Assessment of Response to Hormonal Therapy by Serum Ki-67 as a Biomarker in Non-metastatic Hormone Positive Breast Cancer: A Prospective Study

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Abstract:

Background: Breast cancer has the highest incidence among all female patients with different cancer types. It is a lethal disease which threaten women's health. There are many prognostic and predictive factors that implicated in breast cancer. The expression of Ki-67 is strongly associated with cancer proliferation and is a known indicator of breast cancer prognosis and outcome. Ki-67 expression levels are also useful to inform treatment decision making in some cases. As a result, measurement of Ki-67 is routinely done during pathological tumor evaluation.

Methods: This study is a prospective study; included eighty-eight of newly diagnosed patients presented to the Outpatient Clinic in South Egypt Cancer Institute, Assiut University with primary breast carcinoma all of them hormonal positive regardless Her2 status. From (1/2022 to 12/2023) before starting hormonal treatment and after 6 months of starting treatment. The study was approved by the Institutional Review Board of the SECI, Assiut University (approval No: 582). An informed written consent was taken from all cases. Serum concentration of Ki-67 was measured using a commercially available Ki-67 ELISA Kit and tissue expression of Ki-67 was assessed by immunohistochemical technique.

Results: In this study, clinical significance of serum Ki-67 as a prognostic indicator in breast cancer was evaluated among eighty-eight hormone- positive early breast cancer cases. More than half of them (56) cases are luminal A breast cancer and (32) cases of them are luminal B, no Her2 enriched breast cancer or triple negative, more than half of them (55%) received Tamoxifen and (45%) received Femara, (31) cases of them are stage I, (37) cases are stage II and (20) cases are stage III, more than half of cases showed lower expression of Ki 67, Higher expression for ki67 (≥20%) detected in 32 cases. Moreover, the relationship between serum Ki-67 by ELISA with clinical, pathologic characteristics and patient outcome were detected.

Our study showed that the median (range) value of serum Ki-67 was 0.70 (0.01 – 2.54) ng/ml. serum Ki-67 showed higher median values with increasing age, postmenopausal status, increasing tumor size, nodal affection, higher grade, estrogen and progesterone receptors positive tumors but of no statistical significance. In our study, higher serum Ki-67 level was more frequently associated with HER2-positive and it is statistically significant (p value 0.036). Our study demonstrated that the median time to progression was 10 months. According to Kaplan-Meier analysis, the DFS rate at 20 months was 82.6%. Regarding Overall survival: OS was calculated from date of diagnosis till time of death from any cause. The median OS couldn't be reached at the end of study, no deaths so 1-year OS is 100%.

Conclusion: The results of our study support the finding that serum Ki-67 may be considered a valuable biomarker and add a prognostic information to classical prognostic factors

Keywords: Breast cancer, Serum ki-67, Prognosis

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Introduction:

Breast cancer is the most common cancer in females reaching one third of all cancer types. Breast cancer has a high metastatic potentiality leading to high mortality. The incidence of Breast cancer is increasing annually, seriously affecting the life and health of women. Developments in prognostic and predictor indicators have changed Breast cancer treatment with comprehensive systemic mode of treatment based on surgery, chemotherapy and endocrine, targeted, radiation and immune therapies. Breast cancer becomes among the malignant tumors with greater likelihood for a curative intent [1].

Among prognostic indicators of Breast cancer is Ki-67 that would be an important step in breast cancer management, Ki67 is a nuclear protein that is an excellent marker of active cell proliferation in the normal and tumor cell populations. It has been proposed as a useful clinical marker for breast cancer subtype classification, prognosis, and prediction of therapeutic response, many studies detect Ki-67 by ELISA (Enzyme-Linked Immunosorbent Assay) [2].

In neoadjuvant and adjuvant hormonal treatment downstaging of breast tumors using neoadjuvant hormonal treatment for ER-positive disease is most frequently practiced in elderly women who may not be tolerate chemotherapy. In addition, Ki67 expression after 6 months of hormonal therapy has been found to be more strongly prognostic than baseline values, because it is a derivative of both the prognostic value of baseline Ki67 and the suppressive effect of hormonal therapy on Ki67 in responsive patients [3]

Compared with other markers, Ki-67 immuno-staining is a convenient method for detecting the proliferating index. Ki-67 immunohistochemistry (IHC) is a rapid and inexpensive method that used in almost all pathological laboratories. Ki-67 levels are associated with positive prognosis [4, 5]

The proper Ki-67 cut-off value in hormone responsive breast cancer is carried out according to the following ki-67 risk classes: Low Risk (Ki-67 ≤ 14%); Intermediate Risk (Ki-67 15% - 20%); High Risk (Ki-67 > 20%) [6].

Baseline Ki67 and its change after short-term endocrine treatment have predictive value of recurrence-free survival [3].

Because Ki-67 is associated with cells that are rapidly growing and dividing, it is sometimes considered a good marker of proliferation (rapid increase in the number of cells) [7].

The aim of our study

In this prospective study, we aimed to:

- Investigating the serum level of ki67 protein baseline and after 6 months of hormonal treatment.in breast cancer patients.
- Detecting correlation between serum Ki67 and clinicopathological characteristics in newly diagnosed non- metastatic hormonal positive Brest cancer patients.
- Predicting response to hormonal therapy and to demonstrate the consistency between serum level of ki67 protein by ELISA detection in breast cancer patients.

Primary end point

Detecting level of Ki-67 protein by serum ELISA before (baseline) and after short course hormonal therapy (6 months treatment) and compare Ki 67 at presentation and after 6 months and compare the serum and tissue Ki-67 that was assessed by immunohistochemical technique.

Secondary end points

To evaluate

- Overall survival (OS)
- Disease free survival (DFS)

Patients and Methods:

This study is a prospective study; included 88 patients presented to the Outpatient Clinic in South Egypt Cancer Institute (SECI), Assiut University with primary breast carcinoma from January /2022 to December /2023.

The criteria for selecting the patients were: Age; more than 18 years, non metastatic hormonal positive breast cancer patients and no serious comorbidities as liver, renal or heart failure,

All patients were subjected to full history taking, clinical examination, laboratory investigations and imaging studies. The laboratory investigations included complete blood picture and serum chemistry. Breast, chest and abdomen imaging studies were done, Immunohistochemical staining, Nodal status, Tumer size also detected.

Blood sample:

Venous blood samples were collected from all participants before starting any therapy using a serum separator tube, the sera were separated by centrifugation at 3000 rpm for 10 min and stored at -20°C for further evaluation.

Serum ki-67 detection:

ki-67 serum level was evaluated according to the manufacturer's instructions using Human Ki67P (Ki-67 Protein) ELISA Kit (Cat: ELK2793, ELK Biotech, China)

Statistical analysis:

Data was collected and statistical calculations were performed with SPSS version 22. Categorical and continuous variables were analyzed using chi square, Mann–Whitney U tests. All statistical analysis was based on two-tailed hypothesis tests where $P<0.05$ was considered to indicate a statistically significant difference.

Ethical considerations:

The study was approved by the Medical Ethical Committee – south Egypt cancer institute (Approval

No: 582). Written informed consent was obtained from all participants before enrollment in this study.

Results:

In this study, clinical significance of serum Ki-67 as a prognostic indicator in breast cancer was evaluated among eighty-eight hormone- positive early breast cancer cases. More than half of cases (56 cases) are luminal A breast cancer and (32) cases of them are luminal B, no Her2 enriched breast cancer or triple negative, more than half of cases (55%) of them received Tamoxifen and (45%) received Femara, (31) cases of them are stage I, (37) cases are stage II and (20) cases are stage III. More than half of cases showed lower expression of Ki 67, Higher expression for ki67 (≥20%) detected in (32) cases. Moreover, the relationship between serum Ki-67 by ELISA with clinical, pathologic characteristics and patient outcome were detected.

Demographic and Clinico-pathological characteristics of the studied breast cancer cases are summarized in Table 1. The median age of studied patients was 48 years with range from 28-79. A total of 49 patients were premenopausal and 39 were postmenopausal. Based on the American Joint Committee on Cancer TNM staging, 31 patients had stage I cancer, 37 patients had stage II cancer and 20 patients had stage III. Among the studied cases, we found that seventy nine cases diagnosed with invasive ductal carcinoma, eight cases had invasive lobular carcinoma and one case had mucinous carcinoma,

The association between the level of Ki-67 and a number of conventional prognostic and predictive factors is shown in Table 2. Our study showed that median serum Ki-67 showed higher values with increasing age, postmenopausal status, increasing tumor size, nodal affection, lymphovascular affection and higher grade but of no statistical significance. Median serum Ki-67 expression in estrogen receptor and progesterone receptor positive tumors showed higher values than estrogen and progesterone negative tumors but it does not show statistical significance. In our study, higher serum Ki-67 expression was more frequently associated with HER2-positive and it is statically significant (p value 0.036).

Tissue ki-67 and clinicopathologic parameters:

Regarding tissue ki67 by immunohistochemistry, our study demonstrated that higher expression for ki67 (≥20%) in (32) patients are associated with increasing age, postmenopausal status, smaller tumor size, nodal affection, lymph vascular invasion but of no statistical significance.

Table 1: Demographic and clinicopathologic characteristics of studied patients

Clinico-pathological characteristic	N	(%)
Age (year)		
Mean ± SD	49.41 ± 12.61	
Median (range)	48 (28 – 79)	
• < 45 years	35	(39.8)
• ≥ 45 years	53	(60.2)
Menopausal status		
• Premenopausal	49	(55.7)
• Postmenopausal	39	(44.3)
Side		
• Right	32	(36.4)
• Left	55	(62.5)
Surgery		
• MRM	64	(72.7)
• BCS	24	(27.3)
Types of pathology		
• IDC	79	(89.8)
• ILC	8	(9.1)
• Mucinous IC	1	(1.1)
Stage		
• Stage 1	31	(35.2)
• Stage 2	37	(42.0)
• Stage 3	20	(22.7)
T staging		
• T0	1	(1.1)
• T1	13	(14.8)
• T2	59	(67.0)
• T3	15	(17.0)
N staging		
• N0	21	(23.9)
• N1	28	(31.8)
• N2	23	(26.1)
• N3	15	(17.0)
• Nx	1	(1.1)
LVI		
• Absent	33	(37.5)
• Present	55	(62.5)
Grade		
• Grade 1	5	(5.7)
• Grade 2	70	(79.5)
• Grade 3	13	(14.8)
CIS (carcinoma in situ)		
• Absent	36	(40.9)
• Present	52	(59.1)
Perineural Invasion		
• Absent	46	(52.3)
• Present	42	(47.7)
ER		
• Negative	10	(11.4)
• Positive	78	(88.6)
PR		
• Negative	11	(12.5)
• Positive	77	(87.5)
Her2neu		
• Negative	72	(81.8)
• Positive	16	(18.2)
Ki-67 tissue expression		
• (≥20%)	32	(36.3)
• (<20%)	56	(63.6)

Qualitative data are presented as number (percentage).

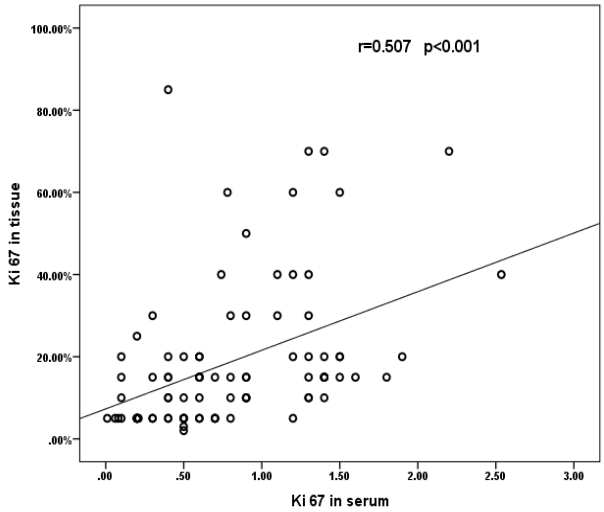

Fig. (1): Scatter plot diagram showing the correlation between serum and tissue KI-67 levels among the studied breast cancer cases

Outcome analysis:

During follow-up, four out of eighty-eight patients (4.5%) developed focal hepatic metastasis. The median time to progression was 10 months. According to Kaplan-Meier analysis, the DFS rate at 20 months was 82.6%.

Regarding Overall survival: OS was calculated from date of diagnosis till time of death from any cause. The median OS couldn't be reached at the end of study, no deaths so 1-year OS is 100%.

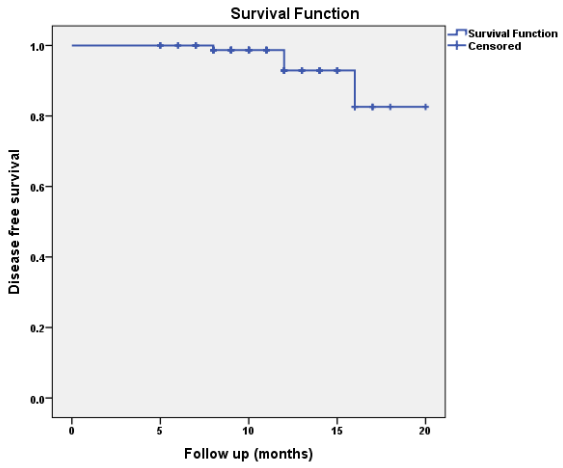

Fig. (2): Disease free survival curves among the studied breast cancer cases.

Discussion:

Breast cancer (BC) is the most common cancer and the second most common cause of death in women. The global estimated new cases in 2023 were 287,580 women diagnosed and 43,250 women died from BC [8].

Early diagnosis and treatment of breast cancer leads to improvement of the prognosis and increase survival rate. Ki-67 is a proliferative marker can be used along with other markers such as ER, PR, and HER2 in breast cancer management [1].

In this study, we aimed to measure the serum level baseline and after 6 months of hormonal treatment, compare the result with tissue expression of Ki-67 and to analyze the association between its level with other prognostic factors of the studied cases and patients' outcome.

Our study showed that median serum Ki-67 showed higher values with increasing age, postmenopausal status, increasing tumor size, nodal affection, lymphovascular affection and higher grade but of no statistical significance.

In agreement with our findings, a study by Ragab, Samy [9] which included 92 patients with primary breast carcinoma showed no statistically significant association between serum level of Ki-67 and the age of patients, nodal affection, pathological types, size, and grade of the tumor.

On contrary, a retrospective study by Kanyılmaz, Yavuz [10] which conducted on 590 women with breast cancer, found no correlation between patient age, menopausal status, and Ki-67 index.

Also, a study by Abbas and Hamdy [11] which included 120 breast cancer patients found significant association between Ki-67 level with increasing age, tumor size and lymph node affection. This different result may be explained by difference in patient's characteristics (higher stage) as 40 % of cases was in T4 stage, N3 was 38.3 % of studied patients and the percentage of the studied cases aged 40 years old or more was 90%.

Also, Ren, Wei [12] concluded that serum ki67 level in the breast cancer patients was closely related to lymph node metastasis but was not obviously related to other clinico pathological features. The high expression of ki67 in breast cancer tissues was related to tumor size, histological grade and lymph node metastasis.

Regarding tissue ki67 by IHC, our study demonstrated that higher expression for ki67 (≥20%) is associated with increasing age, postmenopausal status, smaller tumor size, nodal affection, lymphovascular invasion but of no statistical significance.

A similar finding was demonstrated by Petrelli, Viale [13] who found that a higher Ki-67 index was significantly correlated with increasing tumor size and lymph node positivity.

In our study, median serum Ki-67 level in estrogen receptor and progesterone receptor positive tumors showed higher values than estrogen and progesterone negative tumors but it does not show statistical significance. Higher serum Ki-67 level was more frequently associated with HER2-positive and it is statistically significant (p value 0.036).

Table 2: Relation between baseline "pretreatment" serum Ki-67 and Clinic-pathological characteristic of the studied breast cancer cases

Demographic characteristic	Cases (No.)	Serum Ki-67 Median (range) (ng/ml)	P value
Age (year)			0.130
• < 45 years	35	0.60 (0.01 – 2.20)	
• ≥ 45 years	53	0.85 (0.06 – 2.54)	
Menopausal status			0.608
• Premenopausal	49	0.60 (0.01 – 2.54)	
• Postmenopausal	39	0.78 (0.06 – 1.90)	
Stage			0.742
• Stage 1	31	0.74 (0.20 – 2.54)	
• Stage 2	37	0.70 (0.01 – 2.20)	
• Stage 3	20	0.60 (0.10 – 1.90)	
T staging			0.526
• Early	73	0.70 (0.01 – 2.54)	
• Advanced	15	0.90 (0.10 – 1.90)	
N staging			0.802
• Negative	21	0.60 (0.20 – 2.54)	
• Positive	67	0.70 (0.01 – 2.20)	
LVI			0.479
• Absent	33	0.60 (0.06 – 2.54)	
• Present	55	0.78 (0.01 – 2.20)	
Grade			0.389
• Grade 1	5	0.50 (0.20 – 1.30)	
• Grade 2	70	0.70 (0.01 – 2.54)	
• Grade 3	13	0.80 (0.10 – 2.20)	
CIS			0.293
• Absent	36	0.85 (0.06 – 1.60)	
• Present	52	0.60 (0.01 – 2.54)	
Perineural Invasion			0.713
• Absent	46	0.70 (0.08 – 2.54)	
• Present	42	0.70 (0.01 – 2.20)	
ER			0.968
• Negative	10	0.60 (0.20 – 1.50)	
• Positive	78	0.70 (0.01 – 2.54)	
PR			0.349
• Negative	11	0.50 (0.10– 2.20)	
• Positive	77	0.78 (0.01 – 2.54)	
Her2neu			**0.036**
• Negative	72	0.60 (0.01 – 2.54)	
• Positive	16	1.25 (0.10 – 1.50)	

Quantitative data are presented as median (range), significance defined by $p < 0.05$

Table 3: Relation between KI-67 tissue level and the clinico-pathological details of the studied breast cancer cases

Variable name	Tissue Ki-67< 20		Tissue Ki-67 ≥ 20		P value
Age (year)					0.178
• < 45 years	26	(44.8)	9	(30.0)	
• ≥ 45 years	32	(55.2)	21	(70.0)	
Menopausal status					0.221
• Premenopausal	35	(60.3)	14	(46.7)	
• Postmenopausal	23	(39.7)	16	(53.3)	
Stage					0.711
• Stage 1	22	(37.9)	9	(30.0)	
• Stage 2	24	(41.4)	13	(43.3)	
• Stage 3	12	(20.7)	8	(26.7)	
T staging					0.259
• Early	50	(86.2)	23	(76.7)	
• Advanced	8	(13.8)	7	(23.3)	
N staging					0.933
• Negative	14	(24.1)	7	(23.3)	
• Positive	44	(75.9)	23	(76.7)	
LVI					0.728
• Absent	21	(36.2)	12	(40.0)	
• Present	37	(63.8)	18	(60.0)	
Grade					0.156
• Grade 1	5	(8.6)	0	(0.0)	
• Grade 2	43	(74.1)	27	(90.0)	
• Grade 3	10	(17.2)	3	(10.0)	
CIS					0.212
• Absent	21	(36.2)	15	(50.0)	
• Present	37	(63.8)	15	(50.0)	
Perineural Invasion					0.759
• Absent	31	(53.4)	15	(50.0)	
• Present	27	(46.6)	15	(50.0)	

Qualitative data are presented as number (percentage), significance defined by $p < 0.05$.

Table 4: The correlation between the serum and tissue KI-67 levels among the studied breast cancer cases, our study shows that an increase in tissue KI-67 associated with increase in serum KI-67.

		Ki 67 in serum
Ki 67 in tissue	r	0.507
	P	**<0.001**

Significance defined by $p < 0.05$, r=correlation coefficient

Table 5: The level of serum KI-67 among the studied breast cancer cases from baseline to after six months of follow up. Our study shows significant decreasing serum KI-67 after 6 month of hormonal therapy (**p value 0.001)**

	Median (range)	**P value**
Serum Ki-67 (ng/ml)		**0.001**
• At baseline	0.70 (0.01 – 2.54)	
• After 6 months	0.58 (0.03 – 5.36)	

Quantitative data are presented as median (range), significance defined by $p < 0.05$.

Table 6: Disease free survival according to clinico-pathological details of the studied breast cancer cases (n=88)

DFS (one years)	Estimate ± SE	P value
Age		0.378
< 45 years	91.5 ± 6.0%	
≥ 45 years	93.8 ± 6.1%	
Menopausal status		0.112
Premenopausal	87.9 ± 6.9%	
Postmenopausal	100.0 ± 0.0%	
Stage		0.824
Early	91.3 ± 5.0%	
Advanced	100.0 ± 35.4%	
ER		0.632
Negative	100.0 ± 0.0%	
Positive	92.4 ± 4.4%	
PR		0.411
Negative	100.0 ± 0.0%	
Positive	92.0 ± 4.7%	
Her2neu		0.740
Negative	94.9 ± 3.8%	
Positive	83.3 ± 15.2%	
Ki in serum (ng/ml)		0.375
< 0.70	92.0 ± 5.8%	
≥ 0.70	93.8 ± 6.1%	
Ki in tissue (%)		0.549
< 20.0%	93.5 ± 4.7%	
≥ 20.0%	91.7 ± 8.0%	

Additionally in the line with our result Li, Wu [14] reported that there was no correlation between Ki-67 index with ER positivity. While Shokouh, Ezatollah [15] and Kanyılmaz, Yavuz [10] found that high Ki-67 expression was correlated with HER2 positivity.

On the other side, Kontzoglou, Palla [16] demonstrated higher Ki-67 expression was more frequently associated with HER2-negative. Also, Stathopoulos, Malamos [17] who reported HER2-negative had significantly higher Ki-67 values. These differences may be explained by different patient characteristics and different method of Ki67 detection.

Another interesting finding reported by Ren, Wei [12] showed that high expression of ki67 in breast cancer tissues was related to estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). This difference may be explained by different method of detection and different characteristics of studied sample

The major finding of our results is that significant decreasing serum Ki67 after 6 months of hormonal therapy among studied breast cancer cases (p value 0.001) (which confirm response to hormonal therapy).

In agreement with our results, Dowsett et al. [3] concluded that a reduction in Ki-67 predicts tumor shrinkage to hormonal agents in breast cancer. Thus, a substantial reduction of Ki-67 LI after a short-term challenge test of a 6 month of hormonal treatment might be a simple and unexpensive way to select women with (ER)-positive BC who may not benefit from adjuvant chemotherapy

Similar results reported by Ellis et al. [18] had been shown that higher Ki-67 after 6 months of hormonal treatment with either tamoxifen or anastrozole or their combination was associated with shorter recurrence-free survival.

The results of most studies that investigating the impact of Ki-67 on survival was variable. Some studies showed prognostic effect of Ki-67 expression on survival outcome but others did not demonstrate any correlation. In our study we found no relationship between serum Ki-67 level and DFS. Consistent with our results, A study by Soliman and Yussif [19] found that there was no statistically significant correlation between Ki-67 index with DFS of their studied patients. Also, Zenzola, Cabezas-Quintario [20] demonstrated a similar result.

Additionally, Nishimura, Osako [21] demonstrated that high Ki-67 was significantly correlated with a lower survival. A study done by Yerushalmi, Woods [22] involving 12,155 patients showed that Ki-67 positivity denotes a higher risk of recurrence and worse prognosis in early breast cancer patients. Ibrahim, Farolfi [23] confirmed that high Ki-67 is associated

with worse survival rate. While Kanyılmaz, Yavuz [10] found a significant relationship between high expression of Ki-67 and poor DFS. These differences may be explained different sample size, short follow-up time and different method of detection of Ki67.

Conclusion:

Our study showed higher values of serum ki67 with increasing age, postmenopausal status, increasing tumor size, node positivity and higher grade but of no statistical significance. Median serum Ki-67 expression in estrogen receptor and progesterone receptor positive tumors showed higher values than estrogen and progesterone negative tumors but it does not reach statistical significance while higher serum Ki-67 expression was more frequently associated with HER2-positive and it is statistically significant. Regarding ki-67 tissue expression by immunohistochemistry, our study demonstrated that higher expression (≥20%) is associated with increasing age, postmenopausal status, smaller tumor size, nodal affection and lymph vascular invasion but of no statistical significance.

Also, our study shows that significant decreasing serum Ki-67 after 6 months of hormonal therapy (was correlated with tumor response). Accordingly, ki67 can be used to evaluate response to hormonal therapy.

Regarding patient outcome, we found no relationship between serum Ki-67 level and DFS.

Our recommendation is to focus on standardization of cut off value of Ki-67 assessment and increasing sample size to avoid any contradictory results in Ki-67.

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Conflict of interest

None to declare

Authors` contributions

This work was carried out in collaboration between all authors.

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