Pol J Pathol 2003, 54, 2, 101-110 PL ISSN 1233-9687

Bożena Lackowska[1], Aleksander Niezabitowski[1], Janusz Ryś[1], Jan Skołyszewski[2], Andrzej Stelmach[3], Andrzej Gruchała[1], Agnieszka Jaszcz-Gruchała[1]

S-Phase Fraction and Menopausal Status as the Most Important Prognostic Factors of Disease-Free Survival for Node Negative Patients with Breast Cancer. A Prospective Study

[1]Department of Pathology,
[2]Department of Radiation Oncology,
[3]Department of Surgery, Oncology Center, Kraków

The aim of our study was to determine a prognostic value of DNA flow cytometry measurements performed on fresh breast cancer tissues, separately for patients' groups defined by nodal status, with special attention to histological type of tumor. Between 1993 and 1996 samples from 677 patients were analyzed and 457 cases were included in the survival analysis. Two-hundred and nine patients from them were node negative (N0). The median time of follow-up was 74 months. In multivariate analysis of disease-free survival (DFS), S-phase fraction (SPF) and menopausal status were found to be independent prognostic parameters for N0 group. A combination of this factors allowed us to distinguish three groups different in respect of the risk of recurrence. Our results showed that: 1. SPF and menopausal status could be prognostically valuable factors for DFS in N0 breast cancer patients; 2. prognostic value of SPF and ploidy should be evaluated separately for each histological type of breast cancer.

Introduction

Attempts for evaluation of the prognostic significance of DNA ploidy and S-phase fraction (SPF) in breast cancer have been undertaken for many years, but the results remain controversial. Some of the recently published studies suggest that these factors are not more significant than the classically used [3, 23]. In 1999, prognostic and predictive factors for breast cancer patients were stratified into three categories, according to their strength [10]. While a ploidy, defined by flow cytometry, was practically ruled out (category III), SPF was included among factors of category II (for extensive studies).

The objective of our study was to determine a prognostic value of DNA flow cytometry measurements performed on fresh breast cancer tissues, separately for patients' groups defined by nodal status, with special attention to histological type of tumors. Since the status of axillary lymph nodes is of special predictive importance among classic prognostic parameters, it should be used to verify all other potentially prognostic variables [14]. At present, special attention is paid to patients with negative nodes [2, 5, 18, 23, 29], and a majority of published results demonstrate a significant influence of SPF on disease-free [5, 7, 18, 25] and overall survival [5, 25]. Commonly, the cytometric data are referred to all histological types of breast cancer present in the series studied. However, some authors point out that incidence of aneuploidy, and consequently SPF level in particular types of breast carcinoma is significantly different [11].

Material and Methods

A total of 677 unselected breast cancer patients, consecutively operated in the Oncology Center in Kraków between January 1993 and December 1996 were analyzed for DNA content and S-phase fraction. Finally, 457 patients were included in the analysis. 62 women were excluded because of previous treatment for malignant diseases (34 - for breast cancer), and 57 because of the chemo- or radiotherapy administered before mastectomy. Among the remaining 558 patients, 69 were lost to follow-up (time of observation for disease-free interval was shorter than 40 months) and in 5 only simple mastectomy was performed. Twenty-seven patients were excluded because of uninterpretable DNA histogram. In all the remaining patients radical mastectomy was performed; adjuvant chemo- and/or radiotherapy was administered in all patients with positive axillary lymph nodes and only in 30/209(14.4%) patients without lymph node involvement. The median age of patients was 56 years (range 28 - 85 years). At the time of diagnosis 178 of them were pre- and 279 post-menopausal. Median diameter of tumor measured after surgery was 20mm (range 2 - 100mm). Metastases in axillary lymph nodes occurred in 248 cases

TABLE 1

Univariate analysis of disease-free and overall survival for 457 patients with breast cancer

		No of cases	Recurrences					Deaths				
			n	%	Log-rank	Cox		n	%	Log-rank	Cox	
					p	Relative risk	p			p	Relative risk	p
Age	≤40y	102	45	44.1				22	21.6			
	>40y	355	113	31.8	0.0315	0.68	0.0305	71	20.0	0.8702	0.96	0.8719
Tumor size	≤20mm	285	82	28.8				49	17.2			
	20-50mm	158	65	41.1	0.0071	1.57	0.0070	36	22.8	0.1536	1.37	0.1545
	>50mm	14	11	78.6	<0.00001	5.83	<0.00001	8	57.4	0.00003	6.72	<0.00001
SBR grade	GI	58	6	10.3				5	8.6			
	GII	189	69	36.5	0.0002	4.07	0.0010	36	19.1	0.0683	2.31	0.0804
	GIII	140	68	48.6	<0.00001	6.14	0.00002	43	30.7	0.0005	4.32	0.0020
Menopausal status	post-	279	81	29.0				51	12.8			
	pre-	178	77	43.3	0.0019	1.64	0.0019	42	23.6	0.2301	1.28	0.2324
SPF	≤4.4%	94	18	19.2				12	18.3			
	>4.4%	319	128	40.1	0.0006	2.28	0.0011	76	23.8	0.0380	1.88	0.0421
Ploidy[a]	D	144	35	24.3				17	11.8			
	A	313	123	39.3	0.0013	1.81	0.0020	76	24.3	0.0014	2.27	0.0022
Ploidy[b]	B	205	50	24.4				27	13.2			
	W	252	108	42.9	0.00005	1.98	0.00007	66	26.2	0.0007	2.13	0.0009
SPF+	≤4.4%	94	18	19.2				12	12.8			
menopausal	>4.4% post-	191	63	33.0	0.0260	1.79	0.0295	40	20.9	0.1169	1.67	0.1219
status	>4.4% pre-	128	65	50.8	0.00001	3.05	0.00003	36	28.1	0.0149	2.21	0.0178
Node	none	209	35	16.8				18	8.6			
involvement	1-3 N+	121	44	36.4	0.0003	2.24	0.0004	20	16.5	0.0489	1.88	0.1219
	>3 N+	128	79	62.2	<0.00001	5.23	<0.00001	55	43.3	<0.00001	6.46	<0.00001

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

TABLE 2

Multivariate Cox analysis of disease-free and overall survival for 457 patients with breast cancer

	Recurrences		Deaths	
	Relative risk	p	Relative risk	p
Tumor size	3.34	0.0004	5.12	0.00007
Menopausal status	1.63	0.0036		
SPF	1.71	0.0466		
Ploidy[a](B/W)	1.67	0.0070	1.69	0.0395
Node involvement	2.06	<0.00001	2.41	<0.00001

[a]1B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

(121 in 1 - 3 nodes and 127 in >3 nodes) and the median number of metastatic lymph nodes was 4 (range 1 - 23). Two-hundred and nine patients had no axillary metastases.

The median time of follow-up for survivors was 74 months (range 40 - 106 months). Relapses occurred in 158/457(34.6%) patients; 35/209(16.7%) node negative, 44/121(36.4%) 1 - 3 nodes involved and 79/127(62.2%) >3 nodes involved. Median recurrence-free survival was 24 months (range 1 - 95 months). Almost 90% of relapses occurred within first 5 years of observation. At the end of the observation 307 patients were alive without evidence of disease, and 54 patients with recurrent cancer. Ninety-four patients died of breast cancer and 2 of other causes (after 75 and 84 months).

Breast cancers were histologically classified according to the WHO recommendations [1]; infiltrating ductal carcinomas were graded according to Scarf-Bloom-Richardson (SBR) score [16].

DNA analysis was performed on the suspensions of the cell nuclei from fresh tissue specimens. After mincing with scissors, the tissue was disaggregated mechanically. Then, aliquots $1x10^6$ cells were incubated with the staining solution (PI-Calbiochem 50µg/ml, NP-40 and RNA-se A - Sigma 1mg/ml). Analysis was performed on FACScan Becton-Dickinson flow cytometer equipped with DDM and CellFit software. For each DNA histogram at least 10,000 particles were analyzed. The DNA histograms were classified according to principles adopted at DNA Cytometry Consensus Conference 1992 [24]. Interpretable DNA histograms were obtained in 457 cases and DI values were

TABLE 3

Univariate analysis of disease-free and overall survival for 209 node negative patients

		No of cases	Recurrences					Deaths				
			n	%	Log-rank	Cox		n	%	Log-rank	Cox	
					p	Relative risk	p			p	Relative risk	p
Age	≤40y	42	10	23.8				4	9.5			
	>40y	167	25	15.0	0.2083	0.63	0.2121	14	8.4	0.7343	0.82	0.7301
Tumor size	≤20mm	154	25	16.2				12	7.8			
	20-50mm	55	10	18.2	0.7063	1.15	0.7051	6	10.9	0.3998	1.55	0.3922
	>50mm	0										
SBR grade	GI	32	1	3.1				2	6.3			
	GII	83	18	21.7	0.0205	7.37	0.0518	6	7.2	0.9841	0.98	0.9837
	GIII	46	10	21.7	0.0207	7.66	0.0523	6	13.0	0.2125	2.76	0.2278
Menopausal status	post-	133	16	12.0				9	6.8			
	pre-	76	19	25.0	0.0204	2.17	0.0227	9	11.8	0.2495	1.71	0.2591
SPF	≤4.4%	54	1	1.9				1	1.9			
	>4.4%	133	32	24.1	0.0005	14.23	0.0089	16	12.0	0.0510	6.02	0.0819
Ploidy[a]	D	80	7	8.8				3	3.8			
	A	129	28	21.7	0.0112	2.77	0.0161	15	11.6	0.0205	3.98	0.0322
Ploidy[b]	B	107	10	9.4				6	5.6			
	W	102	25	24.5	0.0038	2.82	0.0056	12	11.8	0.0846	2.32	0.0949
SPF+	≤4.4%	54	1	1.9				1	1.9			
menopausal status	>4.4% post-	85	14	16.5	0.0075	9.47	0.0300	8	9.4	0.0846	5.04	0.1275
	>4.4% pre-	48	18	37.5	0.00001	22.44	0.00249	8	16.7	0.0304	7.49	0.0592

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

estimated in all of them. SPF could be evaluated in 413 cases. The median CV value for the whole group amounted to 5.0 (range 1.8 - 7.9).

Ploidy of tumor cells was expressed as diploid/aneuploid (D/A). Further, the optimal borderline values of DI (DNA index) were defined through iterative analysis using the experimentally established values; at the beginning, ploidy could be ordered in seven compartments: 1. DI=1.0 (diploid, 144 cases), 2. 1.0<DI≤1.3 (near-diploid, 32 cases), 3. 1.3<D≤1.6 (triploid, 58 cases), 4. 1.6<DI≤1.8 (hypertriploid, 110 cases), 5. 1.8<DI≤2.1 (tetraploid, 84 cases), 6. DI>2.1 (hypertetraploid, 22 cases), 7. more than one aneuploid stemline (multiploid, 7 cases). Then, a second division was constructed, after preliminary survival analyses performed separately for patients from each DI compartment. In result, two groups differing in prognosis were found: group B (DI≤1.3 or DI>2.1 and multiploid, better prognostically) and group W (1.3<DI≤2.1, worse prognostically).

Proliferative activity was expressed as percentage of cells in S-phase fraction (SPF). Optimal cut-off point, 4.4%, was established by iterative step by step analysis using the experimental values in reference to disease-free survival.

Disease-free and overall survival have been estimated by Kaplan-Meier approach. The differences between survival curves have been tested by log-rank and Cox tests. For multiple analysis Cox proportional hazard model has been estimated. The final list of variables has been selected by stepwise backward procedure, leaving only statistically significant factors in the final model. P-values not greater than 0.05 were considered to be statistically significant.

Results

Disease-free survival (DFS) analysis

The 5-year actuarial survival rate for the whole group of patients was 70%. Results of univariate analysis are presented in Table 1. Ploidy and SPF demonstrated significant but lower influence on survival than the classically used factors. In multivariate Cox analysis (Table 2) node involvement, tumor diameter, menopausal status, SPF and ploidy in B/W categories were found to be independent factors, but SPF and ploidy henceforth were less significant than the clinical and histopathological parameters.

After stratifying the material according to the axillary nodal status the 5-year survival rate amounted to 85%, 70% and 40% for patients with negative nodes (N0), one to three nodes involved (N1 - 3), and more than three nodes involved (N3), respectively. Relevant data of univariate analysis are shown in Tables 3, 4 and 5. Among node negative patients, SPF was the most significant factor influencing recurrence-

TABLE 4

Univariate analysis of disease-free and overall survival for 121 patients with 1 - 3 nodes involved

		No of cases	Recurrences					Deaths				
			n	%	Log-rank	Cox		n	%	Log-rank	Cox	
					p	Relative risk	p			p	Relative risk	p
Age	≤40y	29	13	44.8				6	20.7			
	>40y	92	31	33.7	0.2032	0.65	0.1891	14	15.2	0.5331	0.74	0.5373
Tumor size	≤20mm	67	20	29.9				9	13.4			
	20-50mm	46	17	37.0	0.4214	1.30	0.4231	7	15.2	0.7952	1.14	0.7956
	>50mm	8	7	87.5	0.0003	7.76	<0.00001	4	50.0	0.0029	8.02	0.0007
SBR grade	GI	20	2	10.0				2	10.0			
	GII	52	18	34.6	0.0528	3.74	0.0771	7	13.5	0.7761	1.26	0.7740
	GIII	38	20	52.6	0.0015	7.02	0.0087	10	26.3	0.1276	2.99	0.1573
Menopausal status	post-	71	22	31.0				10	14.1			
	pre-	50	22	44.0	0.0932	1.66	0.0925	10	20.0	0.4060	1.45	0.4078
SPF	≤4.4%	16	4	25.0				1	6.3			
	>4.4%	95	37	39.0	0.3823	1.57	0.3898	17	17.9	0.3355	2.66	0.3423
Ploidy[a]	D	38	15	39.5				8	21.1			
	A	83	29	34.9	0.5730	0.83	0.5697	12	14.5	0.3438	0.65	0.3442
Ploidy[b]	B	54	20	37.0				10	18.5			
	W	67	24	35.8	0.7903	0.92	0.7890	10	14.9	0.5419	0.76	0.5421
SPF+	≤4.4%	16	4	25.0				1	6.3			
menopausal status	>4.4% post-	56	18	32.1	0.7192	1.22	0.7168	8	14.3	0.4226	2.28	0.4366
	>4.4% pre-	39	19	48.7	0.1472	2.14	0.1703	9	23.1	0.2598	3.23	0.2686

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

free survival. For patients with one to three nodes involved, significance was demonstrated for diameter greater than 50mm and histological grade GIII. In the group with more than three nodes involved, only ploidy pattern was statistically significant. In multivariate analysis (Table 6), SPF and menopausal status were found to be independent prognostic parameters for N0 group, diameter and histological grade for N 1 - 3 and ploidy, expressed as B/W, for N>3.

Basing on the multivariate analysis performed among node negative patients, three groups could be distinguished in respect of the risk of recurrence. The low risk group consisted of the patients with tumor SPF lower than 4.4% and whatever menstrual status. The moderate risk group consisted of the post-menopausal patients with tumor SPF greater than 4.4%. And the high risk group consisted of the pre-menopausal women with tumor SPF greater than 4.4%. Recurrence-free survival for these three groups of patients is shown in figure 1.

Overall survival (OS) analysis

The 5-year actuarial survival rate for the whole group of patients was 83%. Results of univariate analysis are presented in Table 1. Node involvement, tumor diameter and ploidy in B/W categories were found to be independent factors in multivariate Cox analysis (Table 2). In the defined nodal groups, 5-year survival rate was 93%, 86% and 59% for N0, N1 - 3, and N>3, respectively. Relevant data of univariate analysis are shown in Tables 3, 4 and 5.

Results of multivariate analysis (Table 6) for node negative patients were a little different than for DFS. Ploidy in categories D/A was found to be the only significant factor for this group, but SPF lost its significance. For two node-positive groups of patients the same factors as in DFS analysis were found to be significant.

Histology and flow cytometry

Ploidy and median SPF values were analyzed for each histological group of breast cancer separately. Frequency of aneuploidy differed significantly between the groups of various histology. Almost all medullary carcinomas were aneuploid, whereas in lobular and mucinous carcinomas only in one third of tumors aneuploid population was found. This fact was reflected in median value of SPF, which was highest for medullary carcinoma. The relevant data are shown in Table 7. Distribution of histological types of breast cancer among defined nodal groups is shown in Table 8. It is worth noting that frequency of medullary carcinoma, rather low (4.6%) in our series of patients, standing a signi-

TABLE 5

Univariate analysis of disease-free and overall survival for 127 patients with more than three nodes involved

		No of cases	Recurrences					Deaths				
			n	%	Log-rank	Cox		n	%	Log-rank	Cox	
					p	Relative risk	p			p	Relative risk	p
Age	≤40y	31	22	71.0				12	38.7			
	>40y	96	57	59.4	0.5396	0.86	0.5476	43	44.8	0.2925	1.39	0.3160
Tumor size	≤20mm	64	37	57.8				28	43.8			
	20-50mm	57	38	66.7	0.3519	1.24	0.3571	23	40.4	0.8774	0.96	0.8781
	>50mm	6	4	66.7	0.3909	1.69	0.3232	4	66.7	0.1816	2.31	0.1234
SBR grade	GI	6	3	50.0				1	16.7			
	GII	54	33	61.1	0.3405	1.65	0.4054	23	42.6	0.0892	3.92	0.1822
	GIII	56	38	67.9	0.2571	1.81	0.3250	27	48.2	0.0862	4.09	0.1669
Menopausal status	post-	75	43	57.3				32	42.7			
	pre-	52	36	69.2	0.2527	1.14	0.2555	23	44.2	0.96361	1.01	0.9639
SPF	≤4.4%	24	13	54.2				10	41.7			
	>4.4%	91	59	64.8	0.4044	1.28	0.4198	43	47.3	0.5103	1.25	0.5241
Ploidy[a]	D	26	13	50.0				6	23.1			
	A	101	66	65.4	0.0561	1.70	0.0813	49	48.5	0.0139	2.62	0.0263
Ploidy[b]	B	44	20	45.5				11	25.0			
	W	83	59	71.1	0.0025	2.09	0.0045	44	53.0	0.0020	2.65	0.0039
SPF+	≤4.4%	24	13	54.2				10	41.7			
menopausal status	>4.4% post-	50	31	62.0	0.5707	1.20	0.5798	24	48.0	0.5068	1.28	0.5182
	>4.4% pre-	41	28	68.3	0.2742	1.45	0.2822	19	46.3	0.6875	1.17	0.6921

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

TABLE 6

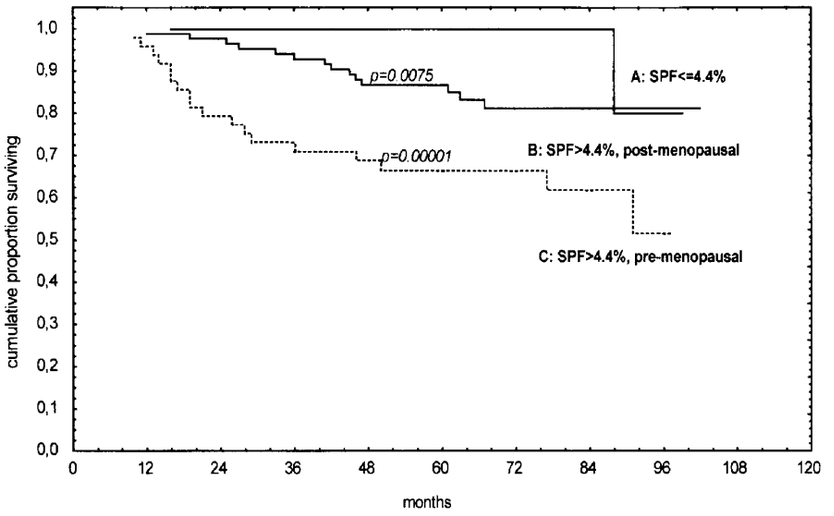
Multivariate Cox analysis of disease-free and overall survival according to nodal status of patients with breast cancer

	Recurrences						Deaths					
	Nodes involved: 0		Nodes involved: 1-3		Nodes involved: >3		Nodes involved: 0		Nodes involved: 1-3		Nodes involved: >3	
	Relative risk	P	Relative risk	P	Relative risk	P	Relative risk	P	Relative risk	P	Relative risk	P
Tumor size			7.11	0.00002					6.34	0.0049		
SBR grade			2.37	0.0072					2.50	0.0477		
Menopausal status	2.40	0.0122										
SPF	14.54	0.0084										
Ploidy[a] (D/A)							3.98	0.0322				
Ploidy[b] (B/)W					2.09	0.0045					2.65	0.0039

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

ficant part (7.7%) of tumors without axillary metastases (Table 8) and growing up to 14.6% in the group of node negative, premenopausal women with highly proliferating tumours (SPF>4.4%, Table 9). It is clear, that this fact would be reflected in survival analysis. Then, we decided to repeat the survival analysis for node negative patients on the biggest group of infiltrating ductal carcinoma, excluding the other histological types of tumors.

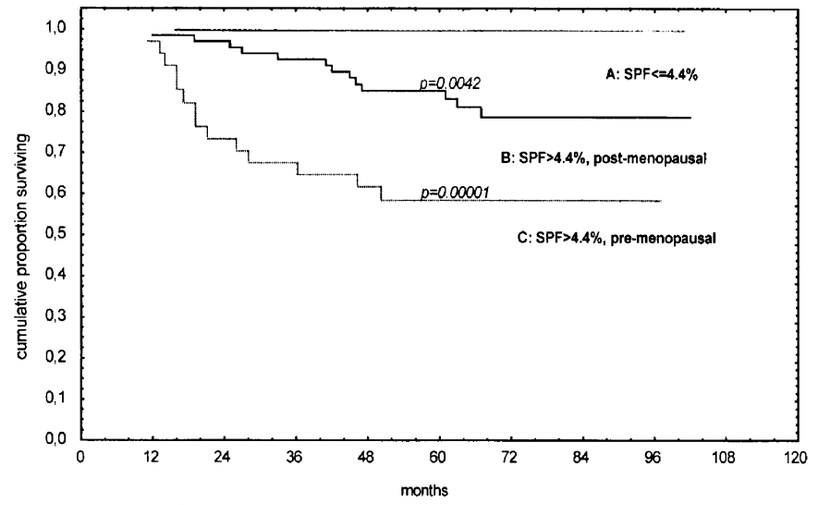
In univariate analysis (Table 10) histological grade, pre-menopausal status, S-phase fraction greater than 4.4% and DI in region 1.3 - 2.1 were found to be the most significant risk factors. None of the patients with SPF lower than 4.4% had any recurrence. For this reason it was impossible to perform Cox regression analysis and to establish a relative risk for S-phase fraction among node negative patients.



Number of patients

A:	54	54	54	54	51	35	24	9	2
B:	85	85	84	81	74	54	34	18	6
C:	48	48	47	44	44	31	22	14	4

Fig. 1. Disease-free survival for 187 patients with node negative breast cancer according to three defined risk groups, without consideration of histological type of the tumor.



Number of patients

A:	39	39	39	39	38	24	18	6	1
B:	69	69	68	65	60	46	29	14	5
C:	34	34	33	31	31	24	17	10	4

Fig. 2. Disease-free survival for 142 patients with node negative ductal infiltrating carcinoma according to three defined risk groups, with exclusion of other histological tumor types.

As could be expected, a prognostic power of combined SPF and menopausal status was raised for groups with SPF>4.4%. (Fig. 2).

Discussion

DNA content and S-phase fraction are auxiliary prognostic parameters, therefore their clinical value should be considered along with the classical prognostic parameters.

Histology

Infiltrating ductal carcinoma is the most frequent type of breast cancer, but the other histological types are also observed, in various number of cases. Survival analyses are usually performed for all patients included in a study. In this paper we showed that distribution of aneuploidy, and also SPF (usually greater in aneuploid than in diploid cases), was different in various histological types of breast cancer. Me-

TABLE 7

Frequency of aneuploid cases and median SPF according to histological types of breast cancer

Histological type	No of cases	Aneuploidy		SPF		
		n	%	n	median %	range
Infiltrating ductal carcinoma	383	272	71.0	345	8.7	0.4-45.3
Intraductal carcinoma	14	9	64.3	13	6.9	2.6-19.2
Lobular carcinoma	22	7	31.8	20	4.0	1.9-41.4
Mucinous carcinoma	9	3	33.3	8	3.7	1.3-22.8
Medullary carcinoma	21	20	95.2	19	15.3	1.6-37.4
Others[a]	8	2	25.0	8	6.7	1.1-14.2
Total	457	313	68.5	413	8.3	0.4-45.3

[a]papillary carcinoma - 2, carcinoma in fibroadenoma - 1, Paget carcinoma - 1, signet ring carcinoma - 1, carcinoma with metaplasia - 2, tubular carcinoma - 1

TABLE 8

Distribution of histological types of breast cancer according to nodal status

Histology	All cases		Nodes involved: 0		Nodes involved: 1-3		Nodes involved: >3	
	n	%	n	%	n	%	n	%
Infiltrating ductal carcinoma	383	83.8	159	76.1	110	90.9	114	89.8
Intraductal carcinoma	14	3.1	10	4.8	2	1.7	2	1.6
Lobular carcinoma	22	4.8	10	4.8	7	5.7	5	3.8
Mucinous carcinoma	9	2.0	7	3.3	0	0	2	1.6
Medullary carcinoma	21	4.6	16	7.7	2	1.7	3	2.4
Others[a]	8	1.7	7	3.3	0	0	1	0.8
Total	457	100.0	209	100.0	121	100.0	127	100.0

[a]papillary carcinoma - 2, carcinoma in fibroadenoma - 1, Paget carcinoma - 1, signet ring carcinoma - 1, carcinoma with metaplasia - 2, tubular carcinoma - 1

TABLE 9

Distribution of histological types of breast cancer between three risk groups of node negative patients defined by SPF and menopausal status. SPF could be determined for 187/209 cases

Histology	SPF≤4.4%		SPF>4.4%, post-menopausal		SPF>4.4%. pre-menopausal	
	n	%	n	%	n	%
Infiltrating ductal carcinoma	39	72.2	69	81.1	34	70.8
Intraductal carcinoma	2	3.7	3	3.5	4	8.3
Lobular carcinoma	7	12.9	2	2.4	0	0
Mucinous carcinoma	3	5.6	2	2.4	1	2.1
Medullary carcinoma	0	0	7	8.2	7	14.6
Others[a]	6	5.6	2	2.4	2	4.2
Total	54	100.0	85	100.0	48	100.0

[a]papillary carcinoma - 2, carcinoma in fibroadenoma - 1, Paget carcinoma - 1, signet ring carcinoma - 1, carcinoma with metaplasia - 2, tubular carcinoma - 1

dullary carcinoma is an example of breast cancer with almost 100% of aneuploid cases, extremely high SPF, signs considered as rather unfavourable, and relatively good prognosis [4]. Medullary carcinoma constituted only a small percentage of breast cancer cases in large studied groups, but its frequency becomes significant in node negative patients. The specific flow cytometric marks of this type of breast cancer could influence final conclusions. Significant correlation between histology and ploidy was found also in the other studies [21, 26]. Then, results of analysis trying to establish prognostic value of ploidy and SPF in mixed (from histological point of view) groups would depend on percentage of specific histological types.

TABLE 10

Univariate analysis of disease-free and overall survival for 159 node negative patients with ductal infiltrating carcinoma. SPF could be determined for 142/159 cases

		No of cases	Recurrences					Deaths				
			n	%	Log-rank	Cox		n	%	Log-rank	Cox	
					p	Relative risk	p			p	Relative risk	p
Age	≤40y	30	7	23.3				2	6.7			
	>40y	129	21	16.3	0.3588	0.67	0.3565	11	8.5	0.8340	1.2	0.8311
Tumor size	≤20mm	125	21	16.8				10	8.0			
	20-50mm	34	7	20.6	0.5733	1.28	0.5696	3	8.8	0.7131	1.3	0.7024
	>50mm	0										
SBR grade	GI	31	1	3.2				2	6.5			
	GII	83	18	21.7	0.02389	7.10	0.0563	6	7.2	0.9175	0.9	0.9146
	GIII	45	9	20.0	0.03418	6.75	0.0700	5	11.1	0.3406	2.2	0.3526
Menopausal status	post-	104	14	13.5				7	6.7			
	pre-	55	14	25.5	0.04769	2.10	0.0492	6	10.9	0.4222	1.6	0.4355
SPF	≤4.4%	39	0	0				0	0			
	>4.4%	103	27	26.2	0.0004			13	12.6	0.0632		
Ploidy[a]	D	57	6	10.5				2	3.5			
	A	102	22	21.6	0.0844	2.16	0.0950	11	10.8	0.0370	4.4	0.0600
Ploidy[b]	B	79	8	10.1				4	5.1			
	W	80	20	25.0	0.0177	2.60	0.0225	9	11.3	0.1088	2.5	0.1249
SPF+	≤4.4%	39	0	0				0	0			
menopausal status	>4.4% post-	69	13	18.8	0.0042			7	10.1	0.0696		
	>4.4% pre-	34	14	41.2	0.00001			6	17.7	0.0455		

[a]D-diploid, A-aneuploid; [b]B-DI≤1.3 or DI>2.1 and multiploid, W-1.3<DI≤2.1

Ploidy

Prognostic value of ploidy remains controversial. Most of studies rule out ploidy as an independent prognostic indicator [3, 12, 15], however some data confirm its predictive role [6, 9, 21]. The differences in patients' survival were more distinct when breast cancers were evaluated according to various ploidy classes [2, 9, 17]. Diploidy was postulated to be associated with longer disease-free survival [6, 8, 13]. Hypodiploidy and hypertetraploidy of breast cancers were regarded as less favourable [2, 9, 13, 25]. Other studies revealed that "medium aneuploidy" (1.50<DI<1.85) is correlated with a shorter relapse-free survival [17].

In this series of ductal infiltrating carcinoma, ploidy considered only in categories diploid (DI=1.0)/aneuploid (DI≠1.0) had significant influence on overall survival only among node negative patients. However, it could be demonstrated, that there exist two sets of DI values related to prognosis: 1. DI≤1.3 or DI>2.1 and multiploid - more favourable, and 2. 1.3<DI≤2.1 - less favourable. Ploidy influenced independently disease-free and overall survival in patients with more than three lymph nodes involved, what is in keeping with the other studies [21, 23].

DI for tumors determined as prognostically worse was in range 1.3 - 2.1 ("medium aneuploidy" and tetraploidy regions). We did not find that the patients with hypertetraploid tumors had worse prognosis.

Proliferative activity

Proliferative activity, when analyzed by flow cytometry, is expressed mainly as the percentage of S-phase fraction (SPF). Many studies demonstrated correlation of tumor diameter, grade and nodal status with SPF [5, 19, 27]. Indicators of proliferative activity are also good predictors of clinical outcome in breast carcinomas; recurrence-free and overall survival or both were associated with the level of proliferation expressed in different ways (as mitosis count, TLI, Ki-67 expression etc.) not only as SPF [5, 6, 15, 26].

In this study SPF was the strongest predictor of relapse for node negative patients, what is in agreement with the findings of others [5, 7, 18, 25]. However, in some studies with long term observations, a prognostic value of SPF for patients without nodal involvement is questioned [22, 23]. Possible cause of failure could be the adoption of mean or median value of SPF as cut-off point [23]. It seems that seeking the optimal cut-off point is the only proper way for

establishing a suitable, prognostically valuable level of SPF. On the other hand, in samples measured for SPF estimation not only cancer cells are present. Thus, gating a subpopulation of cells with cytokeratin expression is suggested as more adequate [22]. However, some observations indicated [28], that in the majority of the most undifferentiated tumors (grade GIII) only a part of cancer cells express cytokeratin. Then, the SPF measured only on cytokeratin-gated population will not be a "real SPF" and it is not surprising that SPF does not correspond with survival.

In our study, the risk of relapse for node negative patients with slowly proliferating tumors was minimal. It seems, that a necessity of adjuvant therapy for this woman is disputable. Among patients with higher proliferating tumors, additional information about menstrual status allowed to stratify this group into two prognostically different subgroups. Both these features taken together characterize the patients with the highest risk of relapses (pre-menopausal with high SPF).

The present study was performed on unselected, consecutive breast cancer patients. The results obtained confirm the possible clinical utility of flow cytometrically determined SPF for node negative, but not node positive patients with breast cancer. Breast cancer is not a homogenous disease, and the histological type of cancer should be taken into consideration before survival analysis.

References

1. *Azzopardi JG, Chepick OF, Hartmann WM:* Histological Typing of Breast Tumors. 2nd ed. Geneva, Switzerland, World Health Organization 1981.
2. *Balslev I, Christensen IJ, Rasmussen BB, Larsen JK, Lykkesfeldt AE, Thorpe SM, Rose C, Briand P, Mouridsen HT:* Flow cytometric DNA ploidy defines patients with poor prognosis in node-negative breast cancer. Int J Cancer 1994, 56, 16-25.
3. *Bergers E, Baak JPA, van Diest PJ, Willig AJP, Los J, Peterse JE, Ruitenberg HM, Schapers RFM, Somsen JG, van Beek MWPM, Bellot SM, Fijnheer J, van Gorp LHM:* Prognostic value of DNA ploidy using flow cytometry in 1301 breast cancer patients: results of the prospective multicenter morphometric mammary carcinoma project. Mod Pathol 1997, 10, 762-768.
4. *Bloom HG, Richardson WW, Field JR:* Host resistance and survival in carcinoma of the breast: A study of 104 cases of medullary carcinoma in a series of 1411 cases of breast cancer for 20 years. Br Med J 1970, 3, 181-188.
5. *Bryant J, Fisher B, Gunduz N, Constantino JP, Emir B*: S-phase fraction combined with other patient and tumor characteristics for the prognosis of node-negative, estrogen-receptor-positive breast cancer. Breast Cancer Res Treat 1998, 51, 239-253.
6. *Camplejohn RS, Ash CM, Gillett CE, Raikundalia B, Barnes DM, Gregory WM, Richards MA, Millis RR:* The prognostic significance of DNA flow cytometry in breast cancer: results from 881 patients treated in a single centre. Br J Cancer 1995, 71, 140-145.
7. *Chassevent A, Jourdan M-L, Romain S, Descotes F, Colonna M, Martin P-M, Bolla M, Spyratos F:* S-phase fraction and DNA ploidy in 633 T1T2 breast cancers: a standardized flow cytometric study. Clin Cancer Res 2001, 7, 909-917.
8. *Ewers SB, Attewell R, Baldertorp B, Borg A, Langstrom E, Killander D:* Prognostic potential of flow cytometry S-phase and ploidy prospectively determinated in primary breast carcinomas. Breast Cancer Res Treat 1992, 20, 93-108.
9. *Fernö M, Baldetorp B, Borg A, Olsson H, Sigurdsson H, Killander D:* Flow cytometric DNA index and S-phase fraction in breast cancer in relation to other prognostic variables and to clinical outcome. Acta Oncol 1992, 31, 157-165.
10. *Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connoly JL, Hayes DF, Edge SB, Lichter A, Schnitt SJ:* Prognostic factors in breast cancer. College of American Pathologist consensus statement 1999. Arch Pathol Lab Med 2000, 124, 966-978.
11. *Frierson HF:* Ploidy analysis and S-phase fraction determination by flow cytometry of invasive adenocarcinomas of the breast. Am J Surg Pathol 1991, 15, 358-367.
12. *Hedley DW:* DNA flow cytometry and breast cancer. Breast Cancer Res Treat 1993, 28, 51-53.
13. *Hedley DW, Clark GM, Cornelisse CJ, Killander D, Kute T, Merkel D:* Consensus review of the clinical utility of DNA cytometry in carcinoma of the breast. Breast Cancer Res Treat 1993, 28, 55-59.
14. *Korzeniowski S, Dyba T, Skolyszewski J:* Classical prognostic factors for survival and loco-regional control in breast cancer patients treated with radical mastectomy alone. Acta Oncol 1994, 33, 759-765.
15. *Kute TE, Quadri Y, Muss H, Zbieranski N, Cirrincione C, Berry DA, Barcos M, Thor AP, Liu E, Koerner F, Henderson IC:* Flow cytometry in node-positive breast cancer: cancer and leukemia group B protocol 8869. Cytometry 1995, 22, 297-306.
16. *Le Doussal V, Tubiana-Hulin M, Friedman S:* Prognostic value of histologic grade nuclear component of Scarf-Bloom-Richardson. Cancer 1989, 64, 1914-1921.
17. *Lykkesfeldt AE, Balslev I, Christensen IJ, Larsen JK, Molgaard H, Rasmussen BB, Thorpe S, Rose C:* DNA ploidy and S-phase fraction in primary breast carcinomas in relation to prognostic factors and survival for premenopausal patients in high risk for recurrent disease. Acta Oncol 1988, 27, 749-756.
18. *Malmstrm P, Bendahl P-O, Boiesen P, Brünner N, Idvall I, Fernö M:* S-phase fraction and urokinase plasminogen activator are better markers for distant recurrences than Nottingham Prognostic Index and histologic grade in a prospective study of premenopausal lymph node-negative breast cancer. J Clin Oncol 2001, 19, 2010-2019.
19. *O'Reilly SM, Camplejohn RS, Barnes DM, Millis RR, Allen D, Rubens RD, Richards MA:* DNA index, S-phase fraction, histological grade and prognosis in breast cancer. Br J Cancer 1990, 61, 671-674.
20. *Pinto AE, Andre S, Nogueira M, Mendonca E, Soares J:* Flow cytometric DNA hypertetraploidy is associated with unfavourable prognostic features in breast cancer. J Clin Pathol 1997, 50, 591-595.
21. *Pinto AE, Andre S, Soares J:* Short-term significance of DNA ploidy and cell proliferation in breast carcinoma: a multivariate analysis of prognostic markers in a series of 308 patients. J Clin Pathol 1999, 52, 604-611.
22. *Prasad AR, Divine G, Zarbo RJ:* Two-color, cytokeratine-labeled DNA flow cytometric analysis of 332 breast cancers. Lack of prognostic value with 12-year follow-up. Arch Pathol Lab Med 2001, 125, 364-374.
23. *Reed DN, Johnson J, Richard P, McCormick S, Shannon N, Mikhail RA, Osuch J, Cerrito PB, McMasters KM:* DNA flow cytometry does not predict 5- or 10-year reccurence rate for T1-2 node-negative breast cancer. Arch Surg 2000, 135, 1422-1426.
24. *Shankey TW, Rabinovitch PS, Bagwell B:* Guidelines for implementation of clinical DNA cytometry. Cytometry 1993, 14, 472-477.

25. *Stål O, Dufmats M, Hatschek T, Carstensen J, Klintenberg C, Rutqvist LE, Skoog L, Sullivan S, Wingren S, Nordenskjold B:* S-phase fraction is a prognostic factor in stage I breast carcinoma. J Clin Oncol 1993, 11, 1717-1722.
26. *Toikkanen S, Joensuu H, Klemi P:* The prognostic significance of nuclear DNA content in invasive breast cancer -a study with long-term follow-up. Br J Cancer 1989, 60, 693-700.
27. *Visscher DW, Wykes S, Kubus J, Crissman JD:* Comparison of PCNA/cyclin immunohistochemistry with flow cytometric S-phase fraction in breast cancer. Breast Cancer Res Treat 1992, 22, 111-118.
28. *Visscher DW, Wykes S, Zarbo RJ, Crissman JD:* Multiparametric evaluation of flow cytometric synthesis phase fraction determination in dual-labelled breast carcinomas. Anal Quant Cytol Histol 1991, 13, 246-252.
29. *Wyss-Desserich MT, Caduff-Joos R, Wyss P, Rageth C, Wight E, Unger C, Walt H, Haller U:* Premenopausal node-negative breast cancer: may adjuvant chemotherapy be indicated by the analysis of nuclear DNA dynamics? Breast Cancer Res Treat 1997, 42, 253-263.

Address for correspondence and reprint requests to:
B. Lackowska M.D., Ph.D.
Department of Pathology, Oncology Center
Garncarska 11, 31-115 Kraków
Fax: (48) (12) 421 20 98
Phone: (48) (12) 423 16 01
e-mail: z5niezab@cyf-kr.edu.pl