas. DNA content was investigated in previously stained, destained and Feulgen stained fine needle aspiration (FNA) material in 35 patients with breast carcinoma. 6 cases were not evaluated due to the suboptimal Feulgen staining. SPF was determined in 20 cases. It could not be determined due to overlapping of different peaks in 9 cases. Cytologic grade was determined based on Papanicolaou stained smears in each case. Immunohistochemical staining for C-erb B-2 oncoprotein was done on paraffin sections in 22 cases. An effort was spent to find out if there is a correlation among DNA index, S-phase fraction (SPF), C-erb B-2 expression and cytologic grade to evaluate the prognostic efficacy of these parameters and to evaluate the usefulness of previously stained cytologic material to determine DNA content. Only SPF and cytologic grade were found to be correlated to each other which suggest that they are

ların DNA analizi için uygun materyal olduğu sonucuna varılmıştır.

Anahtar Sözcükler: Meme neoplasmları, ploidi, C-erb B-2 onkoproteinler, aspirasyon biopsisi.

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Prediction of biologic behavior of breast cancer depends on the prognostic factors. Classic prognostic parameters are no longer adequate to determine the aggressiveness of the primary breast carcinomas. Therefore, there is a need to expand the prognosticators by using new techniques such as DNA ploidy and immunohistochemical studies for oncogene overexpressions in an attempt to obtain additional information about the biologic nature of breast cancer. DNA content of breast cancer has been shown to have a prognostic significance (1-3). While diploid tumors correlates with a good prognosis, aneuploid tumors usually associate with an adverse outcome. High S-phase fraction (SPF) was also shown to be associated with a worse prognosis (2, 3). C-erbB-2 overexpression has been found in 9% to 89.2% of breast carcinomas (1, 4, 5). It was found that C-erbB-2 overexpression was correlated with large tumor size, low estrogen or progesterone receptor level, high DNA index, low benefit of adjuvant tamoxifen and poor clinical prognosis (2, 5-7).

The purpose of this study was to determine DNA content and C-erb B-2 protein expression in breast carcinoma and compare them with tumor grade in an attempt to find out if new techniques give us information which correlates to that of a classic prognostic parameter, cytologic grade, does. We also aimed to investigate the adequacy of previously stained and destained slides as a material for DNA analysis.

Materials and Methods

35 cases with breast carcinoma were included in this study. Breast carcinoma cases with Papanicolaou stained FNA cytologic slides which included adequate isolated cells and paraffin blocks were picked up for this study. Papanicolaou stained slides were destained and

valuable prognosticators. Based on our results, it may be concluded that, previously stained smears are adequate material for DNA analysis.

Key Words: Breast neoplasms, ploidy, C-erb B-2 oncoproteins, aspiration biopsy.

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restained with Feulgen method in 29 cases. A minimum of 200 cells were analysed for DNA content in each case. 100 lymphocytes which were undergone to the same procedure as tumor cells were used as an external reference. This procedure was not repeated in each case. The cells were analysed with SAMBA 4000 cell image analyser. Immunohistochemical staining was performed for C-erb B-2 oncoprotein (Biogenex, USA) on paraffin sections in 22 out of 35 cases. We divided staining intensity into two groups; weak when only less than 15 % of cells in a particular tumor were stained and strong when equal to or more than 15 % of cells in a particular tumor were stained according to the study of Midulla et al (1). Grading of fine needle aspirates was done according to Fisher et al (8). For the purpose of this study cytologic grade of tumors divided into two groups (low grade: grade 1-2 and high grade: grade 3). Statistical analysis was done based on Fisher exact test.

Results

Out of 35 cases two were grade 1, twenty six were grade 2 and seven were grade 3. Six of 35 cases were not evaluated for DNA content due to the suboptimal staining with Feulgen method. Table I shows ploidy status of 29 cases with breast carcinoma. Examples of diploid and single aneuploid histograms are demonstrated in Figure 1 and 2. SPF could be determined only in cases with diploid tumor (three cases) or in cases with single aneuploid tumor (17 cases). It could not be determined due to overlapping of different populations in multiploid tumors. Results of SPF, DNA content, C-erb B-2 staining and cytologic grade are shown in Table II. Comparison of cytologic grade and ploidy status are summarized in Table 3. Correlation between ploidy status and cytologic grade was not significant ($P>0.05$). A linear correlation was found between SPF and cytologic grade ($P=0.0022$) (Table II). C-erb B-2

oncprotein was mostly expressed in the cytoplasmic membrane of neoplastic cells (Figure 3). Benign epithelium around tumor tissue also showed reactivity for this antibody with a lower intensity. We could not find a significant correlation between intensity of C-erb B-2 staining and cytologic grade ($P>0.05$).

Table I. Ploidy status of 29 cases with breast carcinoma.

DIPLOID	Cases	ANEUPLOID	Cases		
	3		26		
		Hypodiploid	18	Multiple	9
		Hyperdiploid	8	Single	17

Table II. Ploidy status, DNA index, SPF and cytologic grade of patients with breast carcinoma.

CASE NO	ANEUPLOID/DIPLOID	DNA INDEX	SPF	C-ERB B-2	CYTOLOGIC GRADE
1.	Aneuploid	0.7	2		2
2.	Aneuploid	2.1	7	Weak	2
3.	Aneuploid	0.7	-		2
4.	Aneuploid	0.8	-	Strong	2
5.	Aneuploid	0.7	9.6		2
6.	Aneuploid	0.6	2		2
7.	Aneuploid	2.4	-	Strong	2
8.	Diploid	1	11.7		2
9.	Aneuploid	1.7	13.3		2
10.	Aneuploid	0.8	7.4	Weak	2
11.	Aneuploid	0.6	20.8	Strong	2
12.	Aneuploid	0.7	28.1	Weak	3
13.	Aneuploid	1.6	21.6		3
14.	Aneuploid	0.7	18.1		2
15.	Aneuploid	0.8	17.9	Strong	2
16.	Aneuploid	1.2	-	Weak	2
17.	Aneuploid	0.5	-	Strong	2
18.	Aneuploid	1.1	-	Weak	3
19.	Aneuploid	0.6	-		2
20.	Aneuploid	0.7	2.3		1
21.	Aneuploid	0.4	14.1		3
22.	Aneuploid	0.7	12.3	Strong	2
23.	Diploid	1	12.9	Strong	2
24.	Aneuploid	1.4	-	Strong	3
25.	Aneuploid	0.3	0	Strong	1
26.	Aneuploid	0.8	18.3	Strong	2
27.	Aneuploid	0.5	13	Strong	2
28.	Aneuploid	0.6	-	Strong	3
29.	Diploid	1	1		2

* C-erb B2 expression of five cases were not included in this table due to the lack of data about ploidy status, DNA index and SPF in these cases.

Table III. Comparison of cytologic grade and ploidy.

	Cytologic Grade 1	Cytologic Grade 2	Cytologic Grade 3
Aneuploid	2	18	6
Diploid	-	3	-

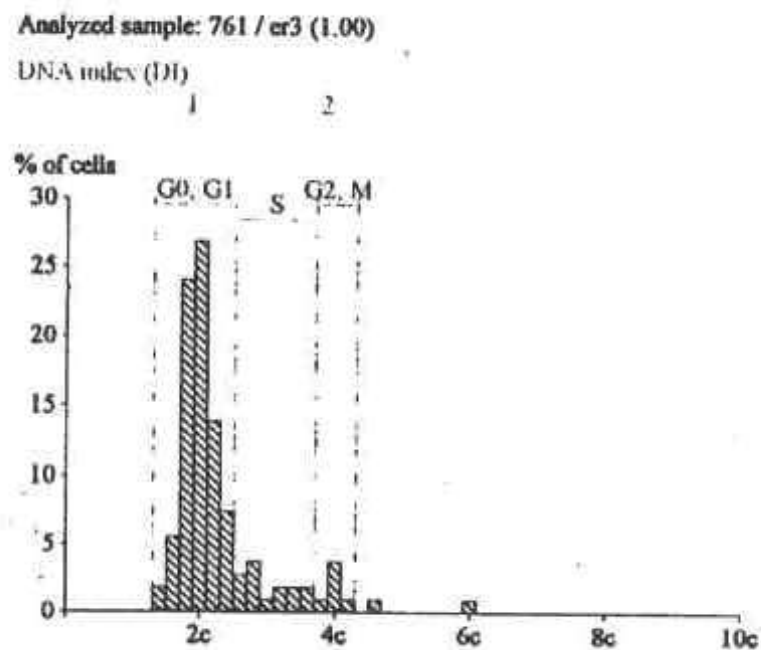


Figure 1. A diploid histogram with a high SPF.

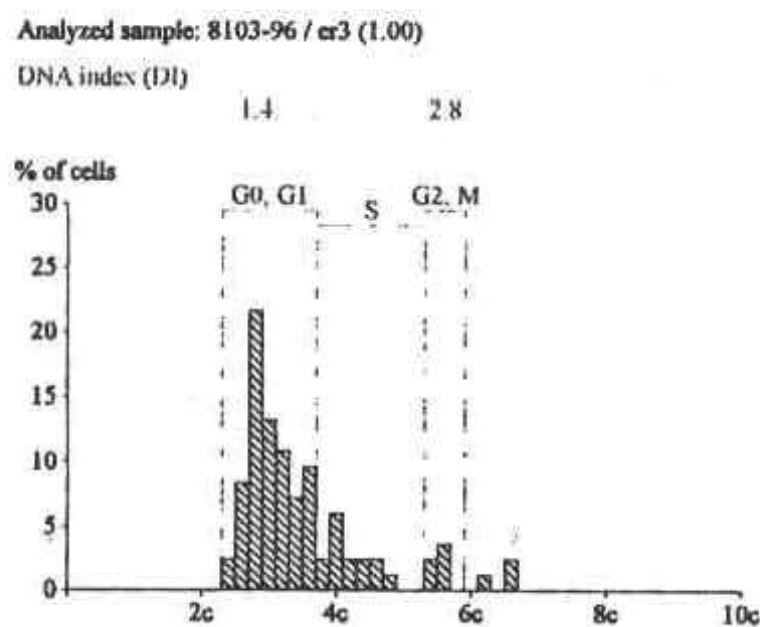


Figure 2. A single aneuploid histogram with a DNA index 1.4. SPF is high.

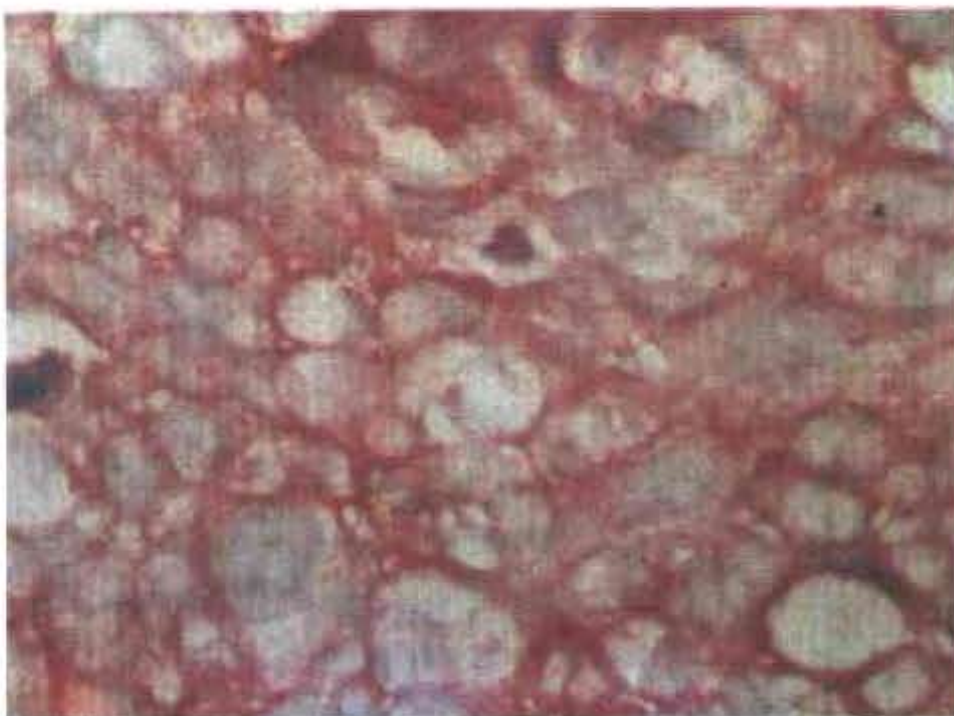


Figure 3. C-erbB-2 expression in breast cancer cells (X 220).

Discussion

DNA content may reflect the biologic aggressiveness of breast cancer (1-3). DNA ploidy has been determined postoperatively on imprints, deparaffinized cell suspensions or tissue sections from surgically excised breast tumors in many studies. Sarker and associates found good correlation between DNA ploidy studies of post-operative fine needle aspirates from breast carcinomas and the results with the ploidy status on histologic specimens from the same lumps (9). The value of DNA analysis on previously stained cytological material is a subject which is under investigation (10). Saad et al found that image analysis on previously stained FNA yields results that favorably compare with those obtained by flow cytometry on paraffin embedded tissue (10). Cytologic grade and ploidy status had been correlated in some studies (11-13) Hangbin et al found a highly significant connection between tumor grade, especially nuclear grade, and SPF (11). Brothrick et al showed that increased tumor grade correlated with aneuploidy (12). Yamamoto used hematoxylin-eosin imprint preparation and needle aspiration smears to evaluate nuclear grade and found that nuclear grade of aneuploid tumors were significantly higher than those of diploid tumors (13). However, DNA ploidy status was not determined on previously stained cytologic slides in these studies. The reason that our study did not reveal a good correlation between cytologic grade and ploidy status may be because of the previously stained cytologic material used for the detection of DNA content or the small number of cases included in this study. New studies with larger series may be needed to evaluate the use of previously stained cytologic material for this purpose.

The C-erb B-2 gene encodes a 185 kd protein with sequence homology to the gene encoding for the growth factor receptor (14). Malignant transformation was found to be associated with amplification of the C-erb B-2 oncoprotein in some malignant tumors (15). C-erbB-2 overexpression has been found in 9% to 89.2% of breast carcinomas (1, 4, 5). It was found that C-erbB-2 overexpression was correlated with large tumor size, lower estrogen or progesterone receptor level, lower DNA index, lower benefit of adjuvant tamoxifen and

poor clinical prognosis (2, 5-7). Correlation of different prognosticators may suggest the prognostic significance of them even if they were not found to be closely associated with clinical outcome in a certain study. Dittadi et al found that tumors with high C-erb B-2 values were mainly aneuploid while tumors with intermediate or low expression had variable degrees of DNA content (2). Midulla et al also stated that aneuploid tumors intensively expressed the C-erb B-2 oncogene (1). Similar to those studies Sinha et al found that breast carcinomas showing overexpression of C-erb B-2 had higher Ki-67 labeling indexes and higher AgNOR counts (16). We

correlated DNA ploidy, SPF, C-erb B-2 immunoreactivity and cytologic grade of each tumor. Only SPF and cytologic grade showed a good correlation ($P=0.0022$). Poor correlations among DNA ploidy, C-erbB-2 protein overexpression and cytologic grade are most probably due to the limited number of cases in this study.

In conclusion, this study suggested that SPF and cytologic grade are valuable prognosticators and previously stained slides may be useful for DNA analysis since we showed a good correlation between cytologic grade and SPF.

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