ORIGINAL ARTICLE

The role of ThinPrep cytology in the investigation of ki-67 index, p53 and HER-2 detection in fine-needle aspirates of breast tumors

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Summary

Purpose: The aim of the current study was to compare the immunocytochemical expression of ki-67, HER-2 and p53 on ThinPrep (TP)-processed smears, obtained by preoperative fine-needle aspiration (FNA) biopsies from primary breast carcinoma with the immunohistochemical results obtained on the corresponding surgical samples.

Methods: FNA biopsies were collected from 119 female patients during a 31-month period. Subsequently, these patients underwent surgical resection of the tumors.

Results: The overall accuracy (OA) of the TP cytology for ki-67, p53 and HER-2 expression was 96, 99 and 97%, respectively. There was a strong positive correlation between immunohistochemistry and immunocytochemistry results for ki-67 (Spearman's test 0.875) for p53 (Cramer's phi test 0.965) and HER-2 (Kendall's tau test 0.891).

Conclusion: This pilot study demonstrates that it is possible to monitor multiple molecular markers by using the TP cytology. Sample collection and storage is simple and permits the assortment of the FNA sample for both morphologic diagnosis and ancillary studies. The accuracy of TP technique in the evaluation of ki-67, p53 and HER-2 expression is comparable to those of the histological evaluation, and could be of paramount importance for the preoperative planning of treatment.

Key words: breast carcinoma, FNA biopsy, HER-2, ki-67, p53, ThinPrep cytology

Introduction

FNA has long been recognized to be a cost-effective method for preoperative diagnosis of breast cancer [1]. It is used most commonly in combination with physical examination and mammography in the so called "triple test" diagnostic triad, which is a highly accurate method for evaluating breast masses [2].

FNA has been used in conjunction with immunocytochemistry to measure molecular markers in patients with primary breast carcinomas [3]. The leading parameters that define treatment recommendations for breast cancer are estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth-factor (HER-2) status. Many other markers have also been assessed, including ki-67 [4].

Clinical studies have shown that breast cancers that contain p53 gene mutations are associated with decreased disease-free and overall survival [5,6]. Moreover, p53 mutations may be involved in the development of multidrug resistance [7].

HER-2 status in patients with breast cancer has become clinically important, not only as a prognostic marker but more importantly as a predictive factor for identifying patients who will benefit from chemotherapy, hormone therapy, and immunotherapy [8,9]. Patients with HER-2 overexpression had a significantly lower overall

survival with twice the mortality rate of women without HER-2 expression [10].

Breast cancers expressing high levels of ki-67, a nuclear marker of cell proliferation, are associated with worse outcomes [11,12]. ki-67 is not included in routine clinical decision-making because of a lack of clarity regarding how ki-67 measurements should influence clinical decisions. Recent studies indicate that changes in ki-67 expression after neoadjuvant endocrine treatment may predict a long-term outcome [13,14].

The use of ThinPrep® (TP) cytology (Hologic, Inc, Marlborough, Mass, USA) for the processing of FNA specimens has gained wide acceptance, and several studies have found a good correlation between TP and paraffin-embedded smears diagnoses [15]. Because such cytologic samples are stored in preservative, they can be a good source of genetic material for various types of analyses.

The aim of the current study was to compare the expression of ki-67, HER-2 and p53 on cytologic samples being placed in CytoLyt®, obtained by preoperative FNA biopsies from primary breast carcinoma with the immunohistochemical results obtained from the corresponding surgical samples.

Methods

Patient population

From April 2003 to November 2005, FNA biopsies were collected from 119 female patients with primary breast cancers who were treated at the Breast unit of the 2nd Department of Propedeutic Surgery of Athens University. The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki and signed informed consents were acquired. Inclusion criteria included: (a) age ≥ 18 years; (b) the patient did not receive neoadjuvant therapy; (c) patients with metastatic tumors were excluded.

FNA cytology

FNA cytology was performed with a 23 gauge needle connected to a 20 mL syringe attached to a Cameco metal holder (Precision Dynamics Corporation, Burbank, CA), as described by Zajicek [16] and Franzen et al. [17], under ultrasongraphic (US) guidance, in palpable breast lesions. The needle was moved back and forth very slightly while being positioned at various angles and different depths within the tumor before being withdrawn. Cellular material was transferred directly into the CytoLyt® solution and processed according to the procedure recommended by the supplier. The specimens were then sent to the Cytopathology Department of the 'Attikon' University Hospital, Athens.

Surgical specimens

The surgical specimens were received fresh in the Pathology Department of 'Laiko' General Hospital, Athens. The specimens were placed into phosphate buffered formalin and were fixed for ~24 h. Representative blocks of tumor embedded in paraffin were taken, cut, and stained with hematoxylin-eosin. Histopathological classification of the tumors was performed.

Immunohistochemical staining

Serial sections (4 μm) were prepared from selected blocks and float mounted on adhesive coated glass slides for HER-2, p53 and ki-67 staining. Primary antibodies included rabbit antihuman c-erbB2 oncoprotein antibody (DAKO) at 1:200 dilution for HER-2, monoclonal mouse antihuman p53 protein antibody (PAb1801; Novocastra, Newcastle, UK) at 1:50 dilution for p53, and monoclonal mouse antihuman ki-67 antibody (MIB-1; DAKO) at 1:100 dilution for ki-67.

Immunostained slides were scored after the entire slide had been evaluated by light microscopy. To determine the score of HER-2 expression, the membrane staining pattern was estimated [18]. The expression status of p53 and ki-67 was assessed according to the estimated proportion of nuclear staining of tumor cells that were positively stained [18]. The scoring system for HER-2, p53 and ki-67 are summarized in Table 1.

TP cytological preparations

The collected FNA specimens were prepared according to the manufacturer's protocol [19]. TP slides were prepared from the PreservCyt® suspended cells using the TP 2000 Automated Slide Processor®. The TP slides were processed by placing a slide and a vial of PreservCyt® on the TP 2000 processor®.

Immunocytochemical staining

Immunocytochemical staining was carried out for HER-2, Ki-67 and p53 using the automated Dako Autostainer. ki-67 immunostaining was performed using the polyclonal antibody (MIB-1; DAKO) at a dilution of 1:100. Immunostaining for p53 was performed using the monoclonal antibody PAb1801 (Novocastra, Newcastle, UK) at a dilution of 1:50. HER-2 immunostaining was performed using the monoclonal antibody (as supplied by DAKO) at a dilution of 1:200. The same scoring system was used as in the immunohistochemical evaluation.

Statistics

The Kolmogorov-Smirnov test was used in order to investigate whether the metric features follow the normal distribution. The Spearman's correlation test, the Cramer's phi and the Kendall's tau tests were applied in order to investigate whether there was a correlation for the ki-67 index, p53 and HER-2 between immuno-

Table 1. Scoring criteria for p53, ki-67 and HER-2

p53			ki-67			HER-2	
Nuclear staining	Score	Interpretation	Nuclear staining	Score	Interpretation	Score	Membrane staining
0	0	Negative	0	0	Negative	0	Negative
<1/10	1	Negative	<1/100	1	Negative	1+	Negative
1/10-1/2	2	Positive	1/100-1/10	2	Positive	2+	Positive
>1/2	3	Positive	1/10-1/2	3	Positive	3+	Positive
			>1/2	4	Positive		

histochemical and immunocytochemical expression. It should be noted that for the above three tests the correlation is stronger when the respective statistic is closer to ±1.

The Kruskal-Wallis test was used in order to examine a possible difference among the different cancer grades for the metric features, while for the categorical features x^2 test for linear trend was applied.

The utility of the TP technique in the evaluation of the of ki-67, HER-2 and p53 expression was studied by determining the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and OA. All of the analyses were performed in SAS 9.1.3 (Cary, NC).

Results

The median age of the study subjects was 50 years (range 36-86). Fourteen out of the 119 (11.7%) patients were premenopausal and 105 (98.3%) postmenopausal. The expression of ki-67, p53 and HER-2 on the TP slides was characterized as ki-67C, p53C and HER-2C and those from the histological sections as ki-67H, p53H and HER-2H.

The population was divided in 5 age groups: 36–45 years (group 1, N=7), 46–55 years (group 2, N=21), 56–65 years (group 3, N=49), 66–75 years (group 4, N=25) and ≥ 76 years (group 5, N=17). The ki-67, p53 and HER-2 expression among the different age groups in the histological and cytological specimens is summarized Tables 2-4, respectively.

Histology

Our cohort consisted of 21 cases with grade 1 ductal carcinoma, 81 cases with grade 2 ductal carcinoma, 13 cases with grade 3 ductal carcinoma and 4 cases with grade 2 lobular breast carcinoma. Twenty-two of the 119 (18.5%) cases had one or more positive lymph nodes after ipsilateral lymph node dissection. These cases were classified as follows: 15 patients had grade 2 ductal carcinoma, 4 patients had grade 3 ductal carcinoma and 3 patients had grade 3 lobular carcinoma. The number of samples showing positive staining for ki-67, p53 and HER-2 were 29 (24.4%), 16 (13.5%) and 23 (19.3%), respectively (Table 5).

TP cytology

The number of samples showing positive staining for ki-67, p53 and HER-2 were 29 (24.4%), 16 (14.3%) and 23 (18.5%), respectively. The immunocytochemistry results for ki-67, p53 and HER-2 are summarized in Table 5. The OA of the TP cytology for ki-67, p53 and HER-2 expression was 96, 99 and 97%, respectively. Sensitivity, specificity, PPV, NPV and OA of the TP cytology for ki-67, p53 and HER-2 are summarized in Table 6.

Correlation between histology and TP cytology

Spearman's correlation test showed a strong positive correlation between histology and TP cytology for ki-67 (correlation coefficient 0.875). There was a strong association between immunohistochemistry and immunocytochemistry results for p53 (Cramer's phi test, correlation coefficient 0.965). Finally, Kendall's tau test showed a strong association between histology and TP cytology for HER-2 (correlation coefficient 0.891).

Discussion

Few studies have investigated the correlation between biologic parameters determined immunocytochemically on preoperative FNAs and the same parameters determined immunohistochemically on corresponding surgical samples [3,20-24], To the best of our knowledge, there have been sporadic reports in the literature which deal with the correlation between the expression of ki-67, HER-2 and p53 on paraffin embedded tissues and on TP processed slides of breast carcinomas [25-29].

ki-67 expression and FNA cytology

Kuenen-Boumeester et al. [30] reported that a correlation between the growth fraction determined by ki-67 in FNA smears and cryostat sections of corresponding tumors was shown, implying that the immunostaining of cytological

smears gives a reliable impression of the growth fraction of a tumor.

Nizzoli et al. [20] reported a concordance of 70% between cytology and histology for ki-67. Moreover, they stated that while on cytologic smears ki-67 is assessed by counting the number of stained cells on a minimum of 500 tumor cells, on histologic specimens ki-67 is evaluated by scoring 10 high-power fields randomly selected from the whole area of the tumor.

Railo et al. [31] reported a series of 98 breast carcinoma patients in whom the ki-67 immunolabelling of FNA and needle-core (NC) biopsy was compared with that of the surgical specimen. According to their results, NC and FNA can be used for preoperative assessment of the proliferative activity and hormonal status in breast carcinoma.

p53 expression and FNA cytology

It is reported that there is good concordance in p53 mutation analysis in FNA samples and matching tissue samples from malignant tumors [32]. Furthermore, higher proportion of tumor cells carrying the p53 mutation are found in the FNAs than in matching tumor samples because malignant cells are known to be less cohesive than normal cells, and are therefore more likely to appear in the aspirates [32].

Ball et al. [33] reported that FNA yields adequate protein for Western blotting studies and could be used as a method to monitor p53 activity in vivo before and during anticancer treatment. Lavarino et al. [34] suggested that FNA may assist clinical decisions by allowing the evaluation of a variety of biological parameters (such as p53) relevant for prognosis and treatment planning.

HER-2 expression and FNA cytology

There are few studies evaluating HER-2 expression on FNA biopsy specimens and validating with data obtained on corresponding paraffin- embedded specimens of the same breast tumor tissue [20,24,35], In general, these studies have shown an overall concordance of more than 90% between HER-2 results obtained by immunocytochemical and immunohistochemical analysis respectively.

Sumiyoshi et al. [35] evaluated the expression of HER-2 in both FNA specimens and tissue sections from 58 cases of invasive breast cancer. They concluded that FNA specimens were suitable for detection of HER-2 overexpression and HER-2 gene amplification in invasive breast cancer.

Moriki et al. [36] demonstrated 100%

Table 2. ki-67 expression among the different age groups in the histological and cytological specimens

Age groups (years)	Immunohisto-chemistry		Immunocyto-chemistry	
	Positive	Nega-tive	Positive	Nega-tive
36-45 (N=7)	6	1	4	3
46-55 (N=21)	10	11	9	12
56-65 (N=49)	5	44	6	43
66-75 (N=25)	6	19	6	19
≥ 76 (N=17)	2	15	3	14
Total	119		119	

Table 3. p53 expression among the different age groups in the histological and cytological specimens

Age groups (years)	Immunohisto-chemistry		Immunocyto-chemistry	
	Posi-tive	Nega-tive	Positive	Nega-tive
36-45 (N=7)	3	4	3	4
46-55 (N=21)	1	20	1	20
56-65 (N=49)	6	43	7	42
66-75 (N=25)	3	22	3	22
≥ 76 (N=17)	3	14	3	14
Total	119		119	

Table 4. HER-2 expression among the different age groups in the histological and cytological specimens

Age groups (years)	Immunohisto-chemistry		Immunocyto-chemistry	
	Posi-tive	Nega-tive	Positive	Nega-tive
36-45 (N=7)	3	4	3	4
46-55 (N=21)	3	18	2	19
56-65 (N=49)	11	38	11	38
66-75 (N=25)	2	23	2	23
≥ 76 (N=17)	4	13	4	13
Total	119		119	

Table 5. Immunohistochemistry and immunocyto-chemistry results for ki-67, p53 and HER-2 expression

Factor	Immunohistochemistry		Immunocytochemistry	
	Positive N(%)	Negative N(%)	Positive N(%)	Negative N(%)
ki-67	29 (24.4)	90 (75.6)	28 (23.5)	91 (76.5)
p53	16 (13.5)	103 (86.5)	17 (14.3)	102 (85.7)
HER-2	23 (19.3)	96 (80.7)	22 (18.5)	97 (81.5)

Table 6. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of the ThinPrep cytology for ki-67, p53 and HER-2 expression

Factor	TP	TN	FP	FN	Sensitivity %	Specificity %	PPV %	NPV %	OA %
ki-67	26	88	2	3	90	98	93	97	96
p53	16	102	1	0	100	99	94	100	99
HER-2	21	95	1	2	91	99	95	98	97

TP: true positive, TN: true negative, FP: false positive, FN: false negative, PPV: positive predictive value, NPV: negative predictive value, OA: overall accurary

concordance between cytology and on the matched histologic sections for HER-2 expression. Nizzoli et al. [20] showed concordance of 84% between immunocytochemical and immunohistochemical analysis on corresponding tissue samples for HER-2 expression.

TP cytology

Gong et al. [36] compared immunocytochemistry results on both ThinPrep and cell-block slides prepared from the same effusion specimens. They suggested that the frequency and intensity of reaction of ki-67 expression on TP were significantly lower than with the cell-block preparation, particularly in malignant effusions.

No study to date has compared the expression ki-67 on TP processed slides of breast carcinomas with the immunohistochemical results obtained on the corresponding surgical samples. Our results were in disagreement with these of Gong et al. [36]. In the current series, Spearman's correlation test showed a strong positive correlation between immunohistochemistry and immunocytochemistry results for ki-67 expression (correlation coefficient 0.875) (Figure 1).

Pollett et al. [27] analyzed p53 mutations in TP-processed breast FNA stored in PreservCyt® for up to 5 years. They demonstrated that DNA suitable for analysis of p53 gene sequence changes could be successfully extracted from TP samples, and that identical alterations were detected in both the cytological and surgical specimens. Tisserand et al. [37] assessed the accuracy of molecular diagnosis techniques using FNA material stored in PreservCyt®. They suggested that TP-processed FNA samples are a viable source of material for various types of DNA, RNA, and protein analysis. In addition, it is feasible breast FNA stored in PreservCyt® to be used for building genetic material banks that will be invaluable for future studies.

Our results were in agreement with these studies [29,37]. In the current series, there was a strong association between immunohistochem-

istry and immunocytochemistry results for p53 (Cramer's phi test, correlation coefficient 0.965) (Figure 2).

Bedard et al. [28] suggested that HER-2 immunostaining with both the HercepTest and TAB250 on TP is unreliable due to low specificity. However, both antibodies have high sensitivity. Moreover, they suggested that positive HER-2 staining would require further validation by either differential polymerase chain reaction (dPCR) of fluorescence in situ hybridization (FISH).

Beatty et al. [23] evaluated HER-2 receptor status by immunocytochemical and immunohistochemical analyses and FISH. HER-2 expression on cytological preparations was insufficiently reliable for clinical use, whereas HER-2 gene amplification determined by FISH demonstrated strong and consistent correlation with HER-2 status on tissue samples.

Pegolo et al. [29] compared the immunocytochemical evaluation of HER-2 on TP- processed FNA with the immunohistochemical analysis performed on the corresponding formalin-fixed paraffin-embedded breast tumor specimens. They found high level of agreement for HER-2 expression on both TP cytology specimens and the corresponding tissue samples and concluded that the TP technique can be routinely used for the biological characterization of invasive breast cancers.

Our results were in disagreement with these studies [23,28,29], In the current series, Kendall's tau test showed a strong association between histology and TP cytology for HER-2 (correlation coefficient 0.891) (Figure 3).

The few studies evaluating ki-67, p53 and HER-2 expression on FNA smears using TP cytology have used various antibodies, different fixation procedures, and different criteria for determining overexpression, which make a direct comparison of results difficult.

Percentages of immunostained cells above and below, but very close to the cutoff point may also account for the disagreement. Based on our results, we conclude that ki-67, p53 and HER-2 expression can be evaluated in FNA mate-
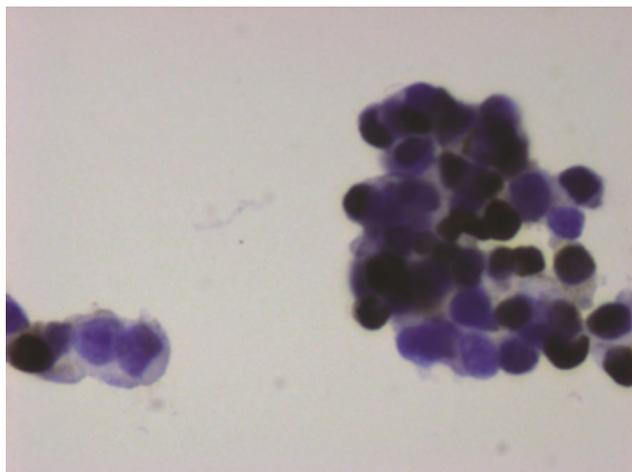
Figure 1. ki-67 expression in a ThinPrep slide (PAP×100).
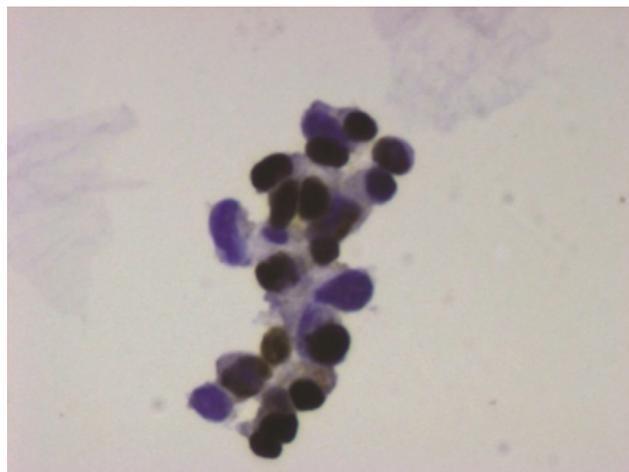
Figure 2. p53 expression in a ThinPrep slide (PAP×100).
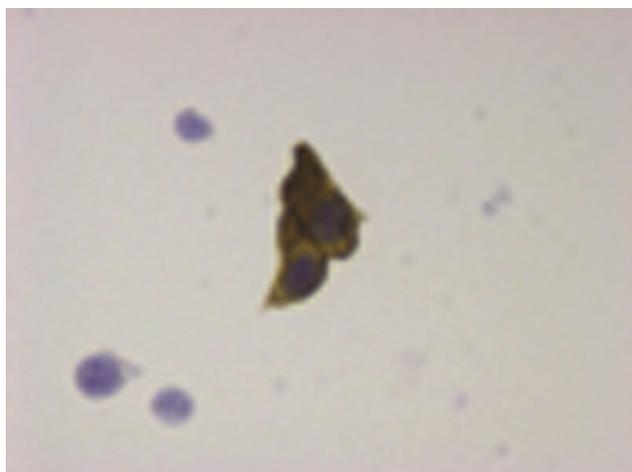
Figure 3. HER-2 expression in a ThinPrep slide (PAP×100).

rial from breast carcinomas by using the TP technique and by applying our tested immunostaining protocol.

In conclusion, this pilot study demonstrates that it is possible to monitor multiple molecular markers by using the TP cytology. Since our study was limited to 119 patients, further studies are required to determine the usefulness and the exact role of TP cytology in the preoperative management of patients with breast cancer.

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