

ORIGINAL PAPER

HIGH KI-67 EXPRESSION IS A MARKER OF POOR SURVIVAL IN APOCRINE BREAST CARCINOMA

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Apocrine carcinoma is a very rare type of breast cancer, which represents 0.5-4% of all breast cancers. The aim of the study was to analyze biological and clinical features of apocrine carcinoma and their influence on patients survival.

The studied group consists of 57 patients, who underwent treatment between 1987 and 2010. Expression of ER, PgR, HER2, AR, GCDFP-15, EGFR, CK 5/6, CK 8/18 and Ki-67 was assessed immunohistochemically on formalin-fixed paraffin-embedded tissue sections. Presence of emboli and extent of lymphocyte infiltration were assessed on haematoxylin-eosin-stained slides.

In the investigated group, 16 cases were ER/PgR positive and 49 were AR-positive. ER/PgR-negative tumours were often characterised by CK5/6 and EGFR positivity. The presence of AR was related to HER-2 and GCDFP-15 expression and tumours with expression of CK5/6 were more likely be EGFR positive and had higher Ki-67 LI. Higher probability of 10-years OS and DFS was observed in patients with tumours characterized by Ki-67 LI < 20% ($p = 0.036$ and $p = 0.009$, respectively). Favourable trend in OS was noted for patients with smaller tumours ($p = 0.053$), without lymph node metastases ($p = 0.074$) and without EGFR expression ($p = 0.060$).

In apocrine breast carcinoma expression of Ki-67 is one of the most important factors influencing patients' survival.

Key words: apocrine breast carcinoma, Ki-67 expression, AR, EGFR.

Introduction

Apocrine carcinoma is rare type of breast cancer, which represents 0.5 to 4% of all breast cancers [1, 2, 3] with evident apocrine differentiation in more than 90% of cancer cells [2]. Microscopically, apocrine tumour cells are distinguishable as large cells with abundant acidophilic, granular cytoplasm and nuclei with prominent nucleoli [2, 3].

It is reported that apocrine carcinomas are usually androgen receptor (AR)-positive and oestrogen/

progesterone (ER/PgR)-negative tumours [5]. However, this phenotype (AR+/ER-/PgR-), which is very often observed in intraductal apocrine carcinoma is more variable in invasive carcinomas [3, 4]. Farmer *et al.* on the basis of microarray data distinguished molecular apocrine group, defined as AR+/ER- [6]. Based on morphological features, another term was introduced: pure apocrine carcinoma [7]; however, some authors define this category as tumours characterised by apocrine differentiation with AR+/ER-/PgR- immunohistochemical (IHC) profile [8, 9].

These two definitions are overlapping because some authors use immunochemistry phenotype AR+/ER-/PgR- to define molecular apocrine breast carcinoma [10, 11].

The clinicopathological and biological characteristics of apocrine carcinoma are intensively studied; however, comparing data might be a problem because some authors investigate differently characterized groups: apocrine carcinoma/pure apocrine carcinoma (based on only morphological feature) [4,

8, 12, 13, 14, 15, 16, 17, 18] or with additional AR+/ER-/PgR- IHC profile [8, 9, 19], molecular apocrine [10, 20, 21, 22, 23, 24], or the subgroup of apocrine carcinomas in triple-negative breast carcinoma subtype [25]. The expression of a number of biological markers have been investigated, mainly: AR, ER, and PgR [4, 8, 9, 11, 13, 16, 17, 18, 19, 20, 24, 25] as well as epidermal growth factor receptor 1 and/or 2 (EGFR, HER2) [8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 22, 23, 25], proliferation rate based on assessment of Ki-67 expression [8, 10, 11, 16, 18, 19, 20, 25], gross cystic disease fluid protein-15 (GCDFP-15) [8, 13, 18, 21, 23], p53 [10, 11, 13, 16, 18, 19], CK5/6 [18, 20, 22] and bcl2 [4, 16]. In intraductal apocrine carcinoma and in apocrine invasive carcinoma gains of 1p, 1q, and 2q and losses of 1p, 17q, 22q, 16q, and 12q were detected [26].

Some authors noted the same prognosis for invasive apocrine carcinoma and invasive ductal carcinoma [17] and others did not observe differences between molecular apocrine and others subtypes of breast carcinoma [20]. However another study stated that molecular apocrine breast carcinoma (MABC) subgroup developed distant metastasis earlier than nonMABC subgroup, and patients with MABC had poorer prognosis [11]. Therefore the search for markers useful for treatment selection is still important.

Herein we analysed expression of ER, PgR, HER2, AR, GCDFP-15, EGFR, CK5/6, CK8/18 and Ki-67 in 57 patients with apocrine breast carcinoma defined using morphological classification. Relationships between the parameters listed above and their influence on patients' survival were investigated.

Material and methods

Patients

The studied group consist of 57 patients with apocrine breast carcinoma, who underwent treatment between 1987 and 2010 at Maria Skłodowska-Curie Memorial Institute – Oncology Center, Cracow Branch. Those patients accounted for 0.5% of all breast carcinoma patients treated during that period. Apocrine carcinoma was defined according to the new WHO classification of breast tumours revised and updated in 2012. The age of patients ranged from 34 to 83 years with a mean value of 58.1 ± 12.0 (SD) years. All patients underwent radical surgery. Radiotherapy, chemotherapy and hormonotherapy were applied after surgery, according to recommendations valid at that time. Clinical and histological characteristics of the patients are presented in Table I.

The study was approved by the Ethical Committee at the Regional Medical Chamber in Krakow (decision from 17th April 2014).

Table I. Clinicopathological characteristics of patients with apocrine breast carcinoma (n = 57)

PARAMETER/CATEGORY	N (%)
pT	
pT1	29 (50.9)
pT2	24 (42.1)
pT3	2 (3.5)
pT4	1 (1.8)
pT unknown	1 (1.8)
pN	
pN0	27 (47.4)
pN1	16 (28.1)
pN2	7 (12.3)
pN3	6 (10.5)
pN unknown	1 (1.8)
Grade	
<i>In situ</i> with microinvasion	3 (5.3)
2	24 (42.1)
3	28 (49.1)
unknown	2 (3.5)
Surgery	
Mastectomy	47 (82.5)
Halsted	2
Patey	36
Madden	9
BCS	10 (17.5)
Hormonal therapy	
Not administered	26 (45.6)
Administered	31 (54.4)
Radiotherapy	
Not administered	38 (66.7)
Administered	19 (33.3)
Chemotherapy	
Not administered	26 (45.6)
Administered	31 (54.4)

BCS – breast conserving surgery

This study was conducted on archived tissues, with no direct patient contact, no modification of diagnostic or treatment procedures, and full confidentiality of patients' personal data. Formal consent is not required for this type of study.

Methods

The study was performed using archival formalin-fixed paraffin-embedded (FFPE) tissues. Tumour specimens were reexamined by a pathologist (J.W.) to confirm histological evaluation and to assess tumour grade. Apocrine carcinoma was defined according to morphological features listed in the 2012 WHO classification. The presence of emboli and extent of lymphocytic infiltration (TILs) were assessed on hematoxylin-eosin-stained (HE) slides. The magnitude of infiltration was assessed using International TILs Working Group recommendation [27] with the authors' modification: tumours were stratified into two groups: low (0-10% of stromal TILs) and high (> 10% of stromal TILs).

Immunohistochemistry staining

Expression of investigated proteins was assessed immunohistochemically. The primary antibodies used were as follows: AR (SP107, 1:100, Cell Marque), CK5/6 (D5/16 B4, 1:50, DakoCytomation, Agilent Technologies, Inc), CK5 (XM26, 1:80, Thermo Fisher Scientific), CK8/18 (NCL-5D3, 1:200, Leica Biosystems), EGFR (H11, 1:200, DakoCytomation, Agilent Technologies, Inc), ER (SP1, 1:250, Thermo Fisher Scientific), PR (SP2, 1:100, Thermo Fisher Scientific), Ki-67 (MIB, 1:100, DakoCytomation, Agilent Technologies, Inc), GCDFP (NCL-GCDFP15, 1:20, Leica Biosystems), and HER2 (HercepTest, DakoCytomation, Agilent Technologies, Inc). The sections from formalin-fixed paraffin-embedded tissues (4 μ m) were mounted on SuperFrost Plus (Menzel-Gläser, Germany) slides. Proteinase K was used to unmask epitope in the case of EGFR and CK8/18 proteins, for AR, CK5/6 and CK5 incubation with TRS pH6 (50 min, 96°C, DakoCytomation) and for the rest heating in citrate buffer pH6 in microwave oven (2 \times 600 W) was applied. After incubation with primary antibody, for protein visualization, detection system BrighVision Immunologic or Ultra-vision (Thermo Fisher Scientific) and DAB (Vector Laboratories, Inc., Burlingame, USA) were used. Hematoxylin was applied for nuclear counterstaining.

Due to insufficient amount of tissue in paraffin blocks or small fragments of tumour tissue, which has precluded obtaining reliable results, whole panel of IHC staining was not available for some cases.

IHC evaluation

Tumours were considered as ER or PR (Fig. 1A, B) immunopositive if nuclear staining was observed in more than 1% of tumour cells. Overexpression of HER2 (Fig. 1C) was assessed using HercepTest (Dako Denmark A/S, Glostrup Denmark) and evaluated according to recommended standards [28]. In case of: AR, GCDFP-15, EGFR, CK 5/6 and CK 8/18 expression (Fig. 1D-H), tumours were classified as positive if staining was detected in more than 10% of tumours cell. In most cases strong expression of above mentioned proteins was observed in > 50% cells. Finally, Ki-67 labeling index (Ki-67 LI) was calculated as the percentage of tumour cells with Ki-67 nuclear staining (Fig. 1I).

Statistical analysis

For continuous variables descriptive statistics were used to determine mean values and standard deviation of means (SD). The Mann-Whitney test was applied for evaluation of differences between two groups. Relationships between categorical variables were analysed using Pearson χ^2 test, or in the case of 2 \times 2 tables two-sided Fisher exact test for independence was used in order to avoid problems with small samples.

Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and the log-rank test was used to evaluate the statistical significance of the differences observed between two groups. A Cox proportional hazard model was applied to test independence of variables. In all statistical procedures, p-value below 0.05 were considered significant. The statistical analysis was carried out using STATISTICA v.13 software (TIBCO Software Inc., Palo Alto, CA, USA).

Results

Relationships between molecular and clinical parameters

Emboli were observed in 19 tumours (34.5%) and high lymphocyte infiltration in 15 tumours (28.8%). Almost all cases were CK8/18, GCDFP-15 and AR positive (96.2%, 94.4%, and 90.7%, respectively). Expression of EGFR, HER2 and CK5/6 was noted in approximately half of the tumours (50.0%, 45.5%, and 48.1%, respectively). About one third of tumours were ER or PgR positive (29.1%).

Statistical analysis revealed that the presence of smaller tumours (pT1) was related to less frequent lymph node involvement (p = 0.001, pN0 vs. pN+) and more frequent presence of G2 tumours (p = 0.012, G2 vs. G3). Absence of emboli was more frequent in tumours without lymph node metastasis

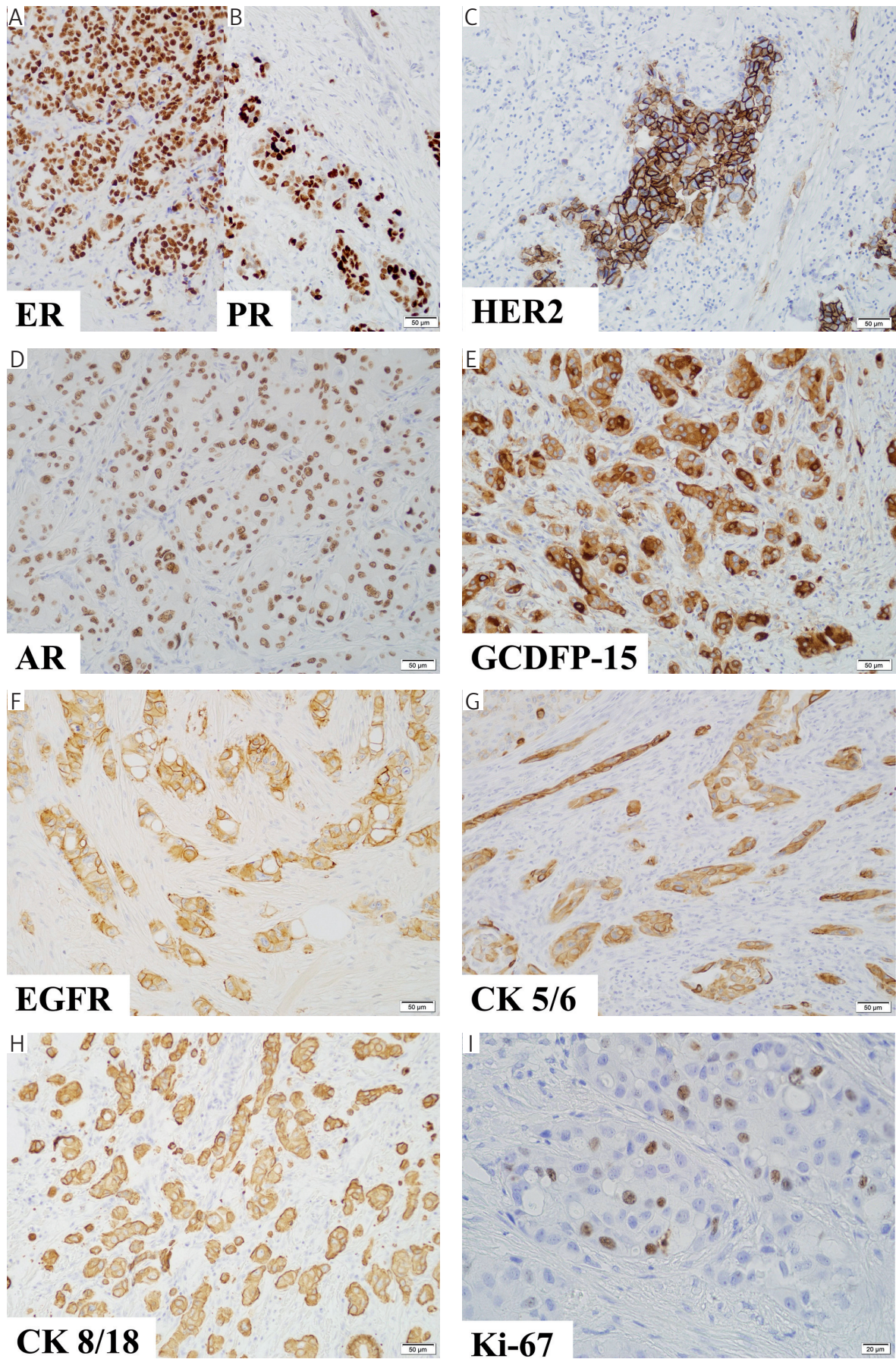


Figure 1. Examples of staining pattern of investigated proteins. A-H) magnification 20×; I) magnification 40×

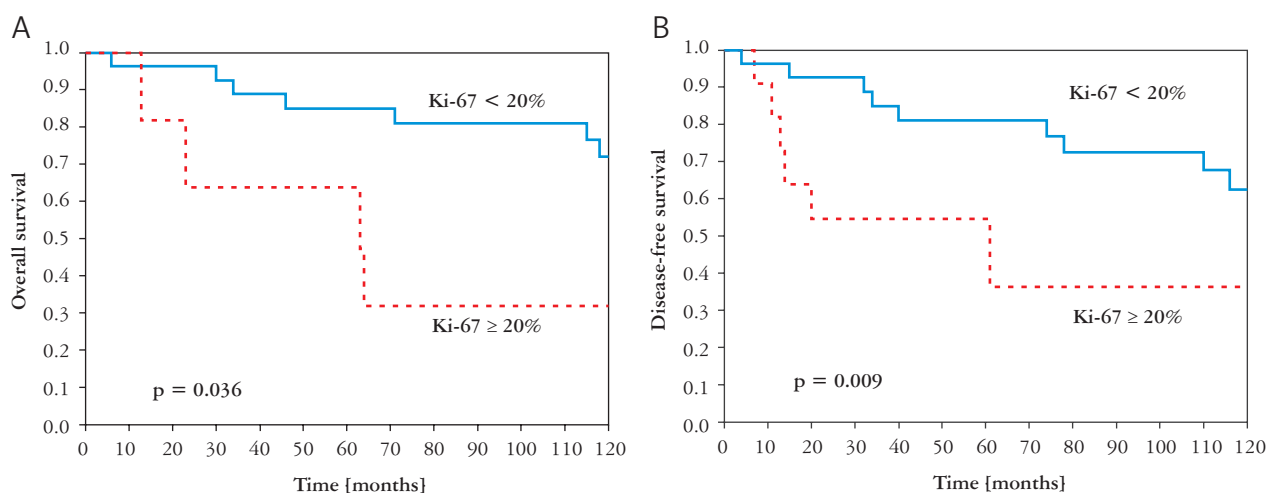


Figure 2. Survival of patients with apocrine breast cancer stratified by Ki-67 expression: A) overall survival; B) disease-free survival

($p = 0.011$, pN0 vs. pN+). Less prominent lymphocyte infiltration was observed in the group of tumours with pN0 + pN1 ($p = 0.003$).

The ER/PgR-negative tumours were more often characterised by CK5/6 and EGFR positivity ($p = 0.014$, and $p = 0.006$, respectively; Table II). Presence of androgen receptors was related to HER-2 and GCDP-15 expression ($p = 0.061$, and $p = 0.021$, respectively; Table II). Additionally, tumours with expression of CK5/6 were more likely to be EGFR positive ($p < 0.001$; Table II). Expression of CK5/6 was also related to higher Ki-67 LI ($p = 0.017$, Table II).

Survival analysis

In studied group mean time of follow-up was 99.4 months (range 6-285 months, median 109 months). During this time 22 patients died and 15 deaths were cancer related. Locoregional recurrence developed in 8 patients and distant metastases were observed in 13 cases. The probability of 10-years overall survival in the whole group was 66.2% and disease-free survival 62.7%. For Ki-67 LI, a value of 20% was established as cut-off point using the minimal p-value method from the log-rank test.

In univariate analysis, longer OS was observed in patients with tumours characterised by Ki-67 LI $< 20\%$ ($p = 0.036$; Table III, Fig. 2A) and a favourable trend was noted for patients with smaller tumours ($p = 0.053$; Table III), without lymph node metastases ($p = 0.074$; Table III) and without EGFR expression ($p = 0.060$; Table III). In the case of DFS only Ki-67 LI $< 20\%$ was a statistically significant favourable factor ($p = 0.009$, Table III; Fig. 2B), and a trend was not observed for any variables.

Cox multivariate analysis confirmed that Ki-67 LI $< 20\%$ is independent positive prognostic factors influencing overall and disease-free survival of patients with apocrine carcinoma (Table IV).

Discussion

The analysed group comprised 57 apocrine carcinomas treated between 1987 and 2010 at Maria Skłodowska-Curie Memorial Institute – Oncology Center, Cracow Branch. Apocrine carcinoma was defined according to 2012 WHO morphological criteria. This group accounted for 0.5% of all breast cancer patients treated during that time period, which is within the reported range [1, 2, 3].

As we mentioned before comparing biological features between different studies can be problematic. Firstly, because apocrine carcinoma is such a rarely occurring subtype of breast carcinoma, the analysed groups were rather small. Therefore, differences concerning a few cases can strongly influence the final results. Secondly, the authors selected groups using various definitions: apocrine carcinoma/pure apocrine carcinoma (based only on morphological feature) or with additional AR+/ER-/PgR- IHC profile or molecular apocrine which can also be differently defined. In order to have better insight and better understanding of this diversity and problems concerning comparing of studies, we have prepared Table V, which contains information about classifications used, the number of patients, and the investigated parameters. Our analysed group of 57 apocrine carcinomas was defined according to morphological features and is one of the largest studied groups (Table V). Studies concerning molecular apocrine carcinoma usually are conducted on larger groups. But even within studies

Table II. Relations between studied parameters

PARAMETER	CATEGORY	ALL N	KI-67LI	EMBOLI		EGFR		CK 5/6		CK 8/18		GCDFP-15		AR		HER2		ER/PR	
				N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		MEAN ±SD		-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
pT	pT1-2	53	15.3 ±8.3	34	18	26	25	28	23	2	48	3	48	5	46	29	23	37	15
	pT3-4	3	15.2 ±12.7	2	1	1	2	0	3	0	3	0	3	0	3	1	2	2	1
pN	pN0	27	16.2 ±8.8	21	4	11	13	11	13	2	21	2	22	3	21	15	10	17	8
	pN+	30	14.6 ±8.1	15	15 ^d	16	14	17	13	0	30	1	29	2	28	15	15	22	8
G	2	24	15.1 ±9.2	18	6	13	10	14	9	0	22	2	21	2	21	14	9	16	7
	3	28	15.6 ±8.2	15	13	12	16	11	17	1	27	1	27	3	25	13	15	20	8
TIL	Low	37	15.2 ±7.9	22	15	19	18	21	16	1	35	3	33	3	33	22	14	24	12
	High	15	15.8 ±9.9	11	4	6	8	5	9	0	14	0	15	1	14	5	10	12	3
ER/PR	- and -	39	15.6 ±8.1	25	14	14	24 ^e	15	23	2	36	2	37	5	34	23	16		
	+ or +	16	14.6 ±9.3	10	5	12	3	12	3 ^g	0	15	1	14	0	14	7	9		
HER2	-	30	16.2 ±8.0	20	10	14	15	14	15	2	27	2	28	5	25				
	+	25	14.4 ±8.9	15	9	12	12	13	11	0	24	1	23	0	23 ⁱ				
AR	-	5	23.1 ±7.3 ^a	4	1	3	2	0	5	1	4	2	3						
	+	49	14.4 ±8.1	31	18	23	25	27	21 ^h	1	46	1	47 ⁱ						
GCDFP-15	-	3	22.7 ±7.9	3	0	1	2	1	2	1	2								
	+	51	14.9 ±8.3	32	19	25	25	26	24	1	49								
CK 8/18	-	2	17.1 [*]	2	0	1	1	1	1										
	+	51	15.3 ±8.4	33	19	25	26	26	25										
CK 5/6	-	28	11.7 ±6.1 ^b	17	11	22	6												
	+	26	18.3 ±8.9	18	8	5	21 ^f												
EGFR	-	27	14.3 ±8.4	19	8														
	+	27	16.3 ±8.5	16	11														
Emboli	absent (-)	36	17.1 ±8.6 ^c																
	present (+)	19	12.2 ±7.3																

* only one case in the group, p-value from Mann-Whitney test: ^a 0.067, ^b 0.017, ^c 0.063, p-value from two-sided Fisher exact test: ^d 0.011, ^e 0.006, ^f p < 0.001, ^g 0.014, ^h 0.023, ⁱ 0.021, ^j 0.061

Table III. 10-years overall and disease-free survival according to studied parameters

PARAMETER	N	10-YEARS			
		OS		DFS	
		%	p*	%	P
pT					
pT1-2	53	67.2	0.053	63.2	0.241
pT3-4	3	33.3		33.3	
pN					
pN0	27	77.5	0.074	75.2	0.113
pN+	30	56.3		51.5	
G					
G2	24	75.2	0.371	62.0	0.309
G3	28	51.7		56.1	
Emboli					
Absent (-)	36	69.8	0.683	65.4	0.390
Present (+)	19	54.7		50.0	
TIL					
Low	37	56.7	0.272	60.8	0.805
High	15	72.7		54.0	
Ki-67 LI					
< 20%	27	72.0	0.036	62.5	0.009
≥ 20%	11	31.8		36.4	
ER/PgR					
-	39	65.1	0.958	62.2	0.993
+	16	64.2		57.5	
AR					
-	5	60.0	0.776	60.0	0.905
+	49	65.4		60.9	
GCDFP-15					
-	3	33.3	0.182	33.3	0.193
+	51	66.3		62.1	
CK 5/6					
-	28	76.1	0.189	60.5	0.324
+	26	49.8		61.5	
EGFR					
-	27	78.9	0.060	60.3	0.666
+	27	47.8		62.0	
HER-2					
-	30	63.6	0.976	62.1	0.240
+	25	65.8		59.6	

* log-rank test was used for comparison of two groups; OS – overall survival; DFS – disease-free survival; TIL – tumour infiltrating lymphocytes

concerning apocrine carcinoma defined by morphological features, authors used different scales and cut off points for expression of proteins. In the case of HER2 it was probably caused by changes in the

ASCO/CAP recommendations. In our group ER/PgR and HER2 positivity was observed in 29.1% and 45.5% respectively, which is within observed range (Table V).

Table IV. Cox proportional hazards for predictor of survival

VARIABLE	RR	95% CI	P
OVERALL SURVIVAL			
pT			
pT1+2		1.9-61.2	0.007
pT3+4	10.8		
Ki-67			
< 20%		1.3-13.3	0.017
≥ 20%	4.1		
DISEASE-FREE SURVIVAL			
pT			
pT1+2		2.6-93.9	0.003
pT3+4	15.6		
Ki-67			
< 20%		1.1-9.0	0.038
≥ 20%	3.1		

Apocrine carcinoma is assumed to be ER/PgR-negative and AR-positive. Some authors combining morphological features with this IHC phenotype created pure apocrine carcinoma subtype, while others use IHC phenotype to distinguish molecular apocrine. In our group 90.1% of the tumours were AR-positive; however, one third were ER/PgR positive. This is consistent with reports noting that the phenotype (AR+/ER-/PgR-) is characteristic for benign apocrine proliferation, very often observed in intraductal apocrine carcinomas, but less frequent in invasive carcinomas [3, 4]. Expression of EGFR, GCDFP-15 was similar to the results reported by other authors. However, we observed expression of CK5/6 more frequently than other authors investigating similarly defined apocrine groups. Similar results to ours concerning CK8 expression were reported by Guo *et al.* [22].

The relationship between ER/PgR lack of expression and EGFR expression was reported not only in apocrine carcinomas [10, 18] but also in other breast carcinoma subtypes [29].

Because GCDFP-15 expression is reported to be a frequent feature of apocrine carcinoma [4, 13, 14] as well as AR expression, it is not surprising that the relationship between the presence of these two proteins was observed not only in our study but also by others authors [21]. Lehman-Che *et al.* suggested that molecular apocrine subtype could be better defined using IHC phenotype ER- and HER2+ or GCDFP15+ [23].

One the most important factors from a clinical point of view is patients' survival. Table VI represents a review of studies concerning the survival of patients with apocrine carcinoma. Usually the studies investigated the differences between apocrine/

molecular apocrine carcinomas and other breast carcinoma groups (defined by the authors; Table VI) [7, 8, 10, 11, 12, 17, 20, 23, 24, 25, 30, 31]. In our study the probability of 10-years overall survival was 66.2% and disease-free survival was 62.7%. This is in agreement with observation of other authors, who reported 6-years OS for apocrine carcinoma of 72% against 52% for invasive ductal carcinoma [7], or 5-year disease-free survival and OS 66% and 77%, respectively for molecular apocrine [23]. The estimated 10-year OS in the group studied by di'Amore was 52% [31]. However, higher values of OS, at around 80%, for apocrine and invasive ductal carcinoma [17] or non-apocrine carcinoma [30] were noted in Japanese women after 10 years observation. Comparison between our result concerning survival of apocrine carcinoma and other subtypes of invasive ductal cancer is difficult due to wide range of estimated OS and DFS for those phenotypes [20, 24, 32, 33]. It is also worth mentioning that those subtypes could be differently selected, and different treatment. protocols could influence probability of survival [33].

Only a few studies have investigated survival according to biological factors in apocrine carcinomas groups [10, 20, 21, 22, 23, 30]. The problem concerning analysis of survival is very complex, especially in the case of apocrine carcinomas classified according to morphological features. Because this type of cancer is so rare, groups consist of cases collected over a long time (Table VI). It is obvious that during that time, treatment regimens might change considerably and new drugs might be implemented, which could influence patient survival. Nevertheless, in our study longer OS and DFS were observed in patients with tumours characterised by low Ki-67 LI (< 20%), and a favourable trend was observed for OS in cases with smaller tumours, without lymph node metastases and without EGFR expression. Similar results acquired Liu *et al.*, who reported better DFS and OS for patients with lower Ki-67 LI (< 20%) tumours, however in that study a molecular apocrine subgroup was investigated [10]. Guo *et al.* did not find a relationship between OS and Ki-67, but those authors investigated molecular apocrine carcinoma and used lower cut-off point (> 14%) [22]. Ki-67 is a well-known biological factor, which, along with ER, PgR, and HER2, can be useful in the assessment of molecular breast cancer subtypes. St. Gallen's recommendation from 2013 stated that cut-off point of Ki-67 ≥ 20% could be used to define luminal B, however, a lack of methodological standarization was also mentioned [34]. Two recent meta-analyses concerning Ki-67 in breast cancer, confirmed that high Ki-67 LI is unfavourable prognostic factor [35, 36]. Both studies also addressed the issue of cut-off point. Liu *et al.* demonstrated that the most appropriate Ki-67 cut-off point for predicting chemosensitivity, may be a value close to ≥ 19% in hormone

Table V. Apocrine carcinoma – review of studies concerning biological factors

AUTHOR TYPE NAMED AND DEFINED BY AUTHORS	N	PERCENTAGE OF CASES WITH EXPRESSION OF							
		AR	ER/PgR	HER2 (...)/F#	EGFR	K-67	GCDFFP-15	P53	OTHERS
APOCRINE CARCINOMA DEFINED ACCORDING TO MORPHOLOGICAL FEATURES									
Honma <i>et al.</i> (2005) [4] Apocrine carcinoma	38	50 (≥ 10%)	5.3/5.3 (≥ 10%)	13.2 3+			65.8 (≥ 50)		Bcl-2: 2.6% (≥ 10%)
Japaze <i>et al.</i> (2005) [7] Pure apocrine carcinoma	37		61.1/58.3						
Dellapasqua <i>et al.</i> (2013) [8] Apocrine carcinoma	72	95.7 (≥ 10%)	30.6/23.6 (≥ 1%)	34.7 (> 10%)/F				27.8 (≥ 30%) 34.7 (< 14%)	
Kaya <i>et al.</i> (2008) [13] Apocrine carcinoma	13	53.8	38.5/23.1 (> 10%)	84.6 (> 10%)			100 (> 50%)	38.5 (> 10%)	
Kasashima <i>et al.</i> (2012) [14] Apocrine carcinoma	48	89.6 (> 10%)	10.4/6.3 (> 10%)	25.0 (> 30%)/F			95.8 (> 50%)	50.0% (> 5%)	PSA: 2.1% (> 10%); 5 α-reductase: 62.5% (> 10%)
Mills <i>et al.</i> (2016) [15] Pure apocrine carcinoma	20	100.0 (≥ 1%)	0.0/0.0% (≥ 1%)	10.0 F					
Matsuo <i>et al.</i> (2002) [16] Apocrine carcinoma	24		17/17 (> 10%)	33.0 (> 10%)				29.0 (> 10%)	Bcl-2 25 (> 25%)
Tanaka <i>et al.</i> (2008) [17] Apocrine carcinoma	57		15.8/21.1 (≥ 10%)	14.6 (≥ 10%)					
Vranic <i>et al.</i> (2015) [18] Apocrine carcinoma	37	89.2 (> 1%)	24.3/2.7 (> 1%)	32.4 (> 10%)/F		41.2		43.0 (≥ 20%)	33.3 (> 10%) E-cadherin: 88.6%; Topo2α: 34.3; MUC-1: 88.2; CK5/6: 11.4 (> 10%); CK14: 0; p63: 5.7; Cav-1: 5.9; PTEN: 17.1; cyclin D1: 50%
Vranic <i>et al.</i> (2010) [9] Apocrine carcinoma	55	87.3 (> 10%)	23.6/- (> 5%)	54 (> 30%)		62 (> 1)			
Montagna <i>et al.</i> (2013) [25] Apocrine (distinguished from triple negative breast cancers)	29		(≥ 1%)	(> 10%)				20.7 (≥ 30%)	
Takeuchi <i>et al.</i> (2004) [30]	33		33.3/27.3						
Wysocka <i>et al.</i> (2018) [presented study]	57	90.7 (> 10%)	29.1 (> 1%)	45.5 (> 10%)		50.0 (> 10%)	94.4 (> 10%)	28.9 (≥ 20%)	Emboli: 34.5%, TIL: 28.8, CK5/6: 48.1 (> 10%), CK8/18: 96.2 (> 10%)

Table V. Cont.

AUTHOR TYPE NAMED AND DEFINED BY AUTHORS	N	PERCENTAGE OF CASES WITH EXPRESSION OF							
		AR	ER/PgR	HER2 (.)/F#	EGFR	K-67	GCDFP-15	P53	OTHERS
APOCRINE CARCINOMA DEFINED ACCORDING TO MORPHOLOGICAL FEATURES AND/OR IHC PHENOTYPE									
Dellapasqua <i>et al.</i> (2013) [8] Pure apocrine carcinoma: ER-PgR-AR+	44	- (≥ 10%)	- (≥ 1%)	40.9 (> 10%) ^F	31.8 (≥ 30%) 31.8 (< 14%)				
Vranic <i>et al.</i> (2015) [18] IHC-apocrine carcinoma (ER-PgR-AR+)	27	- (> 1%)	- (> 1%)	29.2 (> 10%) ^F	56.0 (≥ 20%)	42.0	41.7% (> 10%)	E-cadherin: 92%; Topo2α: 37.1; MUC-1: 88; CK5/6: 16.0; CK14: 0; p63: 4.0; Cav-1: 8.0; PTEN: 12.0; cyclin D1: 44.0	
Vranic <i>et al.</i> (2010) [9] Pure apocrine carcinoma (ER-PgR-AR+)	38	> 10%)	> 5%)	57 (≥ 30%)	76 (> 1)				
Tsutsumi <i>et al.</i> (2012) [19] Apocrine-type carcinoma Only IHC: ER-PgR-AR+	44	> 1%)	> 1%)	52 (≥ 30%)	94.3	Mean Ki67 LI: 39%	71.4 (≥ 10%)	75 CK5/6: 60% (> 10%); CK14: 14.3 (> 10%)	
MOLECULAR APOCRINE									
Liu <i>et al.</i> (2018) [10] Molecular apocrine – IHC: ER- PgR-AR+	200	> 10%)	> 1%)	38.5 (≥ 10%)	86.5	69.5 (≥ 20%)	-	38.5 (≥ 10%)	
Cha <i>et al.</i> (2012) [20] Molecular apocrine – IHC: ER- and AR and/or GGT1+	26	19.2 (> 1%)	- (> 1%)	38.5 (≥ 30%) ^F	23.1 (≥ 1%)	11.5 (≥ 30%)			GGT1: 100% (> 1%); CK5/6: 15.4 (> 1%)
Darb-Esfahani <i>et al.</i> (2014) [21] Molecular apocrine IHC HR-/AR+	56	IRS ≥ 4		42.9*			67.9		
Guo <i>et al.</i> (2015) [22] Molecular apocrine IHC: ER-PgR-AR+	90		> 1%)	51.1 ^b / _F	60	56.7 (≥ 14%)		58.9 (≥ 10%)	CK5/6: 32.2%; CK8: 93.3; CK44: 67.8; CK166: 82.2; bcl-2: 24.4; BRCA1: 71.1
Lehmann-Che <i>et al.</i> (2013) [23] Molecular apocrine: qRT-PCR signature *	58	58 (≥ 10%)	7/3 (≥ 10%)	67 ^a / _F	30		57 (≥ 5%)		FOXAI: 90% (≥ 10%); CK5/6: 11 (≥ 5%); CK17: 5 (≥ 5%);
Lakis <i>et al.</i> (2014) [24] Molecular apocrine IHC: ER-PgR-AR+	103	> 1%)	> 1%)	68 (≥ 30%)	46.5 (≥ 1%)	82.4 (≥ 14%)			CK5: 22.2 (≥ 1%)

IAC – infiltrating apocrine carcinoma; * qRT-PCR signature: absence of ESR1 overexpression and overexpression of AR and FOXA and overexpression of 3 from 5 genes related to AR pathway (Arg2, ALCAM, SPDEF, TTF3, UGT2B28A)
() percentage assumed as 3) IHC (overexpression of HER2)/amplification or HER2 gene confirmed by FISH; ^a ASCO/CAP2007; ^b HER2-positive if IHC score 2+ (≥ 10%) or 3+ (≥ 30%).

Table VI. Apocrine carcinoma – review of studies concerning survival

AUTHOR	(YEARS OF SAMPLES COLLECTION, IF NOTED)	N	RESULTS
Japaze <i>et al.</i> (2005) [7]	(1991-2001)	37	OS significantly better for pure invasive apocrine carcinoma vs. invasive ductal carcinoma not otherwise specified
Dellapasqua <i>et al.</i> (2013) [8]	(1997-2005)	72	Pure apocrine (morphological features and IHC: ER–PgR–AR+) worse DFS comparing to pure ductal and apocrine-like (morphological features of apocrine and IHC other than ER–PgR–AR+). OS – NS
Liu <i>et al.</i> (2018) [10]	(2007-2011)	200	Molecular apocrine (IHC: ER–PgR–AR+) – DFS better for: EGFR-negative, HER2-negative and with lower Ki-67 index (< 20%) cases. OS better for: HER2-negative and with lower Ki-67 index (< 20%) cases
Dreyer <i>et al.</i> (2013) [12]	(1999-2009)	14	DFS apocrine vs. others subtypes of triple negative breast cancer – NS, too small groups to draw conclusion
Kasashima <i>et al.</i> (2012) [14]	(1990-2010)	48	Recurrence-free survival shorter in 5 α -reductase positive apocrine carcinomas
Tanaka <i>et al.</i> (2008) [17]	(1995-2005)	57	OS and relapse-free survival – no significant differences between invasive apocrine carcinoma and invasive ductal carcinoma
Cha <i>et al.</i> (2012) [20]	(2001)	26	No differences in DFS and OS between molecular apocrine (IHC: ER– and AR and/or GGT1 positive) and other breast cancers phenotypes. Molecular apocrine group – tendency for poorer DFS and OS for HER2-positive and basal markers-negative cases
Darb-Esfahani <i>et al.</i> (2014) [21]		56	No differences between DFS and OS between molecular apocrine (IHC: HR–/AR+) groups with and without GCDFP-15 expression
Guo <i>et al.</i> (2015) [22]	(2002-2013)	90	Poorer OS of patients with HER2-positive molecular apocrine (IHC: ER–PgR–AR+) breast cancer. NS for stratification according to grade, lymph node metastases, expression of: EGFR, CK5/6, CK8, CD44, CD169, P53, bcl2, BRCA1, Ki-67
Lehmann-Che <i>et al.</i> (2013) [23]	(1996-2008)	58	No differences in DFS and OS between molecular apocrine (qRT-PCR signature *) and basal-like cancers. DFS – no differences in molecular apocrine group stratified according to AR, HER2,GCDFP-15 expression
Lakis <i>et al.</i> (2014) [24]		103	Luminal and molecular apocrine (IHC: ER–PgR–AR+) better OS than HR-negative group (DFS- on the border of significance)
Montagna <i>et al.</i> (2013) [25]	(1997-2005)	29	Similar OS and DFS between triple negative infiltrating ductal carcinoma and apocrine carcinoma (morphological features)
Liu <i>et al.</i> (2016) [11]	(2004-2005)	205	Distant metastasis-free survival, DFS and OS significantly worse in the group of molecular apocrine breast cancer (MABC) (ER–/PR–/AR+) then in non-MABC
Takeuchi <i>et al.</i> (2004) [30]	(1980-2001)	33	No differences in survival between apocrine and non-apocrine invasive ductal carcinoma. No differences in apocrine group stratified according to tumour size, ER, PgR expression. Worse prognosis for cases with lymph node metastases, lymphatic involvement, vascular involvement
d'Amore <i>et al.</i> (1988) [31]	(1952-1982)	34	No differences in OS between apocrine carcinoma and matched control patients with infiltrating ductal carcinoma
Wysocka <i>et al.</i> (2018) [presented study]	(1987-2010)	57	In apocrine carcinoma group: better OS for cases with low Ki-67 and trend for patients with smaller tumours, without lymph node metastases and EGFR-negative. DFS – better for cases with low Ki-67

DFS – disease-free survival; OS – overall survival; NS – lack of statistically significant differences

* qRT-PCR signature: absence of ESR1 overexpression, overexpression of AR and FOXA and overexpression of 3 from 5 genes related to AR pathway (Arg2, ALCAM, SPDEF, TTF3, UGT2B28A)

receptor-positive patients [35]. A second study which included more than 64 000 patients stated that although the Ki-67 threshold with the greatest prognostic significance is still unknown, a cut-off > 25% is related to higher probability of death compared with lower expression rates [36].

Because many studies have demonstrated, that EGFR expression might be feature of more invasive breast carcinoma phenotype, it is not surprising that also in our studied apocrine carcinoma group the expression of EGFR tended to be related with poorer OS. Similarly, Liu *et al.* in a molecular apocrine tumours group noted relation between shorter DFS and EGFR expression [10]; however, other study did not confirm connection between OS and EGFR [22].

We noted that HER2 expression was not related to OS or DFS. Conversely, some authors observed that HER2-positivity was associated with shorter survival [10, 20, 22], however all those studies were conducted in molecular apocrine groups defined by IHC phenotype (Tables V and VI). In a molecular apocrine group defined by qRT-PCR signature, Lehmann-Che *et al.* did not report this relation [23].

Similarly to others authors, we also did not noted relation between GCDFP-15, CK5/6, AR, ER, and PgR expression and survival [21, 22, 23, 30].

Conclusions

In the studied group of apocrine carcinomas, defined by morphological features, the ER/PgR-negative tumours were more often characterised by CK5/6 and EGFR positivity. Additionally, the presence of androgen receptor was related to HER-2 and GCDFP-15 expression. Data suggest that in breast apocrine carcinomas, like in other breast cancers, high Ki-67 proliferation index is one of the most important factors related to shorter OS and DFS survival. Expression of other investigated proteins (ER, PgR, HER2, AR, GCDFP-15, EGFR, CK 5/6, CK 8/18) was not statistically significant for OS and DFS

It is worth mentioning that there is a problem in the comparison of studies concerning apocrine carcinomas, due to the different types of classification applied by authors. In the literature, the following terms are used presently: apocrine carcinoma, pure apocrine carcinoma, and molecular apocrine carcinoma, which are defined differently by authors and could overlap. Sometimes the same “term” could mean differently defined groups or different “terms” are applied to describe the same group. It seems that there is an urgent need for unification of classification of different apocrine carcinoma subgroups.

Clinical practice points:

1. Our data suggest that in breast apocrine carcinomas one of the most important factor related

to shorter survival, like in other breast cancers, is high Ki-67 proliferation index.

2. There is a problem concerning the clear definition of apocrine carcinoma, pure apocrine carcinoma, molecular apocrine carcinoma, because in different studies the same “name” could mean differently selected groups or different “names” describe the same group. It seems that there is urgent need for unification of these classifications.

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