Immunohistochemical Expression and Potential Value of c-MYC in Non-invasive and Invasive Mammary Carcinoma

Marwa Mahmoud Hassan[a]*, Afaf Taha El Nashar[b], Maisa Hashem Mohammed[b]

[a]Department of Pathology, Qena Oncology Center, Qena, Egypt.

[b]Department of Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt.

Abstract

Background: Breast cancer (BC) is the most prevalent cancer diagnosed globally. In Egypt, BC is the most common type of female cancer, about 22,000 new cases diagnosed every year. The most common variants of breast carcinoma are invasive duct carcinoma (IDC) and invasive lobular carcinoma (ILC). Non-invasive BC has malignant cells within the ducts without stromal invasion. Molecular subtypes of BC are including Luminal A, Luminal B, HER2 enriched and basal like subtypes. c-Myelocytomatosis (MYC) protein is a transcription factor and has role in DNA synthesis, cellular proliferation, differentiation and immortalization. c-MYC in BC cells can promote tumor progression by facilitating invasion and metastasis.

Objectives: Evaluation of c-MYC in BC and its relation with clinicopathological parameters and molecular subtypes.

Patients and methods: 52 cases of BC were histopathologically evaluated using a standard H&E stain and assessed immunohistochemicaly for c-MYC protein expression.

Results: c-MYC expression and higher tumor grade showed a significant association (p-value =0.040 and molecular subtypes, especially TNBC (p-value=0.010).

Conclusion: Elevated c-MYC expression is related to poor prognostic pathological parameters of BC.

Keywords: BC; c-MYC expression; Immunohistochemistry.

DOI: 10.21608/SVUIJM.2025.362398.2122

Correspondence: marwa.m.hassan95@gmail.com

Received: 1 February,2025.

Revised: 25 February, 2025.

Accepted: 4 March, 2025.

Published: 8 March, 2025

Cite this article as Marwa Mahmoud Hassan, Afaf Taha El Nashar, Maisa Hashem Mohammed. (2025). Immunohistochemical Expression and Potential Value of c-MYC in Non-invasive and Invasive Mammary Carcinoma. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 1, pp: 535-544.

Introduction

BC incidence and mortality have increased in both developing and developed countries in the past three decades. About 2.3 million new cases of BC in women were diagnosed worldwide in 2020, according to the World Health Organization (WHO) **(Lv et al., 2023).**

In Egypt, the incidence rate of BC is lower than the global incidence rate; however the mortality rate is higher **(Azim et al., 2023).**

Invasive breast carcinoma involves two main forms; invasive duct carcinoma (IDC) and invasive lobular carcinoma (ILC). IDC is the commonest type and shows infiltration of malignant cells beyond basement membrane. ILC represents 10% of all breast cancers and shows infiltration of surrounding breast tissue in single cell pattern **(Chotai et al., 2020).**

IDC has no specific gross picture and shows great variations in size from a few millimeters to huge mass. Microscopically, malignant cells are arranged in tubules, ducts and occasionally in solid sheets with desmoplastic intervening stroma. Nuclei are uniform with inconspicuous nucleoli or highly pleomorphic with prominent nucleoli **(Tuzlali, 2019).**

ILC appears with irregular borders but sometimes may not be defined and breast tissue looks normal with only firm consistency. Microscopically, tumor cells have the arrangement of single files and encircle the mammary duct in targetoid pattern. They are small and uniform cells showing cellular discohesion due to loss of the E-Cadherin protein. This protein has a role in adhesion of cells together **(Christgen et al., 2021).**

Non-invasive breast carcinoma can be of ductal type (ductal carcinoma in situ, DCIS) or of lobular type (lobular carcinoma in situ, LCIS) **(Rosai, 2011).**

DCIS is presented by proliferation of neoplastic ductal cells that are bordered by myoepithelial cells and surrounded by intact basement membrane. According to nuclear grade, differentiation and presence of necrosis, DCIS can be divided into three grades: low, intermediate, and high grade **(Brčić I and Balić, 2017).**

LCIS develops in terminal duct lobular units, where the lobular cells may include the major ducts in a pagetoid pattern and distend the mammary lobules. Small lobular cells have eccentric plasmacytoid nuclei and little cytoplasm **(Shaaban, 2021).**

The transcription factor c-MYC is found in the nucleus of cells and is associated with the basic-helix-loop-helix-leucine zipper family. It controls the division, growth, metabolism, and death of cells. In many human malignancies, it is commonly dysregulated **(Madden et al., 2021).**

c-MYC stimulates cancer cell growth by inducing a substantial rise in ribosomal and protein biogenesis. c-MYC promotes cellular survival by specific influence on DNA replication. c-MYC overexpression can affect host endothelial cells leading to tumor micro-environment reprogramming to induce angiogenesis **(Dhanasekaran et al., 2022).**

Our study aimed to determine the immunohistochemical expression of c-MYC and the relationship between its expression and clinicopathological parameters in non-invasive and invasive BC.

Patients and methods

The Sohag University Faculty of Medicine's Ethics Committee accepted this study, and it was assigned a registration number: Soh-Med-24-3-15MS. A retrospective study was conducted on fifty two formalin-fixed paraffin-embedded (FFPE) tissue blocks of IDC and ILC which were collected from the archives of the

Surgical Pathology Laboratory of Qena Oncology Center in the period from January 2022 to December 2023.

4 µm thick paraffin-embedded fixed-formalin- tissue sections were placed on slides coated with 3-aminopropyltriethoxysilane (APES). Sections were deparaffinized in Xylene and rehydrated using graded alcohols. Tissue slices were incubated with primary mouse monoclonal c-MYC antibody for 30 minutes at room temperature and washed with phosphate buffer saline (PBS) solution. Tissue sections were incubated with a biotinylated goat secondary antibody for 20 minutes at room temperature then they were washed in PBS twice. Diammoniobenzidine (DAB) chromogen was applied for five to ten minutes.

Counterstaining was done with Mayer's haematoxylin, followed by clearing and mounting.

Immunohistochemical scoring

IRS, or immunoreactive score, was used. According to **Blanccato et al. (2004),** IRS was calculated by multiplying an estimate of the staining intensity (intensity score; IS) by an estimate of the proportion of immunoreactive cells (quantity score; QS). The following is how staining quantity is scored: No staining = 0, 1–25% of stained cells = 1, 26–50% of stained cells = 2, 51–75% of stained cells = 3, and 75–100% of stained cells = 4. On a scale of 0 to 3, staining intensity is rated as follows: No staining = 0, Weak = 1, Moderate = 2, and Strong = 3. Negative scores are 0, mildly positive scores are 1, 2, 3, and 4, moderately positive scores are 6 and 8, and very positive scores are 9 and 12 **(Blancato et al., 2004).**

Statistical analysis

SPSS version 20 (Statistical Software package version 20) was used to analyze the data. The range, mean ± standard deviation (SD), and median were used to depict quantitative data. Data was analyzed using student t-test to compare means of two groups and ANOVA for comparison of the means of three groups or more. Chi-square and Mann-Whitney tests were employed to compare groups when the data was categorical. The STATA or Excel programs were used to create the graphs. If the P value was less than 0.05, it was deemed significant.

Results

(Table.1) summarizes the clinicopathological characteristics of the 52 BC cases that were a part of this study. Patients were between the ages of 37 and 84 (mean ± SD and median 57.17 ± 12.26 and 59 years, respectively).

Table 1. Clinical and pathological parameters of the studied cases

Parameters		**Frequency**	**Percentage**
Age (years)	≤60	32	61.5%
	>60	20	38.5%
	Mean ± SD	57.17 ± 12.260	
	Median	59 (37-84)	
Tumor grade	Grade 1	4	7.7%
	Grade 2	39	75%
	Grade 3	9	17.3%
Tumor stage	T1	16	30.8%
	T2	25	48.1%
	T3	8	15.4%
	T4	3	5.8%

Nodal metastasis	Positive	34	65.4%
	Negative	18	34.6%
Histopathological types	Ductal carcinoma	47	90.4%
	Lobular carcinoma	5	9.6%
In situ presence	Yes	25	48.1%
	No	27	51.9%
Molecular subtypes	Luminal A	16	30.8%
	Luminal B	27	51.9%
	Her2-enriched	6	11.5%
	Triple-negative	3	5.8%
Ki-67	High ≥14	36	69.2%
	Low <14	16	30.8%
Multifocality	Multifocal	9	17.3%
	Unifocal	43	82.7%
Laterality	Unilateral	47	90.4%
	Bilateral	5	9.6%
PNI	Positive	23	44.2%
	Negative	29	55.8%
LVI	Positive	34	65.4%
	Negative	18	34.6%

Immunohistochemical expression of c-MYC

c-MYC protein expression in tumor cells appeared as brownish nuclear staining. Using immunoreactive score, c-MYC expression was positive in 37/47 of cases with ductal carcinoma **(Fig.1)** and 3/5 of cases with lobular carcinoma **(Fig.2).** 21/25 (84%) of in situ component showed positive c-MYC expression **(Fig. 3).** Negative expression of c-MYC was found in 12/52 (23%) of invasive tumors and 4/25 (16%) of in situ tumors **(Fig.1C).** 38/48 (79%) of grade I and grade II cases showed positive c-MYC expression. 3/3 (100%) of T4 cases and 26/34 (76.5%) of positive LN metastasis showed positive c-MYC expression. 17/23 (73.9%) of cases with positive PNI revealed positive c-MYC expression while 6/23 (16.1%) showed negative PNI. 25/34 (73%) of cases with positive LVI had positive c-MYC expression. (4/5) 80% and (5/9) 55.6% of bilateral and multifocal tumors showed positive c-MYC expression, respectively **(Table. 2).**

Table 2. Evaluation of c-MYC expression in clinicopathological parameters

Parameters		**Immunoreactive score**	
		Positive (n= 40)	Negative (n=12)
Age (years)	**Mean ± SD**	56.80 ± 12.149	58.42 ± 13.09
Grade	1 (4)	2	2
	2 (39)	32	7
	3 (9)	6	3
Tumor Stage	T1 (16)	14	2
	T2 (25)	17	8
	T3 (8)	6	2
	T4 (3)	3	0
Perineural invasion	Positive (23)	17	6
	Negative (29)	23	6

Histopathology	Ductal (47)	37	10
	Lobular (5)	3	2
In situ presence	Yes (25)	21	4
	No (27)	19	8
Nodal status	Positive (34)	26	8
	Negative (18)	14	4
Lymphovascular emboli	Positive (34)	25	9
	Negative (18)	15	3
Multifocality	Multifocal (9)	5	4
	Unifocal (43)	35	8
Laterality	Unilateral (47)	36	11
	Bilateral (5)	4	1

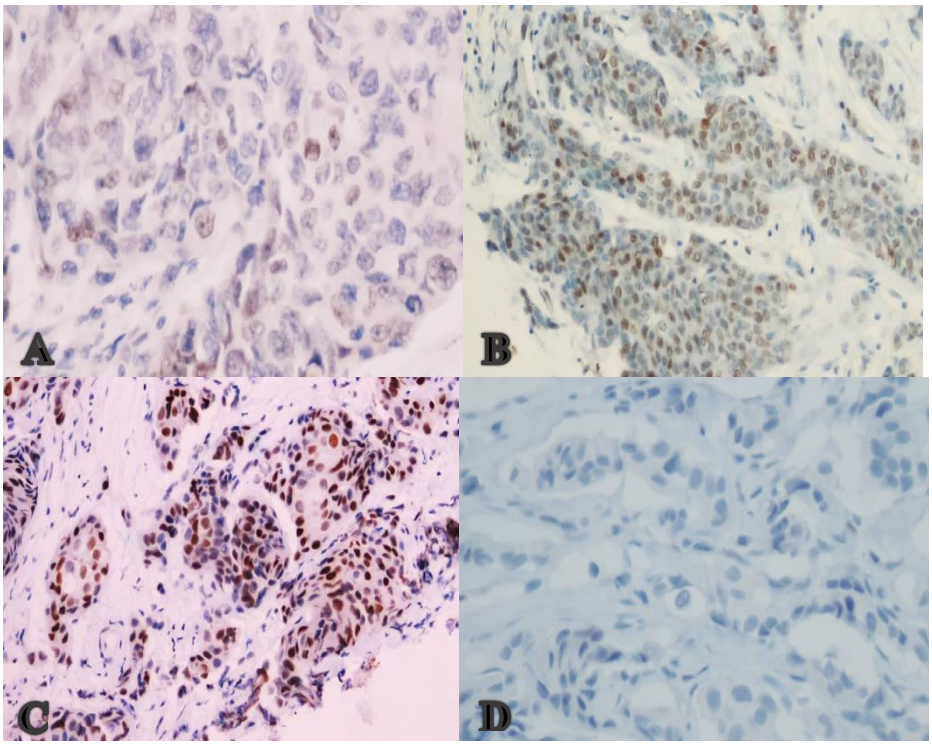

Fig. 1. Positive and negative c-MYC expression in IDC. A: Mild c-MYC nuclear expression in IDC (X400). **B:** Moderate c-MYC nuclear expression in IDC (X200)**. C:** Strong c-MYC nuclear expression in IDC (X200)**. D:** Negative c-MYC expression (X200)

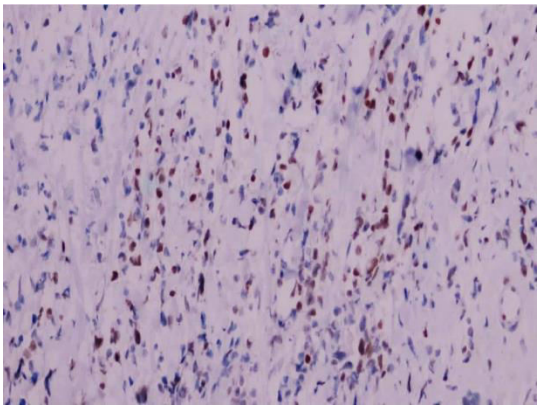

Fig. 2. Positive c-MYC nuclear expression in ILC (X200)

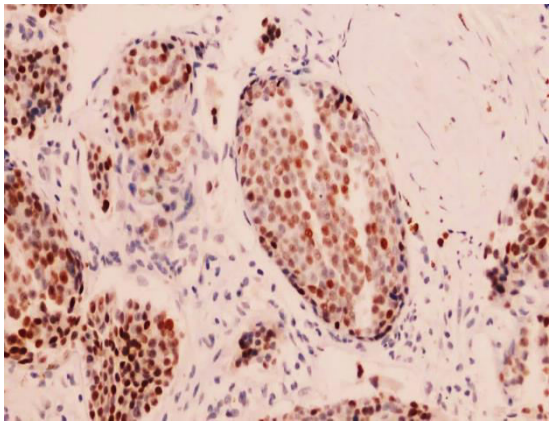

Fig.3. Positive c-MYC nuclear expression in DCIS (X200)

c-MYC expression and tumor grade had significant association with each other; the median IRS was significantly higher among grades II and III compared to grade I p-value=0.040. However, statistical evaluation of c-MYC expression in relation to T-stage or nodal metastasis, histopathological subtype, multifocality, laterality, presence of LVI, PNI or insitu component showed no significance **(Table. 3).**

Table 3. Association between c-MYC expression and clinicopathological parameters

Parameters		IR score Median (IQR)	P value
Tumor grade	G1	0.50 (0-1)	**0.040***
	G2, G3	2.50 (1-6)	
T-stage	T1	2 (1-6)	0.527
	T2, T3, T4	2 (0-4)	
Histopathology	Ductal	2 (1-6)	0.863
	Lobular	4 (0-7)	
In situ presence	Yes	3 (1-6)	0.138
	No	2 (0-4)	
Multifocality	Unifocal	2 (1-6)	0.485

	Multifocal	2 (0-6)	
Laterality	Unilateral	2 (1-6)	0.826
	Bilateral	3 (1-5)	
Nodal status	Positive	2 (0.75-6)	0.915
	Negative	2.50 (0.75-6)	
Lymphovascular invasion	Positive	2 (0-4.50)	0.340
	Negative	3.50 (1-6)	
Perineural invasion	Positive	2 (0-4)	0.852
	Negative	2 (1-6)	

Mann-Whitney test . *means significant

c-MYC expression showed significant association with molecular subtypes. 66.67% of Triple-negative tumors had a strong c-MYC expression compared to 3.7% in Luminal B and 0% in Luminal A subtypes p-value=0.010. Also, Ki-67 index showed significant association with molecular subtypes. 100% of triple-negative and Her-2 enriched tumors had high Ki-67 compared to 12.5% among the Luminal A subtypes p-value<0.001. Molecular subtypes did not significantly correlate with tumor stage, grade, multifocality, laterality, PNI, LVI, or nodal metastases (p-value >0.05) **(Table. 4).**

Table 4. Relation between molecular subtypes, c-MYC expression and clinicopathological parameters

Parameters		**Molecular subtypes**				**P value**
		Luminal A (n= 16)	Luminal B (n=27)	Her-2 enriched (n=6)	Triple-negative (n=3)	
c-MYC expression	Negative	5	5	2	0	**0.010***
	Mild	8	14	3	1	
	Moderate	3	7	1	0	
	Strong	0	1	0	2	
Grade	I	3	1	0	0	0.316
	II	12	19	5	3	
	III	1	7	1	0	
Tumor Stage	T1	7	7	1	1	0.774
	T2	6	14	4	1	
	T3	2	5	0	1	
	T4	1	1	1	0	
Perineural invasion	Positive	9	15	3	2	0.973
	Negative	7	12	3	1	
Nodal status	Positive	5	9	3	1	0.922
	Negative	11	18	3	2	
Lymphovascular	Positive	5	9	3	1	0.922

emboli	Negative	11	18	3	2	
Multifocality	Multifocal	4	3	1	1	0.588
	Unifocal	12	24	5	2	
Laterality	Unilateral	15	24	5	3	0.816
	Bilateral	1	3	1	0	
Ki-67	High	2	25	6	3	**<0.001***
	Low	14	2	0	0	

Chi-square test ; *means significant

Discussion

This study included 52 specimens of invasive mammary carcinoma with co-exist in situ component. The age range of the studied cases was 37 to 83 years old and the median age was 59 years old. These results were close to the findings of **Giaquinto et al. (2022)** who reported that the age range of their studied cases was 35-90 years old with a median age of 62 years old.

Regarding histological type, 90.4% of cases had invasive duct carcinoma while 9.6% of cases had invasive lobular carcinoma. These findings were close to **Zhao (2021)** who reported that 84.2% and 9.6% of their studied cases had IDC and ILC respectively.

In our study, 48% of cases had in situ component. These results were near to those reported by **Heng et al. (2017)** who found that 45.5% of cases had DCIS component.

In this study 16/52 (30.8%) of studied cases had tumor size of ≤ 2 cm, while 36/52 (69.2%) of cases had tumor size of > 2 cm which was similar to the results of **Elkin et al. (2005)** who found that 67% of their cases had tumor size of > 2 cm. While **Catacchio et al. (2019)** reported that 64.6% of their studied cases had tumor size of ≤ 2 cm. This may be due to wide spread use of screening programs in developed countries.

Regarding tumor grade; the current study had 7.7% of cases grade I, 75% grade II and 17.3% grade III tumor. There was similarity to what recorded by **Elkhodary et al. (2014)** and near to those recorded by **Wang et al. (2022)** who reported that 12% of cases grade I, 65% grade II and 23% grade III.

Current study showed a significant association between c-MYC expression and tumor grade p-value=0.040. This aligns with many researches **(Blancato et al., 2004; Green et al., 2016; Qu et al., 2017).**

Regarding molecular subtypes; luminal A and B subtypes represented 43/52 (82.6%), HER2-enriched subtype 6/52 (11.5%) and TNBC 3/52 (5.3%) of the studied cases in the present study. These results were in agreement with **Abdelaziz et al. (2023)** who recorded that 82.7% of cases were luminal A and luminal B subtypes while 17.3% of cases were non luminal subtypes.

In the current study there is significant association between c-MYC expression and molecular subtypes (p-value=0.010); 66.67% of Triple-negative tumors had a strong c-MYC expression. This was consistent with **Green et al. (2016)** who revealed a relation between elevated c-MYC expression and different molecular subtypes of BC (p-value<0.001).

There is no significant relation between c-MYC expression and histopathological types of BC which is consistent with **(Burkhardt**

et al., 2010). However, **Green et al. (2016)** reported a significant relation between histopathological types and c-MYC expression. This may be due to difference in sampling; this study includes IDC and ILC only but the other study includes IDC, ILC and other histopathological types.

There is no significant association between c-MYC expression and patient age, tumor stage, PNI or LVI. That is in agreement with **(Green et al., 2016).** However, 100% of cases with T4 stage showed c-MYC expression that is close to **Todorović-Raković et al. (2012)** who stated that most cases of T4 breast cancer showed c-MYC expression. In our study; negative c-MYC expression was found in the older cases. This is correlated with **Todorović-Raković et al. (2012)** who found that negative c-MYC expression was more in post-menopausal women.

Conclusion

The current findings indicate that aggressive molecular subtypes and higher tumor grades are linked to positive c-MYC expression. c-MYC is a potentially helpful biomarker for determining the aggressiveness of tumors.

References

- **Abdelaziz MA, Ebrahim EM and El-Din Zikry MS. (2023).** Evaluation of Pattern of Relapse in Luminal Breast Cancer. Al-Azhar International Medical Journal, 4(3): 9.
- **Azim HA, Elghazawy H, Ghazy RM, Abdelaziz AH, Abdelsalam M, Elzorkany A, et al., (2023).** Clinicopathologic features of breast cancer in Egypt—contemporary profile and future needs: a systematic review and meta-analysis. JCO Global Oncology, 9: e2200387.
- **Blancato J, Singh B, Liu A, Liao DJ and Dickson RB. (2004).** Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridisation and immunohistochemical analyses. British journal of cancer, 90(8): 1612-1619.
- **Brčić I and Balić M. (2017).** Molecular Classification of Breast Cancer. Mechanisms of Molecular Carcinogenesis, 2: 1-22.
- **Burkhardt L, Grob TJ, Hermann I, Burandt E, Choschzick M, Jänicke F, et al., (2010).** Gene amplification in ductal carcinoma in situ of the breast. Breast cancer research and treatment, 123: 757-765.
- **Catacchio I, Silvestris N, Scarpi E, Schirosi L, Scattone A and Mangia A. (2019).** Intratumoral, rather than stromal, CD8+ T cells could be a potential negative prognostic marker in invasive breast cancer patients. Translational oncology, 12(3): 585-595.
- **Chotai N, Kulkarni S, Chotai N and Kulkarni S. (2020).** Invasive Mammary Carcinoma. Breast Imaging Essentials: Case Based Review: 91-110.
- **Christgen M, Cserni G, Floris G, Marchio C, Djerroudi L, Kreipe H, et al., (2021).** Lobular breast cancer: histomorphology and different concepts of a special spectrum of tumors. Cancers, 13(15): 3695.
- **Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM and Felsher DW. (2022).** The MYC oncogene—the grand orchestrator of cancer growth and immune evasion. Nature reviews Clinical oncology, 19(1): 23-36.
- **Elkhodary TR, Ebrahim MA, Hatata EE and Niazy NA. (2014).** Prognostic value of lymph node

ratio in node-positive breast cancer in Egyptian patients. Journal of the Egyptian National Cancer Institute, 26(1): 31-35.

- **Elkin EB, Hudis C, Begg CB and Schrag D. (2005).** The effect of changes in tumor size on breast carcinoma survival in the US: 1975–1999. Cancer: Interdisciplinary International Journal of the American Cancer Society, 104(6): 1149-1157.
- **Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, et al., (2022).** Breast cancer statistics, 2022. CA: a cancer journal for clinicians, 72(6): 524-541.
- **Green AR, Aleskandarany MA, Agarwal D, Elsheikh S, Nolan CC, Diez-Rodriguez M, et al., (2016).** MYC functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. British journal of cancer, 114(8): 917-928.
- **Heng Y J, Lester SC, Tse GM, Factor RE, Allison KH, Collins LC, et al., (2017).** The molecular basis of breast cancer pathological phenotypes. The Journal of pathology, 241(3): 375-391.
- **Lv L, Zhao B, Kang J, Li S and Wu H. (2023).** Trend of disease burden and risk factors of breast cancer in developing countries and territories, from 1990 to 2019: Results from the Global Burden of Disease Study 2019. Frontiers in Public, 10:1078191.
- **Madden S K, de Araujo A D, Gerhardt M, Fairlie D P and Mason JM. (2021).** Taking the Myc out of cancer: toward therapeutic strategies to directly inhibit c-Myc. Molecular Cancer, 20(1): 3.
- **Qu J, Zhao X, Wang J, Liu X, Yan Y, Liu L, et al., (2017).** MYC overexpression with its prognostic and clinicopathological significance in breast cancer. Oncotarget, 8(55): 93998.
- **Rosai J. (2011).** Rosai and Ackerman's surgical pathology e-book. Elsevier Health Sciences.
- **Shaaban AM. (2021)**. Why is LCIS important—pathological review. Current Breast Cancer Reports, 13: 132-140.
- **Todorović-Raković N, Nešković-Konstantinović Z and Nikolić-Vukosavljević D. (2012).** C-myc as a predictive marker for chemotherapy in metastatic breast cancer. Clinical and experimental medicine, 12: 217-223.
- **Tuzlali S. (2019).** Pathology of breast cancer. Breast Disease: Diagnosis and Pathology, 1: 201-220.
- **Wang Y, Acs B, Robertson S, Liu B, Solorzano L, Wählby C and Rantalainen M. (2022).** Improved breast cancer histological grading using deep learning. Annals of Oncology, 33(1): 89-98.
- **Zhao H. (2021).** The prognosis of invasive ductal carcinoma, lobular carcinoma and mixed ductal and lobular carcinoma according to molecular subtypes of the breast. Breast Cancer, 28: 187-195.