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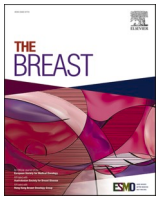

Could macrocytosis predict survival In advanced breast cancer patients that were treated with CDK 4–6 inhibitors?

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A B S T R A C T

Introduction: Cyclin Dependent Kinase (CDK) 4–6 inhibitors are the recommended first-line treatment option for hormone-positive metastatic breast cancer (MBC). They show their effects by causing cell cycle arrest in G1-S phase. Neutropenia is the most common haematological side effect. In the literature, data on the association between CDK 4–6 inhibitors and macrocytosis are limited. We aimed to investigate the effect of macrocytosis on survival.

Methods: We retrospectively analysed 133 patients with de novo hormone positive MBC using CDK 4–6 inhibitors in first line treatment. Mean Corpuscular Volume (MCV) > 100 was considered macrocytosis and patients were divided into two groups; MCV<100 and MCV >100. The association of macrocytosis with clinicopathological features, Progression Free Survival (PFS) and Overall Survival (OS) were evaluated.

Results: 42 patients were receiving palbociclib and 81 patients were receiving ribociclib. Median OS was determined as 33 months and median PFS was determined as 22 months. Macrocytosis ever rate was 45.8 % during follow-up. Macrocytosis was observed in 4.2 % of the patients in the first month, 16.7 % in the third month, 41.6 % in the sixth month and 42.2 % in the twelfth month. ER receptor level, ki-67, macrocytosis at 6–12 months and macrocytosis-ever which were found to affect OS as a result of univariate Cox regression analysis, were evaluated with multivariate Cox regression models and it was observed that they had significant effect on PFS and OS.

Conclusion: Macrocytosis may be a useful biomarker for the prediction of PFS and OS in MBC patients receiving CDK 4–6 inhibitors.

1. Introduction

Breast cancer remains the most frequently diagnosed cancer worldwide [1]. Hormone receptor (HR)-positive, HER2-negative metastatic breast cancer represents approximately 70 % of the histological subtypes of invasive breast cancer and this group can express both estrogen and progesterone receptors. However, the main pathway affecting cell proliferation is the estrogen signalling pathway. Some drug therapies have been developed to block this pathway and have become one of the main drug groups in hormone-positive metastatic breast cancer (MBC). Aromatase inhibitors, selective estrogen modulators and selective estrogen down-regulators constitute this group of drugs. The main aim of endocrine therapy is to block this pathway, which down-regulates cell proliferation. There may be resistance to the estrogen hormone and tumoral cells may use alternative pathways. A known alternative pathway is the cyclin D-cyclin-dependent kinase 4/6 (CDK4/6) pathway of the CDK4-retinoblastoma pathway [2,3].The CDK 4/6-cyclin D pathway triggers cell cycle progression in response to estrogen signalling [4–6]. By inhibiting this pathway, CDK 4–6 inhibitors aim to increase the efficacy of current endocrine treatment regimens and prevent resistance to treatment. In studies, it has been observed that combinations of CDK 4/6 inhibitors and endocrine therapy provide significant improvement in the survival of patients and these agents, which are well tolerated, cause neutropenia as a class side effect [7–9]. In tumoral cells, inhibition of CDK 4/6 results in cell cycle arrest, which can lead to a mismatch between cytoplasmic and nuclear maturation in erythroid precursors leading to macrocytosis and the development of a new population of large erythrocytes. In the literature, there are also case reports reporting that these agents cause macrocytosis [10,11]. To our knowledge, there are no data on macrocytosis in large patient groups. In our study, we aimed to investigate the effect of macrocytosis on survival in patients receiving CDK 4/6 treatment with real life data (see Figs. 1 and 2).

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2. Material and method

2.1. Patient characteristics

Between January 2018 and September 2023, 133 postmenopausal patients diagnosed with de novo metastatic breast cancer and treated with first-line CDK 4/6 and aromatase inhibitor combination in two oncology clinics in Istanbul were retrospectively evaluated. Baseline data were obtained from databases and medical records. The study included patients with histopathologically confirmed breast cancer, radiologically proven metastatic disease and first-line endocrine therapy plus CDK 4/6 inhibitors. Patients who received less than three months of treatment, had incomplete follow-up data, and whose vitamin B12, folic acid and thyroid stimulating hormone values were not in the normal range were excluded from the study.Thebaselinecharacteristics of thepatientsaredisplayed in Table 1.

Hemogram and biochemistry parameters including Mean Corpuscular Volume (MCV) were recorded as laboratory parameters. MCV>100 was considered macrocytosis and patients were divided into two groups; MCV<100 and MCV >100. During the follow-up of the patients after the initiation of CDK 4/6 inhibitor, 1st, 3rd, 6th and 12th month laboratory values and presence of macrocytosis were recorded. Macrocytosis ever group was defined as patients with macrocytosis at any time, and macrocytosis 6-12 group was defined as patients with macrocytosis at 6th month and/or 12th month. Age at diagnosis, menopausal status, detection of de-novo metastatic disease, hormone receptorlevels, number of metastatic sites, presence of visceral or non-visceral metastasis and grade ≥3 toxicities were evaluated. Progression Free Survival (PFS) and Overall Survival (OS) differences were analysed between the groups divided according to MCV status. In addition, drug-related side effects and dose reductions due to side effects were analysed.

2.2. Treatment

Ribociclib was given at a dose of 600 mg and palbociclib was given at a dose of 125 mg orally, as the initial dose once daily for 21 consecutive days (day 1–21) in a 4-week cycle. Anastrozole or letrozole was administered concurrently. Anastrozole (1 mg) and letrozole (2.5 mg) were given orally on continuous daily schedule.

2.3. Statistical analysis

Thecharacteristics of thepatientswerecomparedwiththeFisherorChi-squared test forcategoricaldataand a *t*-test forcontinuous data. OS wasdefined as the time fromtheinitiation of CDK 4/6i untilmortalityfromanycause. PFS wasdefined as the time fromtheinitiation of CDK 4/6i totheprogressionormortalityfromanycause. No-eventpatientswerecensored at theend of thelastfollow-up. Survivalcurveswereestimatedbythe Kaplan-Meiermethodandcomparedwiththelog-rank test. Coxregression wasusedtoanalysethehazardratiosfor PFS and OS. Statistical testswe retwo-sided, and a p-valuelessthan 0.05 wasconsideredstatistically significant. Thestatisticalanalyseswereconductedusing SPSS version 26.

3. Results

A total of 133 patients from two centers were included in this study (palbociclib = 42, ribociclib = 81).Thebaselinecharacteristics of thepatientsaredisplayed in Table 1. Mean age of the patients was 57.4 +-12.9. All of the patients were postmenopousal. The most common histology was invasive ductal carcinoma (%81). All patients were 100 % ER receptor positive and dual receptor (ER + PR+) positivity was 75 %. Median OS was determined as 33 months and median PFS was determined as 22 months. During follow-up, 23.2 % of the patients died. A summary of patient characteristics is presented in Table 1.

The frequency of macrocytosis increased gradually over time and reached a plateau after roughly 6 months. Macrocytosis ever rate was 45.8 % during follow-up. Macrocytosis was observed in 4.2 % of the patients in the first month, 16.7 % in the third month, 41.6 % in the sixth month and 42.2 % in the twelfth month.

Neutropenia was observed in 48.2 % of patients during treatment. Dose reduction was performed in 22.8 % (n:38) of the patients due to side effects. The association of macrocytosis which developed in the 6th-12th months, and macrocytosis-ever with neutropenia (<1500), was found to be significant ($p < 0.05$) (Table 2) (see Table 3).

Univariate Cox regression analysis results of clinical parameters considered to affect OS and PFS are presented in Table 2. It was observed that ER receptor level, ki-67, macrocytosis at 6–12 months and development of macrocytosis at any time had significant effects on both PFS and OS (Table 2). No significant effect of menopause, pr receptor level, neutropenia, dose reduction and histological subtype on PFS and OS time was found ($P > 0.05$). ER receptor level, ki-67, macrocytosis at 6–12 months and macrocytosis-ever which were found to affect OS as a result of univariate Cox regression analysis, were evaluated with multivariate Cox regression models and the results are presented in Table 2. It was observed that macrocytosis 6–12 months, macrocytosis ever, er receptor level and ki-67 levels had significant effect on OS.

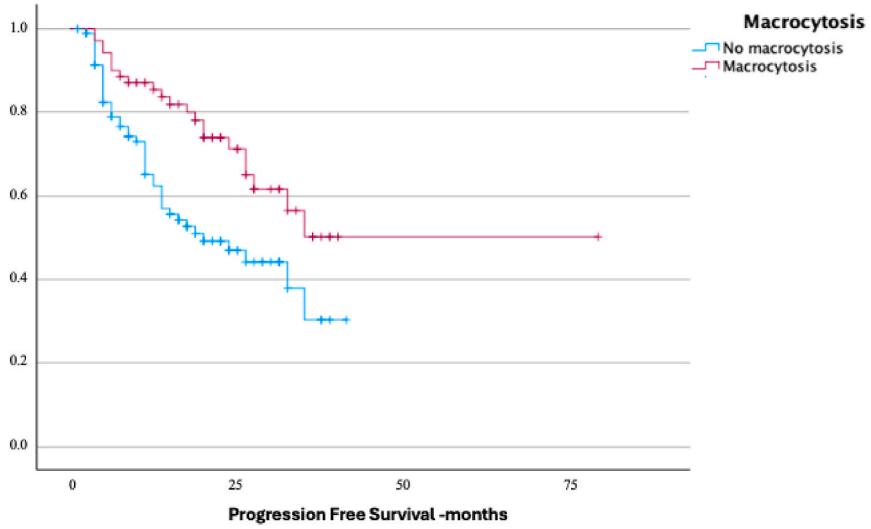

Fig. 1. Progression Free Survival in patients with macrocytosis at 6–12 months and no macrocytosis.

4. Discussion

In our study, we have shown that macrocytosis (MCV>100) in the follow-up of patients with metastatic hormone-positive breast cancer using CDK 4–6 and aromatase inhibitor (AI) combination can predict survival. 6–12. There was a strong correlation between macrocytosis and macrocytosis-ever and OS-PFS, and these patients were found to have longer OS and PFS. We believe that macrocytosis observed in the follow-up of patients receiving CDK 4–6 inhibitor and AI treatment may be useful for their clinical course.

The increase in MCV in patients treated with ribociclib and palbociclib has been reported in small patient groups in the literature [12,13]. There are no studies on this subject with large patient data. In one of these studies, Anampa et al. demonstrated longer PFS in metastatic breast cancer patients with macrocytosis using palbociclib [12]. In our study, similar to this study, we found that patients who received CDK4-6 inhibitor treatment and had macrocytosis had longer OS and PFS. Perhaps; one of the reasons for this correlation may be that the increase in MCV observed during treatment reflects drug-induced cell cycle arrest in haematopoietic cells. Considering the long half-life of red blood cells (115 days) as well as a time-dependent correlation between MCV and palbociclib and ribociclib use, the change in MCV may be a potential pharmacodynamic biomarker of functional CDK4/6 inhibition. It should be emphasised that in our patient group, this effect plateaued at roughly the 6th month and beyond, and it is not possible to predict the association between macrocyte changes and survival in the first months of treatment, probably due to the long erythroid series half-life.

Studies on other cancer types in the literature have also investigated the effect of macrocytosis on survival [14–16]. In a study conducted in renal cell carcinoma patients using Tyrosine Kinase Inhibitors, it was observed that patients with macrocytosis had longer PFS and OS [14]. In studies in which patients who received chemotherapy in the metastatic stage with lung cancer, ovarian cancer and breast cancer diagnoses were analysed, it was observed that the group with macrocytosis had longer survival times [15,16]. In our study, in accordance with the results in other cancer types in the literature, macrocytosis can be used as a survival marker in patients using CDK4-6 inhibitors in metastatic breast cancer.

The mechanism behind the effect of palbociclib and ribocyciclib on MCV remains unclear. Our study was not designed to determine the cause of macrocytosis caused by the use of CDK4-6 inhibitor. However, in order to exclude other causes of macrocytosis, we excluded patients whose vitamin B12, folic acid and TSH values were not at normal values.

Table 1
Patient characteristics.

		n	%
Age (mean)		57,4	
CDK 4/6 inhibitors	Palbociclib	42	31,5
	Ribosiklib	81	68,5
Histological subtype	Ductal carcinoma	107	81,7
	Lobular carcinoma	22	15,9
	Other	4	2,4
Histological grade	1	5	2,4
	2	85	64,6
	3	43	32,9
Bone metastasis	No	36	27
	Yes	97	73
Liver metastasis	No	109	82,1
	Yes	24	17,9
Lung metastasis	No	100	75
	Yes	33	25
Brain metastasis	No	127	94,4
	Yes	6	5,6
Macrocytosis ever	No	72	54,2
	Yes	61	45,8
Macrocytosis 6/12 months	No	75	56,7
	Yes	58	43,3
Neutropenia	No	68	51,8
	1000–1500	26	14,2
	500–1000	34	25
	0–500	4	3
Dose reduction	No	95	77,2
	Yes	38	22,8
Exitus	No	103	76,8
	Yes	30	23,2

Anampa et al. hypothesised that MCV changes trigger the development of a new population of large erythrocytes in palbociclib [12]. They found that palbociclib treatment significantly inhibited the number of glycophorin-A positive erythroid cells, leading to decreased erythroid colony formation and decreased erythroid differentiation. This may lead to reversible macrocytosis mimicking Myelodysplastic syndrome.

In pivotal studies of CDK 4/6 inhibitors; it has been shown that neutropenia may occur as a side effect [7–9]. Dose titration is recommended according to the neutropenia grade in the follow-up. Studies have shown that survival was not affected despite the recommended dose reduction in patients who developed neutropenia [17,18]. In our study, there was a significant correlation between the 6th month macrocytosis in patients with neutropenia ($p < 0.05$). When analysed statistically, macrocytosis at the 6th month is most common in patients

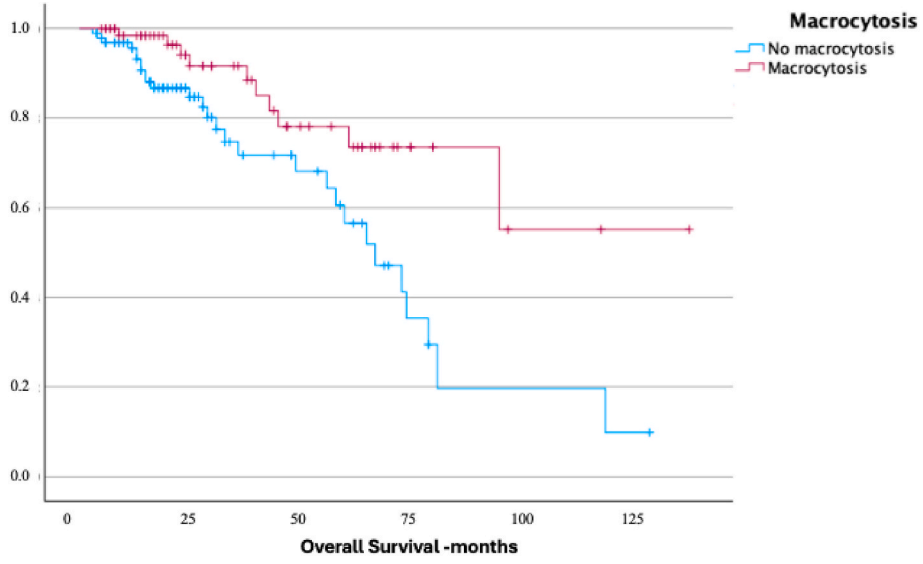

Fig. 2. Overall Survival in patients with macrocytosis at 6–12 months and no macrocytosis.

Table 2
The association of macrocytosis with neutropenia.

	Neutropenia										p
	No		1500 LLN		1000–1500		500–1000		0–500		
	n	%	n	%	n	%	n	%	n	%	
3rd month macrocytosis	7	9,3	1	12,5	8	30,8	11	21,6	1	25,0	0,067
6th month macrocytosis	19	27,9	5	83,3	13	52	20	42,6	2	50,0	0,024*
12th month macrocytosis	14	28,0	3	60,0	11	55,0	17	45,9	1	33,3	0,141
Macrocytosis ever	22	29,3	5	62,5	18	69,2	28	54,9	2	50,0	0,001*

Table 3
Univariate and multivariate analysis of parameters regarding progression free survival and overall survival.

Parameter	Univariate HR				Multivariate HR	p
	Progression Free Survival	p	Overall Survival	P		
Dose reduction	0.841(0.473–1496)	0.549	0.591 (0.241–1.448)	0.25		
Neutropenia	1.019(0.63–1.647)	0.939	1.041 (0.525–2.068)	0.908		
Macrocytosis(6/12m)	0.478 (0.286–0.798)	0.005	0.38 (0.184–0.785)	0.009	0.416 (0.191–0.905)	0.027
Macrocytosis - ever	0.568 (0.347–0.931)	0.025	0.543 (0.279–1.054)	0.054		
Dual Positivity (ER-PR)	0.698 (0.4–1.219)	0.207	1.124 (0.540–2.341)	0.754		
ER %	0.992(0.974–1.01)	0.372	1.035 (1.001–1.071)	0.045	1.066 (1.019–1.116)	0.005
PR %	2.526 (1.32–4.831)	0.030	0.999 (0.99–1.008)	0.829	0.93 (0.983–1.002)	0.124
HER 2 positivity	2.526 (1.32–4.831)	0.005	0.585 (0.172–1.99)	0.391	0.275 (0.048–1.564)	0.145
K-i67 %	1.018 (1.006–1.03)	0.004	1.02 (1.006–1.035)	0.005	1.018(1.003–1.033)	0.018

with a neutrophil count below 1500. There was also a significant correlation between neutropenia and the presence of macrocytosis ($p < 0.05$). This suggests that cell arrest induced by CDK4-6 inhibition partially co-acts on neutrophil and erythrocyte pathways. However, while the association between macrocytosis and survival is significant, neutropenia does not show such a feature and the survival relationship of macrocytosis persists despite dose reduction.

In the hormone positive type of MBC, CDK4/6 inhibitors are the recommended first-line treatment option. Determining which patients will benefit from this treatment option is important for the patient's life expectancy. In this respect, cheap, practical and reliable parameters are needed in our daily oncological practice. Therefore, with this study, we tried to show that macrocytosis at follow-up may be helpful in terms of prognosis.

4.1. Limitations

The limitations of the present study are the small sample size, the inclusion of only post menopausal patients, its retrospective design and the exclusion of patients receiving adjuvant endocrine therapy. Therefore, prospective studies with a larger number of patients are needed.

5. Conclusion

The findings of this study provide evidence of an association between macrocytosis and survival in metastatic breast cancer patients treated with first-line CDK 4/6 inhibitors. Therefore, we think that patients with macrocytosis have longer PFS and OS.

CRediT authorship contribution statement

Ilkay Gültürk: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rumeysa Colak:** Formal analysis, Data curation. **Caner Kapar:** Methodology, Investigation, Funding acquisition. **Murad Guliyev:** Validation, Supervision, Software, Gulru Birce Sonmezoz, Resources, Project administration, Methodology. **Cigdem Yıldırım:** Formal analysis, Data curation, Conceptualization. **Mesut Yilmaz:** Visualization, Supervision, Conceptualization.

Financial disclosures

None.

Disclosure

The authors have stated that they have no conflict of interest.

Clinical practice points

In the group of patients with hormone-positive metastatic breast cancer, CDK 4–6 inhibitors are recommended as first line in the guidelines. It has been shown to prolong survival in pivotal studies, and hematological side effects, especially neutropenia, have been reported. There is very little data in the literature that it causes macrocytosis. In our clinical practice, we observed macrocytosis in some patients. We designed this study to evaluate the effect of macrocytosis on predicting response to treatment and survival.

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