International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Epidemiology, risk factors and molecular subtypes of Breast Carcinoma

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Abstract

Breast cancer (BC) is the leading cause of cancer-related death among women and the most commonly diagnosed cancer worldwide. The 5-year overall survival (OS) from BC has increased steadily in most of the developed countries. The incidence rates of BC vary worldwide, with high rates in North America, Northern and Western Europe, intermediate rates in South America and Southern Europe, and low rates in Africa and Asia. In Egypt, BC represents about 37.7% of total cancer cases among women. Gene amplification is a frequent device leading to the overexpression of oncogenes in human cancers. At present, the prognosis, classification, and treatment of BC are dependent on tumor histological grading, tumor stage, as well as 3 major protein markers: Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor type 2 (HER2). Despite the efforts to improve the effectiveness of the combination of surgical approach, radio-chemotherapy, endocrine-therapy, and human epidermal growth factor receptor-2 (HER-2)-based targeted therapy, there is an immense clinical need for new therapeutic strategies and molecular targets.

Keywords: Breast cancer, Estrogen receptor, Progesterone receptor, Human epidermal growth factor receptor type 2, Cyclin D1

Full length article **Corresponding Author*, e-mail: drdosoky2000@gmail.com Doi # https://doi.org/10.62877/35-IJCBS-24-25-19-35

1. Introduction

Breast cancer is currently the most frequently diagnosed female cancer and is a leading cause of cancer-related deaths in women [1] In Egypt, BC accounts for 38% of all female cancers according to the National Cancer Institute, Cairo university [2] and for 23.9% of all malignancies according to Pathology-based Cancer registry, Ain Shams faculty of medicine [3]. At present, the prognosis, classification and treatment of BC are dependent on tumor histological grading, tumor stage, as well as 3 major protein markers: Estrogen receptor (ER), Progesterone receptor (PR) and HER2 [4]. Despite the efforts to improve the effectiveness of the combination of surgical approach, radio-chemotherapy, endocrine-therapy, and human epidermal growth factor receptor-2 (HER-2)-based targeted therapy, there is an immense clinical need for new therapeutic strategies and molecular targets [5].

2. Epidemiology

Breast cancer is the most commonly diagnosed cancer among women in the vast majority of countries worldwide, representing a quarter of all cancers diagnosed in women [6]. Female BC incidence rates vary by more than 10-fold among the different countries, with the highest rates in Western Europe and the United States and the lowest rates in Africa and Asia (with the exception of Israel, which has a rate among the highest ones [7]. Mortality rates vary about 4-fold. The highest mortality rates are found in the United States among Black women, whereas the lowest are in Korean women [8]. Higher BC incidence in high income countries (HICs) reflects the use of BC screening as well as higher prevalence of BC risk factors, including excess body weight, physical inactivity, alcohol consumption, as well as reproductive and hormonal factors, such as a long menstrual history, use of hormone replacement therapy (HRT) or oral contraceptives (OC) and nulliparity or later age at first birth [9]. In contrast to rising or stable incidence patterns, BC mortality rates are decreasing in many HICs. These declines are attributed to early detection and improved treatment [9]. Breast cancer incidence rates are expected to further increase within many less developed countries due to longer life expectancy coupled with the adoption of a more ‘‘westernized’’ life- style, less physical activity, and delays in childbearing [10]. The higher mortality within the less developed countries is explained by their inability to afford testing for individual molecular events or multi-parameter profiles and to provide expensive therapies directed against ER, HER2 or other emerging targets [11]. In Egypt, BC

accounts for 23.9% of all malignancies according to the pathology-based cancer registry of Ain-Shams faculty of medicine, and for 19.94% of all malignancies and 38% of total female malignancies, according to Cancer Pathology Registry of the National Cancer Institute (Helal et al., 2015. BC is commonest in Upper Egypt (38.72%) followed by Lower Egypt (33.22%) and then Middle Egypt (26.84%) [12]. Breast cancer is predominantly a disease of aging. More than 40% of the affected patients are older than 65 years. The estimated risk of developing BC is 1/53 before the age of 49 years, 1/43 for 50–59 years old,1/23 for 60–69 and 1/15 for women aged >70 years ([13]. Studies showed that age at diagnosis of BC in Arab countries is a decade younger than that in Western countries [14]. In Egypt, the mean age at presentation for BC is 51 years [14].

3. Etiology and risk factors

A woman's risk of BC is shaped by many different factors over the course of her life. Some of these factors, such as family history, can't be modified, but many others are modifiable [15].

Genetic and Familial Factors

All cancers develop because of mutations in certain genes, such as those involved in the regulation of cell growth or DNA repair. Not all of these mutations are inherited, sporadic mutations can occur in somatic/ tumor cells only, and de novo mutations can occur for the first time in a germ cell (i.e., egg or sperm) or in the fertilized egg during early embryogenesis [16]. Family history is the strongest risk factor for developing BC [17]. Generally accepted criteria for familial BC include:

- At least three breast and/or ovarian cancer cases in a family.
- Two BC cases in close relatives, with at least one diagnosed before age of 50.
- Two BC cases in a family diagnosed before 40 years of age.
- Any female BC with a family history of ovarian cancer or early onset female BC.
- Ashkenazi Jewish ancestry with BC, particularly triple negative breast cancer (TNBC), diagnosed before age 60.
- Breast and ovarian cancer in the same patient.

Hormonal and Reproductive Factors

Several BC risk factors are believed to operate through sex hormones pathways. These risk factors are modestly associated with BC risk. The strongest and most consistent relationship between reproductive risk factors and BC are seen in cancers that express either ER or PR [18]**.** Invasive lobular carcinoma (ILC) being commonly hormone receptor-positive, is strongly associated with these risk factors [19]. Estrogen is thought to promote the growth of ER+ BC, and may also have a role in the early development of both ER+ and ER- BC [20]**.** Two possible mechanisms have been proposed to explain the increased risk:

- Estrogen receptor mediated stimulation of breast cell proliferation with a concomitant enhanced rate of mutations.
- Metabolism of estradiol to genotoxic metabolites with a resulting increase in DNA mutations [21].

Menstrual factors

Early age at menarche is an important BC risk factor, with a 2-year delay in menarche corresponding to a 10% risk reduction. This may be related to higher lifetime exposure to endogenous hormones. Interestingly, women who have early menarche (before age of 12) are also subjects to higher levels of hormone stimulation during a given cycle compared with those having menarche later (after age of 13) [22]. A short menstrual cycle is also associated with an increased risk of BC due to higher lifetime exposure to estrogen [23].

Pregnancy

Pregnancy has a dual effect on BC risk; it transiently increases the risk after child birth (short-term effect) but reduces the risk in later years (long-term effect) [24]. This protective effect is lasting and overall outweighs the transient risk. The short-term adverse effect is thought to result from elevated hormone levels and rapid proliferation of breast epithelial cells during pregnancy. On the long-term, breast epithelial cells undergo differentiation following the first pregnancy. Differentiated cells have longer cell cycles and are thus less sensitive to the effects of carcinogens and have more time to undergo DNA repair [25]**.** However, any pregnancy after the age of 35 increases the risk of BC as the breast tissue at that age is more likely to have clusters of abnormal cells carrying cancer-causing mutations [26].

Lactation

Breast-feeding appears to have a protective effect against the development of BC, with a dose-response relationship. Lactation prevents disordered postpartum involution as the involution in women who have breastfed typically occurs gradually over a period of weeks or months thus returning the lactating gland to its pre-pregnancy state in a more coordinated and less tumorigenic fashion [27].

Oral contraceptives

Although, it is well documented that OC use decreases ovarian and uterine cancer risk**,** it is associated with increased BC risk. This risk decreases with increasing time since last use and is no longer evident 10 years after ceasing use. This risk varies by OC dose and formulation, where high estrogen OC are associated with higher BC risk than low estrogen OC [28].

Hormone replacement therapy

Hormone replacement therapy use is associated with increased risk of BC. The effect appears to be stronger with the use of combined HRT rather than estrogen alone, with current use of HRT, with longer duration of use and with HRT use initiated soon after menopause**.** This elevated risk

disappears at 5 years following cessation of therapy, regardless of treatment duration [29].

4. Molecular subtypes of breast cancer

Gene expression analyses have provided a deeper understanding of the complexity of BC**.** In 2000, the 1st molecular classification of BC identified 4 major subtypes: **I)** ER+/luminal, **II)** HER2+ (HER2-enriched), **III)** Basal-like and **IV)** Normal-like**.** Subsequent transcription profiling studies provided a molecular taxonomy for BC, dividing them into 5 types: luminal A and B, HER2 type, normal breast-like, and basal-like types [30]**.**

Luminal A

Luminal-A is the most common molecular subtype of BC. They are characterized by expression profile reminiscent of the luminal breast epithelial cells [30] with higher levels of ER and lower levels of proliferation related genes. They express luminal CK 8 and 18 and other luminal associated markers including ER, genes associated with ER function such as GATA binding protein 3 (GATA3), B cell lymphoma 2 (BCL2), erbB3 and erbB4. These tumors frequently have low histological grade and include good prognosis special histological types (e.g. tubular, cribriform, mucinous and lobular) [31]**.**

Luminal B

Compared to luminal A tumors, luminal B tumors have higher expression of proliferation related genes (e.g. MKI67) and lower expression of luminal-related genes such as the PR and FOXA1, but not the ER, which is found similarly expressed between the 2 luminal subtypes. At the DNA level, luminal B tumors show higher number of mutations across the genome, higher number of chromosomal copy-number changes and higher TP53 mutations, compared to luminal A tumors [32]**.** Luminal B tumors also show increased expression of growth factor receptor genes, with approximately 20% being HER2 positive by mRNA levels and IHC [32]**.**

HER2 Enriched

The HER2 gene, is a proto-oncogene mapped to chromosome 17q21, encoding the HER2 receptor. Its activation leads to activation of transcription factors that regulate many genes involved in cell proliferation, survival, differentiation, angiogenesis, invasion and metastasis [32]**.** HER2 enriched subtype is characterized by the high expression of HER2- regulated genes and low expression of luminal-related genes**,** with high expression of proliferation-related genes and low expression of basal-related genes (e.g. CK 5 and FOXC1) [33]. At the DNA level, these tumors show the highest number of mutations across the genome, with common TP53 and PIK3CA mutations [34]**.**

Basal Like Subtype

The basal-like breast cancer (BLBC) subtype name is derived from shared gene expression patterns with normal basal epithelial cells including CK 5, 6 and 17, and laminin. They show low expression of the luminal and HER2 gene clusters [35]**.** This subtype is also characterized by relatively high frequency of BRCA1 mutations, increased genomic instability and high expression of the proliferation related genes**.** These tumors are frequently ER, PR and HER2-negative and CK 5⁄6 and epidermal growth factor receptor (EGFR; HER1) positive by IHC [36]**.**

Normal Breast-Like

Normal breast-like BC are poorly characterized, and their clinical significance remains undetermined. They express genes characteristic of adipose tissue presenting an intermediate prognosis between luminal BC and BLBC [37]. They usually don't respond to neoadjuvant chemotherapy [38]. These tumors are classified as Triple Negative (TN) but aren't considered to be BLBC as they are negative for CK5, EGFR and P53 [39].

Claudin low

Claudin-low BC are characterized by low to absent expression of luminal markers and high enrichment for epithelial-to-mesenchymal transition markers, immune response genes, and cancer stem cell-like features [40].

5. Immunohistochemical Surrogates for Classification of Breast Carcinomas

Although, the correspondence between IHC and molecular (i.e., gene expression) classification is not very good. IHC-based molecular classification is now recommended for clinical decision making, as it is neither economical nor practical to use gene expression profiling (GEP) in daily practice. Surrogate approaches are now accepted using more widely available IHC tests for ER, PR and Ki-67 (as a proliferation marker), together with IHC or in situ hybridization (ISH) tests for HER2 overexpression or amplification [41].

Estrogen and Progesterone Receptors

Estrogen receptor, which belongs to the class of steroid hormonal receptors, plays an important role in cell proliferation, survival and invasion of ER+ BC [42]. PR is also a transcription factor, largely controlled by ER and to some degree by growth factors as well**.** Hormone receptor-positive BC, defined on the basis of the IHC, constitutes about 60% of premenopausal BC and 80% of postmenopausal BC [43]**.** The main clinical application of ER and PR is in selecting patients with BC for treatment with endocrine therapy, including selective ER modulators (tamoxifen), third-generation aromatase inhibitors, luteinizing hormone-releasing hormone agonists, pure ER down-regulators (fulvestrant), oophorectomy and other endocrine therapies [44]**.** Higher hormone receptor levels are associated with higher probability of response to endocrine therapy and lesser absolute benefit of chemotherapy [45].

Human Epidermal Growth Factor Receptor Type 2

About 12% to 20% of BC overexpress the HER2 protein and/or have HER2 gene amplification. The main clinical use of HER2 measurement is in predicting the response to anti-Her2 therapy in the neoadjuvant, adjuvant and advanced disease settings. Two diagnostic techniques are currently approved for assigning Her2 status in clinical practice; IHC and ISH [46].

Ki-67

Ki-67 is a nuclear marker for proliferation expressed in all phases of the cell cycle except G0. The cut-point between 'high' and 'low' values for Ki-67 is a source of debate. Although a 14% cutoff was accepted in St Gallen Consensus in 2011**,** the panel accepted a cutoff of > 20% as the new cutoff in the 2013 St Gallen conference [47, 48]. In 2017, the panel agreed that either histologic grading or Ki-67 index can be used to distinguish between the Luminal A- and B-like BC **[**49].

6. Conclusions

Breast cancer is heterogeneous at the molecular level, with different patterns of gene expression leading to differences in behavior and prognosis.

References

[1] L. Wilkinson, T. Gathani. (2022). Understanding breast cancer as a global health concern. The British journal of radiology. 95 (1130) 20211033.

[2] B. El-Helkan, M. Emam, M. Mohanad, S. Fathy, A.R. Zekri, O.S. Ahmed. (2022). Long non-coding RNAs as novel prognostic biomarkers for breast cancer in Egyptian women. Scientific Reports. 12 (1) 19498.

[3] H.A. Atwa, H.M. Ibrahim, E.I. Ismail, I.M. Ibrahim. (2017). ALDH 1A1 and caveolin-1 expression in triple negative breast cancer. Oncology and Translational Medicine. 3 (5) 185-196.

[4] P. Tang, G.M. Tse. (2016). Immunohistochemical surrogates for molecular classification of breast carcinoma: a 2015 update. Archives of pathology & laboratory medicine. 140 (8) 806-814.

[5] G.E. Tanios, M.E. Burow, B. Collins-Burow, D.G. Morrison. (2017). Invasive Breast Cancer Therapy 2017: How Well Are We Hitting the Target?. Resistance to Targeted Therapies in Breast Cancer. 1-34.

[6] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 71 (3) 209-249.

[7] P. Wangkulangkul, S. Laohawiriyakamol, P. Puttawibul, S. Sangkhathat, V. Pradaranon, T. Ingviya. (2023). A Clinical Prediction Model for Breast Cancer in Women Having Their First Mammogram. In Healthcare. 11 (6) 856.

[8] A. Ferro, B. Peleteiro, M. Malvezzi, C. Bosetti, P. Bertuccio, F. Levi, N. Lunet. (2014). Worldwide trends in gastric cancer mortality (1980–2011), with predictions to 2015, and incidence by subtype. European journal of cancer. 50 (7) 1330-1344.

[9] L.A. Torre, F. Islami, R.L. Siegel, E.M. Ward, A. Jemal. (2017). Global cancer in women: burden and trends. Cancer epidemiology, biomarkers & prevention. 26 (4) 444-457.

[10] D.R. Youlden, S.M. Cramb, N.A. Dunn, J.M. Muller, C.M. Pyke, P.D. Baade. (2012). The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. Cancer epidemiology. 36 (3) 237-248.

[11] V.J. Wirtz, H.V. Hogerzeil, A.L. Gray, M. Bigdeli, C.P. de Joncheere, M.A. Ewen, M.R. Reich. (2017). Essential medicines for universal health coverage. The Lancet. 389 (10067) 403-476.

[12] A.S. Ibrahim, N.N. Mikhail. (2015). The evolution of cancer registration in Egypt: from proportions to population-based incidence rates. SECI Oncol. 4 1-21.

[13] E.I. Papageorgiou, J. Subramanian, A. Karmegam, N. Papandrianos. (2015). A risk management model for familial breast cancer: A new application using Fuzzy Cognitive Map method. Computer methods and programs in biomedicine. 122 (2) 123-135.

[14] A.D. Darwish, A.M. Helal, N.A. El-Din, L.L. Solaiman, A. Amin. (2017). Breast cancer in women aging 35 years old and younger: The Egyptian National Cancer Institute (NCI) experience. The Breast. 31 1-8.

[15] Z. Momenimovahed, H. Salehiniya. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer: Targets and Therapy. 151-164.

[16] I. Martincorena, P.J. Campbell. (2015). Somatic mutation in cancer and normal cells. Science. 349 (6255) 1483-1489.

[17] O.A. Stefansson, M. Esteller. (2013). Epigenetic modifications in breast cancer and their role in personalized medicine. The American journal of pathology. 183 (4) 1052-1063.

[18] Y. Feng, M. Spezia, S. Huang, C. Yuan, Z. Zeng, L. Zhang, G. Ren. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & diseases. 5 (2) 77-106.

[19] L. Dossus, P.R. Benusiglio. (2015). Lobular breast cancer: incidence and genetic and non-genetic risk factors. Breast Cancer Research. 17 1-8.

[20] I. Dimauro, E. Grazioli, C. Antinozzi, G. Duranti, A. Arminio, A. Mancini, L. Di Luigi. (2021). Estrogen-receptor-positive breast cancer in postmenopausal women: The role of body composition and physical exercise. International journal of environmental research and public health. 18 (18) 9834.

[21] W. Yue, J.D. Yager, J.P. Wang, E.R. Jupe, R.J. Santen. (2013). Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. Steroids. 78 (2) 161-170.

[22] J. Chang-Claude, N. Andrieu, M. Rookus, R. Brohet, A.C. Antoniou, S. Peock, D.F. Easton. (2007). Age at menarche and menopause and breast

cancer risk in the International BRCA1/2 Carrier Cohort Study. Cancer Epidemiology Biomarkers & Prevention. 16 (4) 740-746.

[23] L. Hilakivi-Clarke, S. De Assis, A. Warri. (2013). Exposures to synthetic estrogens at different times during the life, and their effect on breast cancer risk. Journal of mammary gland biology and neoplasia. 18 (1) 25-42.

[24] L.P. Rweyemamu, G. Akan, I.C. Adolf, E.P. Magorosa, I.J. Mosha, N. Dharsee, F. Atalar. (2021). The distribution of reproductive risk factors disclosed the heterogeneity of receptor-defined breast cancer subtypes among Tanzanian women. BMC women's health. 21 1-13.

[25] K.A. Ban, C.V. Godellas. (2014). Epidemiology of breast cancer. Surgical Oncology Clinics. 23 (3) 409-422.

[26] M.E. Rasheed. (2021). Breast cancer, medical imaging, and cancer genetics. A new genetic concept regarding the causes and prevention strategies of cancer is presented (Doctoral dissertation, University of Bradford).

[27] M.M.M. Hatmal, M.A. Al-Hatamleh, A.N. Olaimat, W. Alshaer, H. Hasan, K.A. Albakri, R. Mohamud. (2022). Immunomodulatory properties of human breast milk: microRNA contents and potential epigenetic effects. Biomedicines. 10 (6) 1219.

[28] T. Karlsson, T. Johansson, J. Höglund, W.E. Ek, Å. Johansson. (2021). Time-dependent effects of oral contraceptive use on breast, ovarian, and endometrial cancers. Cancer research. 81 (4) 1153-1162.

[29] T.K. Yoo, K.D. Han, D. Kim, J. Ahn, W.C. Park, B.J. Chae. (2020). Hormone replacement therapy, breast cancer risk factors, and breast cancer risk: a nationwide population-based cohort. Cancer Epidemiology, Biomarkers & Prevention. 29 (7) 1341-1347.

[30] B. He, L. Bergenstråhle, L. Stenbeck, A. Abid, A. Andersson, Å. Borg, J. Zou. (2020). Integrating spatial gene expression and breast tumour morphology via deep learning. Nature biomedical engineering. 4 (8) 827-834.

[31] R. Ahmed, S. Samanta, J. Banerjee, S.S. Kar, S.K. Dash. (2022). Modulatory role of miRNAs in thyroid and breast cancer progression and insights into their therapeutic manipulation. Current Research in Pharmacology and Drug Discovery. 3 100131.

[32] J.M. Cejalvo Andújar. (2020). Uncovering the molecular and cellular mechanisms of metastatic dormancy in luminal breast cancer.

[33] A. Prat, T. Pascual, C. De Angelis, C. Gutierrez, A. Llombart-Cussac, T. Wang, M.F. Rimawi. (2020). HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. JNCI: Journal of the National Cancer Institute. 112 (1) 46-54.

[34] W. Xiao, G. Zhang, B. Chen, X. Chen, L. Wen, J. Lai, N. Liao. (2021). Characterization of frequently mutated cancer genes and tumor mutation burden in Chinese breast cancer. Frontiers in Oncology. 11 618767.

[35] K.S. Johnson, E.F. Conant, M.S. Soo. (2021). Molecular subtypes of breast cancer: a review for breast radiologists. Journal of Breast Imaging. 3 (1) 12-24.

[36] U. Testa, G. Castelli, E. Pelosi. (2020). Breast cancer: a molecularly heterogenous disease needing subtype-specific treatments. Medical Sciences. 8 (1) 18.

[37] S. Bhattarai. (2020). Prognostic and Predictive Biomarkers in Breast Cancer.

[38] E. Provenzano. (2021). Neoadjuvant Chemotherapy for Breast Cancer: Moving Beyond Pathological Complete Response in the Molecular Age. Acta medica academica. 50 (1).

[39] R.R. Hashmi, V. Chauhan, A. Balaji, S. Sultana. A review of triple negative breast cancer and its advancement of classification.

[40] H. Skálová, N. Hájková, B. Majerová, M. Bártů, C. Povýšil, I. Tichá. (2019). Impact of chemotherapy on the expression of claudins and cadherins in invasive breast cancer. Experimental and Therapeutic Medicine. 18 (4) 3014-3024.

[41] T. Mathew, S. Niyas, C.I. Johnpaul, J.R. Kini, J. Rajan. (2022). A novel deep classifier framework for automated molecular subtyping of breast carcinoma using immunohistochemistry image analysis. Biomedical Signal Processing and Control. 76 103657.

[42] A. Chimento, A. De Luca, P. Avena, F. De Amicis, I. Casaburi, R. Sirianni, V. Pezzi. (2022). Estrogen receptors-mediated apoptosis in hormone-dependent cancers. International journal of molecular sciences. 23 (3) 1242.

[43] T.T. Kunstič, N. Debeljak, K.F. Tacer. (2023). Heterogeneity in hormone-dependent breast cancer and therapy: Steroid hormones, HER2, melanoma antigens, and cannabinoid receptors. Advances in Cancer Biology-Metastasis. 7 100086.

[44] R. Patel, P. Klein, A. Tiersten, J.A. Sparano. (2023). An emerging generation of endocrine therapies in breast cancer: a clinical perspective. NPJ Breast Cancer. 9 (1) 20.

[45] J.F. Robertson, R.J. Paridaens, J. Lichfield, I. Bradbury, C. Campbell. (2021). Meta-analyses of phase 3 randomised controlled trials of third generation aromatase inhibitors versus tamoxifen as first-line endocrine therapy in postmenopausal women with hormone receptor-positive advanced breast cancer. European Journal of Cancer. 145 19-28.

[46] E.A. Rakha, P.H. Tan, C. Quinn, E. Provenzano, A.M. Shaaban, R. Deb, S.E. Pinder. (2023). UK recommendations for HER2 assessment in breast cancer: an update. Journal of Clinical Pathology. 76 (4) 217-227.

[47] A. Lombardi, R. Lazzeroni, L. Bersigotti, V. Vitale, C. Amanti. (2021). The proper Ki-67 cut-off in hormone responsive breast cancer: a monoinstitutional analysis with long-term follow-up. Breast Cancer: Targets and Therapy. 213-217.

[48] B.S. Finkelman, H. Zhang, D.G. Hicks, B.M. Turner. (2023). The evolution of Ki-67 and breast

carcinoma: past observations, present directions, and future considerations. Cancers. 15 (3) 808.

[49] A.F. Maranta, S. Broder, C. Fritzsche, M. Knauer, B. Thürlimann, W. Jochum, T. Ruhstaller. (2020). Do YOU know the Ki-67 index of your breast cancer patients? Knowledge of your institution's Ki-67 index distribution and its robustness is essential for decision-making in early breast cancer. The Breast. 51 120-126.