"EVALUATION OF CD53 MARKER EXPRESSION IN TRIPLE NEGATIVE BREAST CARCINOMA: A HOSPITAL BASED CROSS SECTIONAL STUDY"

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LIST OF ABBREVIATIONS USED

AJCC	-	American Joint Committee on Cancer's system
BLBC	-	Basal like breast carcinoma
BRCA	-	Breast Cancer Genes
CD	-	Cluster of differentiation
CK	-	Cytokeratin
CTL	-	Cytotoxic T lymphocytes
DAB	-	Diaminobenzidine
DCIS	-	Ductal carcinoma in situ
DCs	-	Dendritic cells
DNA	-	Deoxyribonucleic acid
DPX	-	Dibutylphthalate Polystyrene Xylene
EDTA	-	Ethylenediaminetetraacetic Acid
EGFR	-	Epidermal growth factor receptor
ER	-	Estrogen receptor
ErbB family	-	Erythroblastic Leukemia Viral Oncogene Homolog family
FIG	-	Figure
FOXP3	-	Forkhead Box P3 gene
GLOBOCAN	-	Global Cancer Observatory
H and E	-	Haematoxylin and eosin
HER 2	-	Human epidermal growth factor 2 receptor
HRP	-	Horse-radish peroxidase
HSCs	-	Hematopoietic stem cells
IDC	-	Invasive ductal carcinoma
IHC	-	Immunohistochemistry

ILC	-	Invasive lobular carcinoma
IM	-	Immunomodulatory
LCIS	-	Lobular carcinoma in situ
LIQ	-	Lower inner quadrant
LOQ	-	Lower outer quadrant
LPBC	-	Lymphocyte-predominant breast cancer
LVI	-	Lymphovascular invasion
M	-	Mesenchymal
MSL	-	Mesenchymal stem like
NACT	-	Neoadjuvant chemotherapy
NCBC	-	Non-classifiable breast carcinoma
NEG	-	Negative
NK cells	-	Natural killer cells
NPI	-	Nottingham Prognostic Index
NSABP	-	National Surgical Adjuvant Breast and Bowel Project
NST	-	No special type
pCR	-	Pathological complete response
PD-L1	-	Programmed cell death ligand 1 receptor
PNI	-	Perineural invasion
POS	-	Positive
PR	-	Progesterone receptor
SD	-	Standard Deviation
TDLU	-	Terminal duct lobular unit
TEMs	-	Tetraspanin-enriched microdomains
TILs	-	Tumour infiltrating lymphocytes

TLS	-	Tumor-Lymphoid Structures
TNBC	-	Triple negative breast cancer
TNM	-	Tumor, Node, Metastasis
Tris Buffer	-	Tris(hydroxymethyl)aminomethane Buffer
TSPAN	-	Tetraspanin
UIQ	-	Upper inner quadrant
UOQ	-	Upper outer quadrant
WHO	-	World Health Organization

ABSTRACT

Background

Triple-negative breast cancer (TNBC) is a molecular subtype that develops independently of estrogen, progesterone, and HER2 amplification. Recent research highlights the role of immune surveillance in carcinogenesis, suggesting that CD53 could serve as a potential prognostic marker for TNBC. This study aims to examine CD53 expression in TNBC.

Objectives

This study aims to study expression of CD53 as IHC marker and correlate its expression with clinicopathological parameters in TNBC.

Methodology

In this study, 50 immunohistochemically proven TNBC cases were taken and CD53 expression was studied in tumor-infiltrating lymphocytes (TILs) and tumor cells. Various clinicopathological parameters including age group, tumor size, histological grade, lymphovascular invasion (LVI), perineural invasion (PNI), axillary lymph node status, lymphocytic response, Paget's disease and Nottingham Prognostic Index (NPI) were compared with CD53 intensity.

Results

CD53 expression was seen in tumor cells, TILs and normal ducts adjacent to tumor tissue in membrane as well as in cytoplasm. Significant correlation was seen with better prognostic index and higher lymphocytic response in both intra and stromal TILs. There was no significant correlation between clinical parameters and CD53

intensity. Around 11.90% cases in good prognosis category have 3+ intensity, hence showing significant correlation of CD53 intensity in peri-tumoral lymphocytes with Nottingham Prognostic Index.

Conclusion

Our study evaluated CD53 expression and correlation with various clinicopathological parameters. This study is one of the very few studies evaluating potential biomarker CD53 in TNBCs. Higher intensity of CD53 in TILs was associated with parameters like absence of LVI, PNI, Paget's disease, axillary lymph node metastasis and better Nottingham Prognostic Index.

Key words: TNBC, Breast cancer, CD53, TILs, Molecular, Tumor microenvironment, Prognostic

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INTRODUCTION

Breast cancer is the most prevalent cancer among women worldwide. In 2020, approximately 2.3 million new cases were diagnosed, and the disease caused 685,000 deaths. By 2040, breast cancer cases could reach 3 million annually with 1 million deaths, highlighting the need for better prevention and treatment.[1] Breast malignancy caused 10.6% (90,408) of all deaths and 13.5% (1,78,361) of all cancer cases in India in 2020, according to Global Cancer Observatory (GLOBOCAN) statistics.[2]

The prognosis of breast malignancies is influenced by histological subtypes as per 1999 College of American Pathologists Consensus statement.[3,4] However, traditional histopathological classification systems have their drawbacks and novel molecular classifications are helpful in diagnosis, management as well as predicting prognostic outcomes.

Triple negative breast cancer (TNBC), a molecular subtype, develops through a pathway independent of estrogen and progesterone and does not involve human epidermal growth factor 2 receptor (HER2) gene amplification. As a result, it is negative for estrogen receptors (ER), progesterone receptors (PR) and HER2.(5) Development of more effective treatments for TNBC has been challenging as obvious drug targets (such as ER and HER2) are lacking.[5,6]

Recent research has emphasized that host immune surveillance plays a crucial role in affecting carcinogenesis. In initial stage of carcinogenesis, tumour associated antigens can activate stromal microenvironment based on immune cells [histiocytes, natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTL)], which will produce a potent anti- tumour response.[7-9]

The higher responses of tumour infiltrating lymphocytes (TILs) are associated with better prognosis in different malignancies [melanoma, colorectal, oral squamous cell, ovarian, and breast carcinomas] reinforcing positive impact of immune surveillance.[10]

CD53 (cluster of differentiation 53) belongs to the tetraspanin/transmembrane-4 superfamily and is used as a marker for thymocyte selection. It modulates leukocyte migration into the tumor microenvironment and causes proliferation of lymphocytes.[11,12]

Considering relevance of immune responses in cancer pathology, it is suggestive that CD53 can be used as a potential prognostic factor for TNBC. Thus, targeting CD53 expression in TNBC will aid in better understanding of tumour behaviour and further role in immunotherapy. The purpose of this study is to examine expression of CD53, an upcoming potential immunohistochemical (IHC), prognostic marker in TNBC.

AIMS AND OBJECTIVES

Primary objective - To study expression of CD53 as an immunohistochemical marker in Triple Negative Breast Carcinoma.

Secondary objective - To correlate CD53 expression with other clinicopathological parameters.

REVIEW OF LITERATURE

BREAST: EMBRYOLOGY

Breast is considered to be derived from apocrine gland as an epidermal appendage. Branching morphogenesis is defined as process of development of mammary acini and ducts which starts in foetal life; however, development arrests in early childhood and hormonal stimulation during puberty begins further differentiation.[13] The developmental changes can further be divided into prenatal, during infancy and pubertal.

In prenatal stage, breast specific progenitor cells are seen in early 1st trimester, which lead to growth of adjacent areas of epithelial cells in epidermis, extending from foetal axilla to inguinal region forming a line. These two lines or ridges are known as milk lines or mammary ridges or crests.[14,15] With the exception of paired firm epithelial masses that form the principal mammary buds, remaining mammary crest atrophies.[16,17] Mesenchyme secretes regulatory factors causing primary buds to grow into underlying mesenchyme in late 1st trimester.[18,19] Primary mammary bud enlarges and indentations along basolateral margin indicate future secondary outgrowths, which will grow vertically into the mesenchyme surrounding primary bud.[20] These subsequent outgrowths give rise to lactiferous ducts in 2nd trimester.[18] The lactiferous duct has two linings, luminal layer with secretory activity and basal layer differentiating into myoepithelial cells.[21] During 3rd trimester, retro-areolar ampullae, which congregate in mammary pit (depressed epidermis) on the skin above, receive the lactiferous duct drainage and smooth muscle fibres are arranged in a circular and longitudinal pattern to form the nipple.[18] The secondary epithelial buds repeatedly

branch and canalize and vascularized loose connective tissue stroma is formed. Approximately 15-20 lobes of glandular tissue develop by term.[15]

There is typically no discernible difference in the quantity of tissue present in each newborn's breast at birth, regardless of gender.[22] From the age of two until adolescence, typically breast gland is dormant.[23,24] Higher levels of estradiol in female infants cause breast tissue persists, unlike in male infants.[25] Anzbagahan et al. have described stages of maturation based on morphological and functional characteristics.[18,26] (Fig. 1 and 2)

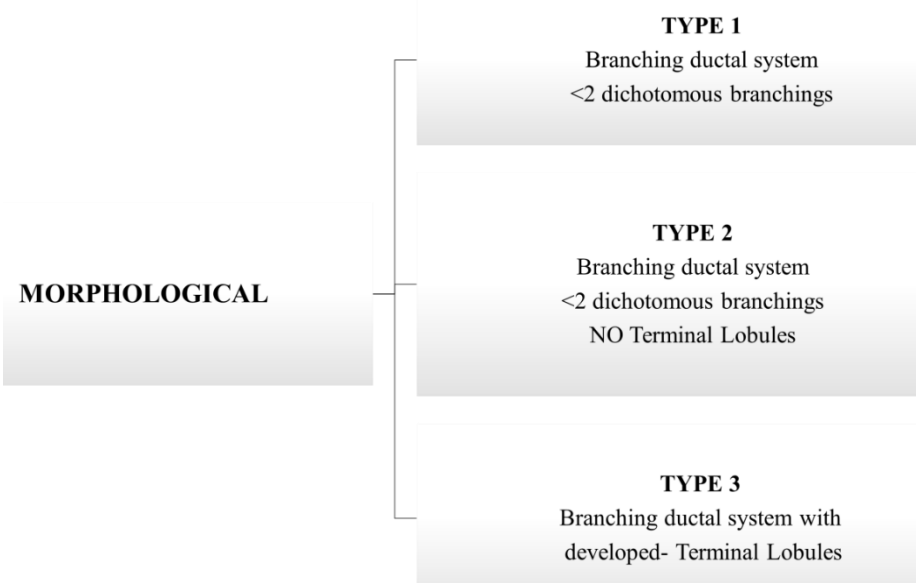


Figure 1- Breast development during infancy as per the morphology

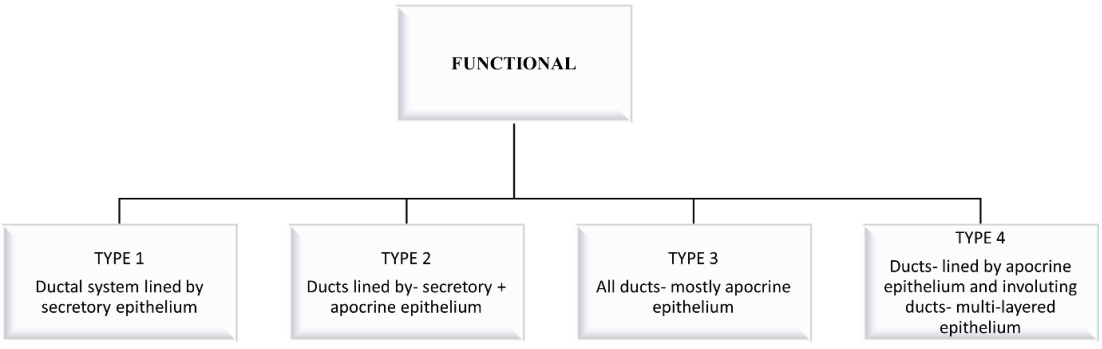


Figure 2- Breast development during infancy as per the function.

Mammary gland development during puberty is driven mainly by estrogen.[27] The gross anatomical stages of mammary gland development are described by Tanner (Table1).[28]

STAGES	FEATURES
STAGE I (Pre-puberty)	Papilla only (elevated nipple) No underlying breast tissue
STAGE II (Thelarche)	Widening of areola Formation of breast bud
STAGE III	Enlargement continues No separate contours
STAGE IV	Secondary breast mound formation
STAGE V	Areola regression Mature breast development

TABLE 1- Tanner Staging [Taken from: Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303.]

At cellular level, number of alveoli keep increasing with every menstrual cycle, however significant increase is seen during pregnancy.[29,30] At the terminal ductal bud, complex and hierarchical branching occurs forming terminal duct lobular unit (TDLU).[18] Side branching and ductal elongation are believed to be caused by progesterone and oestrogen, respectively.[31]

In males, increasing testosterone hormonal levels cause breast development arrest at puberty. Breast development during puberty is influenced by number of factors including race, genetics, nutrition, socioeconomic status and hormonal levels.[32]

MAMMARY GLAND: ANATOMY

Breast glands are modified apocrine sweat glands located on the anterior chest wall. Parenchyma is supported by Cooper's ligaments which are dense fibrous connective tissue.[33-35] Breast lies on deep pectoral fascia which causes tethering of breast to underlying muscles when affected by advanced breast carcinoma.[36]

Breast is composed of two parts, parenchyma comprising of ducts and gland and stroma formed by adipose and fibrous tissue. The adult female breast has 15-20 lobes and 20-40 lobules. Each lobule has TDLU, a functional unit of breast, where milk production takes place after stimulation by prolactin.

The nipple is surrounded by areola, which gets distinctive and more pigmented during puberty. The modified areolar glands or Montgomery glands along with sweat and sebaceous glands cause tiny elevations on surface of areola.

VASCULAR SUPPLY

Breast is supplied by arterial network formed by internal thoracic, intercostal and axillary arteries. Main arteries and their branches are as shown in figure 3.[36] The major artery supplying breast is internal thoracic artery. In around thirty percent of cases, the axillary artery is not very significant, and fifty percent of cases have minimal or no reliance on the intercostal arteries. Within the breast parenchyma, branches of the arterial circulation do not precisely align with the primary duct system.

Venous drainage usually follows arterial circulation in distribution.

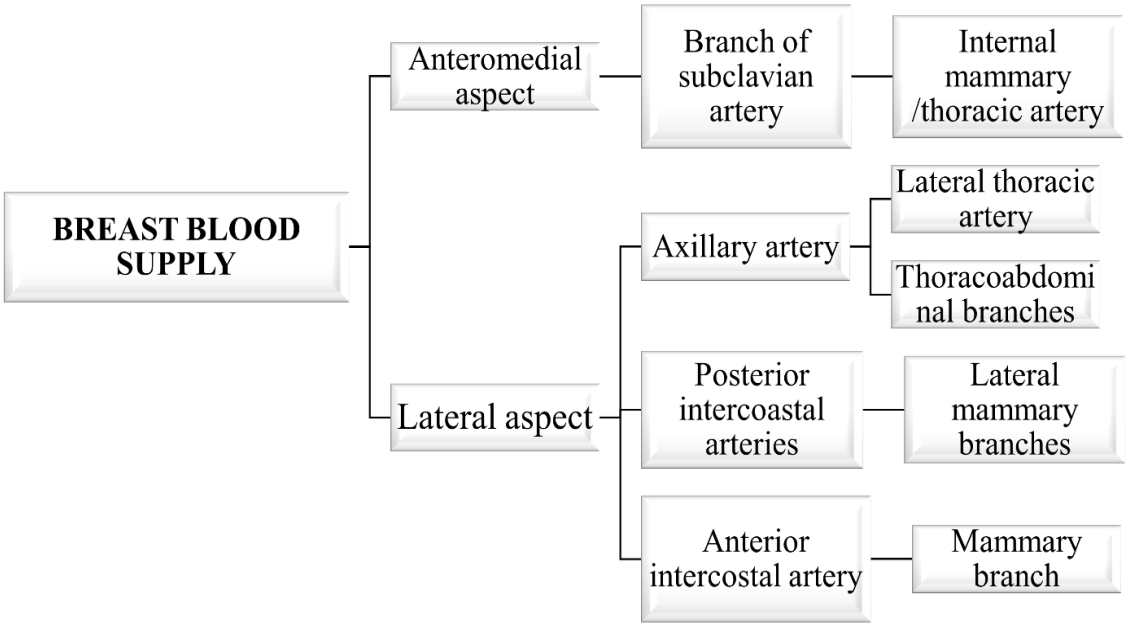


Figure 3- Breast: Blood supply

NERVE SUPPLY

The 4th to 6th intercostal nerves supply sensation to breast. Anterior branch of lateral cutaneous nerve forms extensive plexus within nipple, which is essential for suckling reflex during breast feeding.[37]

LYMPHATIC DRAINAGE OF BREAST

It has been recognized for ages that the breast contains lymphatic outflow to both axillary and extra-axillary locations. Lymph node involvement is a key prognostic indicator in breast malignancies.[38] Superficial lymphatics drain the skin surrounding the breast while deep lymphatics are responsible for draining the nipple-areolar complex and the breast parenchyma.[37]

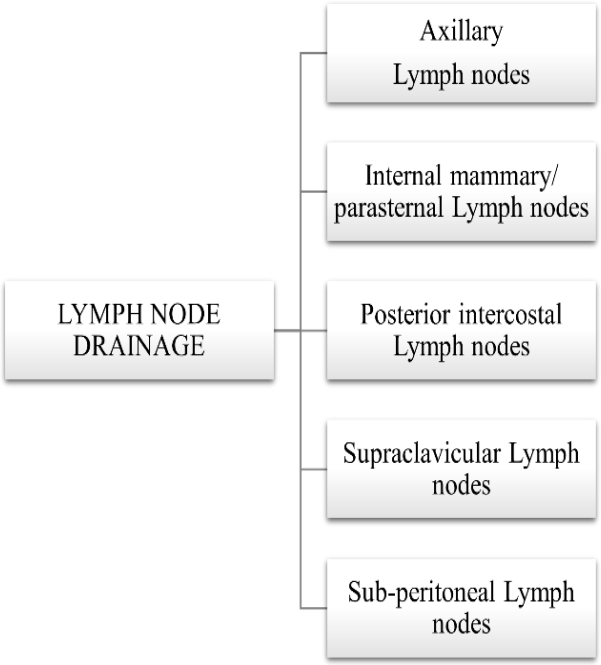


Figure 4- Breast Lymphatics

BREAST PHYSIOLOGY AND FUNCTION

Estrogen stimulates breast growth by promoting proliferation of breast ductal epithelium, myoepithelial cells and stroma; whereas progesterone causes development of the terminal duct and lobuloalveolar units.

Lactogenesis (breast-milk production) is one of the most important functions of mammary gland along with secretion and ejection of milk. The stage one of lactogenesis includes secretion initiation under influence of progesterone and stage two involves secretory activation stimulated by decreased levels of progesterone following delivery and elevated levels of prolactin, cortisol and insulin.[39,40] The suckling reflex causes release of oxytocin.

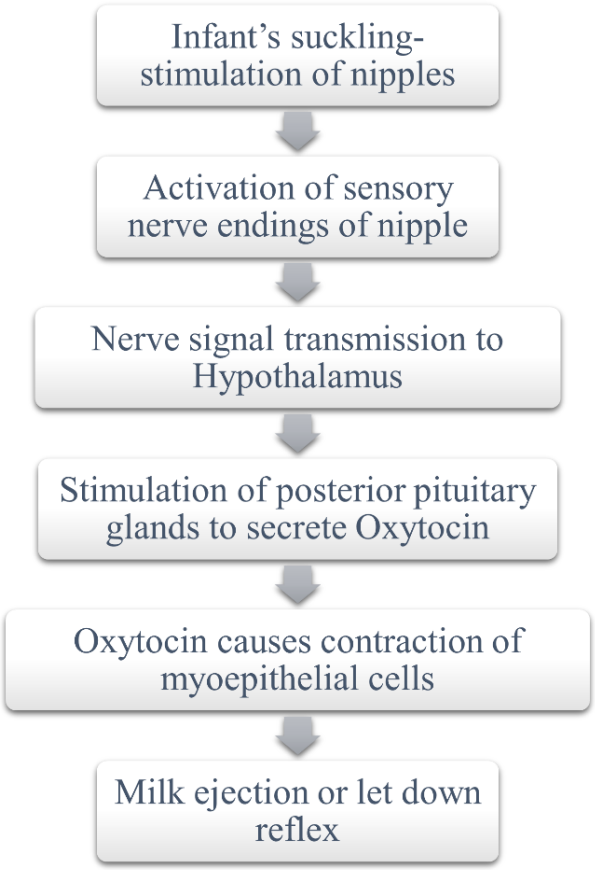


Figure 5- Physiology of suckling reflex

HISTOLOGY OF BREAST

Mammary gland tissue is diverse, combining glandular and connective components that are cyclically regulated by hormones.[41] The histology of breast is influenced by age as well as physiological status (Menstrual status, pregnancy and lactation). The fibrofatty tissue that makes up the majority of the breast contains functional glandular and ductal parts of the breast.

Each lactiferous duct drains a mammary lobe with lactiferous ducts (15-20) exit the breast at nipple forming lactiferous duct orifice which is lined superficially by flattened epithelium and dilated part, also known as lactiferous sinus shows squamocolumnar junction.

Ductal epithelium consists of cuboidal or columnar cells and myoepithelial cells which forming a branching network with their cytoplasmic processes.[42] During breastfeeding, the contraction of myoepithelial cells in lobules and around ducts helps to facilitate the flow of milk. The normal periductal stroma contains elastic fibres, sparse mononuclear inflammatory cells, mast cells and ochrocytes. The subareolar glands of Montgomery are similar to lactiferous ducts in histology. The nipple histology shows stratified squamous epithelium with Toker cells.

Breast glands lined by inner cuboidal epithelium and outer myoepithelium. The terminal duct branches into round acini and forms TDLU. The interlobular stroma has more collagen and less capillaries compared to intra-lobular stroma.

CARCINOMA BREAST: HISTORY

World Health Organization (WHO) defines cancer as collection of diseases characterized by uncontrolled cell proliferation. Galen used term "crab" to describe

cancer in order to represent the dilated veins that emanate from the tumour.[43] Imhotep, Egyptian physician described breast carcinoma as "cool to touch, bulging and spread all over the breast".[44,45] Hippocrates described progression of breast carcinoma stages in 400 B.C., indicating early theories for cancer etiopathogenesis.[45,46] Leonides of Alexandria gave initial principles for wide local excision surgeries in 1st century A.D.[43]

In 19th century, any excision approaches were based on the finding that breast malignancies spread via lymphatics to the guardian axillary nodes.[45] In Liverpool, William Banks dissected axillary lymph nodes in 1882 as part of the extermination concept.[47] In 1894, William S. Halstead performed first radical mastectomy with pectoralis major excision to prevent recurrence.[48]

National Surgical Adjuvant Breast and Bowel Project (NSABP-1985) showed that irradiation lowers the risk of local tumor recurrence.[49] George Beatson demonstrated in 1895 that ovarian excision causes decrease in size of breast tumour lump.[50] Elwood V Jensen identified oestrogen receptors in 1960.[51]

CARCINOMA BREAST-EPIDEMIOLOGY

Breast cancer is among the most common cancers globally with an estimates 2.3 million cases and is 5th leading cause of cancer-related deaths according to GLOBOCAN 2020 data.[52] Females are diagnosed with breast malignancies more often than any other malignancy. In high-income nations, survival rates are higher as compared to in low- and middle-income nations.[53]

According to GLOBOCAN 2018 , new occurrences of cases and deaths in India from breast cancer was roughly 162,468 each year.[54] Current statistics show that

breast cancer develops at younger age in Indian women as compared to those in Western countries.[55]

Effective prevention is crucial, as addressing modifiable risk factors can help reduce breast cancer rates given the significant rise in both morbidity and mortality over recent decades.[56]

CARCINOMA BREAST- RISK FACTORS

The are many risk factors for breast cancer including modifiable and non-modifiable elements. [56] (Table 2)

MODIFIABLE FACTORS	NON-MOODIFIABLE FACTORS
Habits/Addiction (smoking, alcohol)	Gender- Female
High Body Mass Index (Obesity)	Age (elderly)
Drug therapy (Exogenous Hormonal Supplements)	Family History (Carcinoma Breast or Carcinoma Ovary)
Diethylstilbestrol	Race
Artificial light excess exposure	Genetic mutations
Others (Processed food, chemicals)	History of radiation

Table 2- Risk factors [Taken from: Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers. 2021;13(17):4287.]

CLINICAL FEATURES OF CARCINOMA BREAST

About 45% of women with cancer exhibit symptoms, while the remaining women are identified by mammography screening.[57]

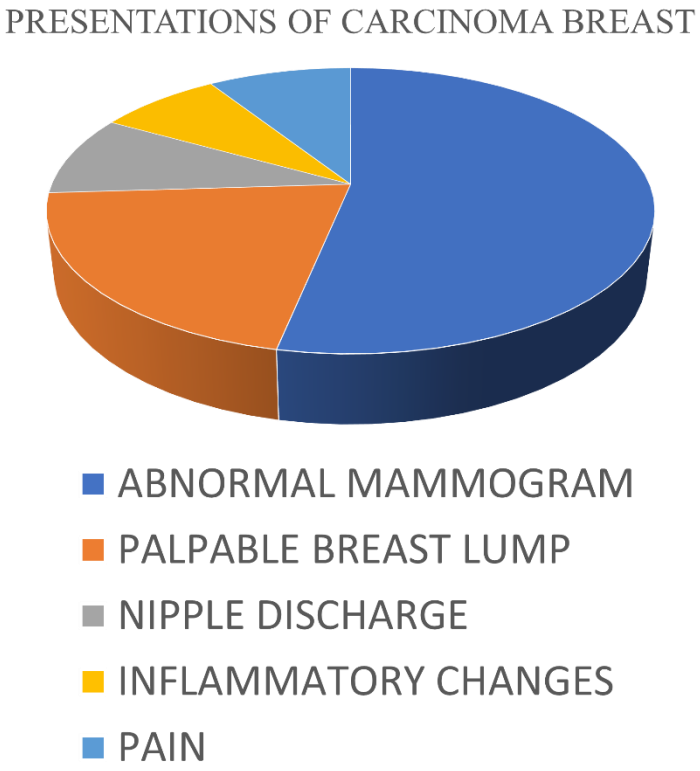


Figure 6- Carcinoma breast: Clinical presentations

CARCINOMA BREAST: PATHOLOGY

Genetic Factors

The risk of breast malignancies increases with duration of exposure to estrogen levels which is a key factor in pathogenesis.[58] Percentage of cases that are of genetic aetiology which is about 5–10%, including breast cancer genes (BRCA1 and BRCA2) mutations.[59,60] BRCA1 and BRCA 2 have important function in deoxyribonucleic acid (DNA) damage response and loss of function leads to cell growth defect and tumour development.[61]Hormonally driven cellular proliferation

during menstrual cycle generates reactive oxygen species causing oxidative DNA damage and replication stress, thereby activating BRCA-1 and BRCA-2 mediated homologous recombination pathway.[61,62]

WHO 5th edition classification

Breast tumors are historically classified by cell type, extracellular secretion, architectural characteristics, and an immunohistochemical profile.[63]

Depending on basement membrane breach, breast carcinoma can be classified as follows (Fig.11)

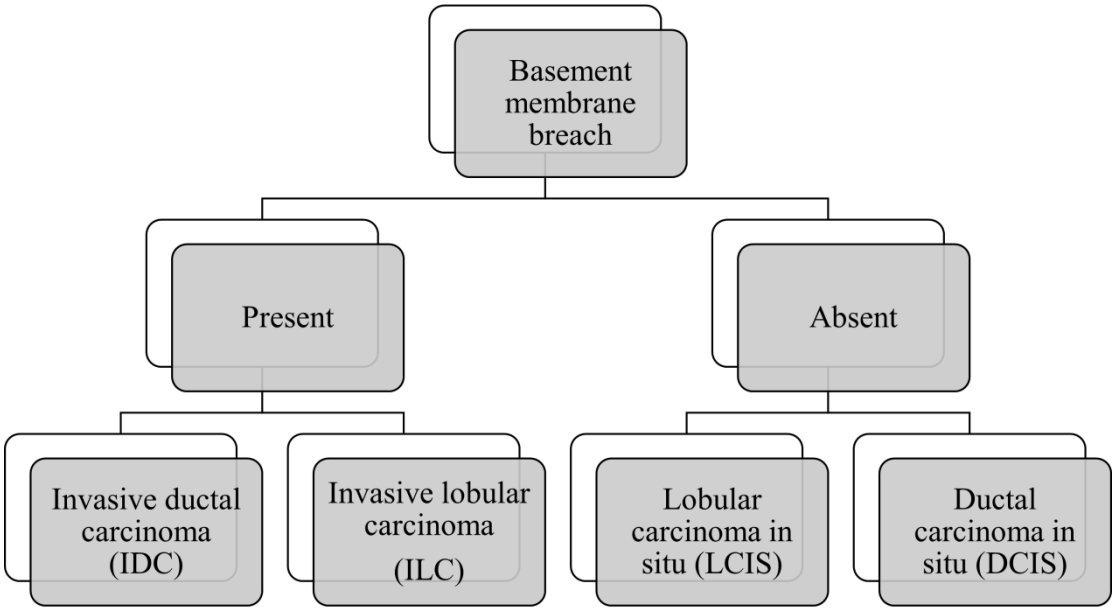


Figure 7 - Histopathological classification of carcinoma breast

Invasive breast carcinoma of no special type (NST) is the most prevalent form accounting representing 50-60% incidence.[64]

WHO 5th edition classification mainly focuses on morphology and divides invasive breast carcinoma.

TNM STAGING

The TNM classification system is based on tumor size, regional lymph nodes metastases, and distant metastases (M). The system developed by American Joint Committee on Cancer's system (AJCC) is most widely used.[65,66]

Histopathological Grading of breast carcinoma

PARAMETER	VALUES	SCORE
Tubule Formation	>75%	1
	10-75%	2
	<10%	3
Nuclear pleomorphism	Mild	1
	Moderate	2
	Severe	3
Mitotic count/10 high power field	0-3	1
	4-7	2
	>8	3

Table 3- Breast Carcinoma Histological Grading [Taken from: Tsang JYS, Tse GM. Molecular Classification of Breast Cancer. Adv Anat Pathol. 2020;27(1):27–35.]

Parameters like tubule formation, nuclear pleomorphism and mitotic index are assessed using Nottingham variation of the Scarff Bloom Richardson grading, which generates a summary score for the grade assignment (Table 3).[63]

Total score:

3-5, grade 1: well differentiated,

6-7 grade 2: moderately differentiated,

8-9 grade 3: poorly differentiated.[67]

MOLECULAR CLASSIFICATION OF CARCINOMA BREAST

In 2000, Perou and Sorlie presented "Molecular Classification" nomenclature demonstrating the variations in gene expression by an extensive investigation.[68]

Gene expression studies classified breast cancer into subtypes, such as luminal A and luminal B (ER positive), basal-like (ER-, PR-, and HER2-negative) and HER2-enriched, highlighting molecular heterogeneity.[68,69]

ER-The two subtypes are Erα and Erβ. Erα is transcription factor in cell cycles as well as proliferation and apoptosis.

Erα activation boosts production of oncogenic molecules causing increased proliferation of cancer cells and DNA damage.[70]

Since estrogen activation of ERα is main pathogenesis for majority of breast carcinomas, hormone therapy is the preferred treatment.[71]

PR- Progesterone is responsible for epithelial cell proliferation during pregnancy. Estrogen binding to the ER enhances PR expression causing increased proliferative effects.[72]

Progesterone induced co-regulatory proteins interacting with endoplasmic reticulum can modulate its transcriptional activity, hence influencing cell growth and survival.[73]

HER2 - ErbB family (Erythroblastic Leukemia Viral Oncogene Homolog family) includes HER1 (epidermal growth factor receptor -EGFR), HER2, HER3, and HER4. 15%–20% of invasive breast carcinomas have amplified HER2 genes leading to overexpression of the HER2 protein and poor prognosis.[74]

Precise determination of HER2 status is first step since targeted treatment is only successful in HER 2 upregulated tumors.[75]

Molecular subtypes of breast carcinoma-To differentiate between different subtypes, a panel comprising hormonal receptors, HER2, Ki-67, EGFR, and basal cytokeratins (CK) might be utilized.[76] The subtypes based on these markers are as follows (Table 4)

	MOLECULAR SUBTYPE	ER	PR	HER2	Ki67	CK5	EGFR
LUMINAL	Luminal A	+	+	-	<14	-	-
	Luminal B PR<20%	+	<20	-	+/-	-	-
	Luminal B Ki67 >14%	+	>20	-	>14	-	-
HER2 POSITIVE	Luminal Her 2 PR Pos	+	+	+	+/-	-	-
	Luminal Her 2 PR Neg	+	-	+	+/-	-	-
	Her2 Enriched	-	-	+	+/-	-	-
TRIPLE NEGATIVE	Basal like (BLBC)	-	-	-	+/-	+	+
	Non-classifiable (NCBC)	-	-	-	+/-	-	-

Table 4- Molecular classification [Taken from: Varga Z, Maccio U. Molecular pathology in breast disease: diagnostic, prognostic, and therapeutic tools. Virchows Arch Int J Pathol. 2024;484(2):247–61.]

Luminal A – This subtype is associated with high expression of hormonal receptor markers along with expression of luminal low molecular weight cytokeratins and include histological types like classical, lobular, tubular and cribriform.[77] Prognosis is usually good with response to endocrine therapy.

Luminal B – There is low to moderate expression of hormonal receptor markers along with luminal low molecular weight cytokeratins expression. This subtype usually invasive breast carcinoma NST and micropapillary variants.[77] As compared to type A, luminal B has less favourable prognosis.

Her 2 Positive – This subtype shows high expression of Her2/neu with comparatively low expression of hormonal receptors. It has unfavourable prognosis and shows response to Transtuzumab (Herceptin).[77]

Basal type -There is low or nil expression of ER, PR and Her2/neu and high expression of basal epithelial genes and cytokeratins and this subtype includes medullary, metaplastic, adenoid cystic, secretory and high grade IDC as histopathological variants.[77]

TRIPLE NEGATIVE BREAST CARCINOMA

TNBCs account for 12–17% of all cases of breast cancer. HER2, PR and ER are not expressed in TNBC, an aggressive form of breast cancer. The ASCO/CAP guidelines indicate that TNBCs typically show ≤1% expression of progesterone and estrogen receptors, and 0 to 1+ expression of HER2, as determined by immunohistochemistry.[78]

It is referred as 'basal-type' cancer due to expression of EGFR, CK5/6, CK14, and CK17.[79,80] However, according to Prat and colleagues, only 70–80% of TNBCs are included in basal-like category. [81]

It is prevalent in females with a mutation in the BRCA 1 gene, which causes breast cancer.[82] Another entity that was discovered using gene expression profiling is known as claudin-low breast cancer.[83]

TNBCs are classified into four transcriptional subtypes: mesenchymal, luminal androgen receptor and two basal subtypes. Along with this based on gene expression heterogeneity and different molecular mechanisms for tumorigenesis, TNBCs are classified in six categories.[84-86]

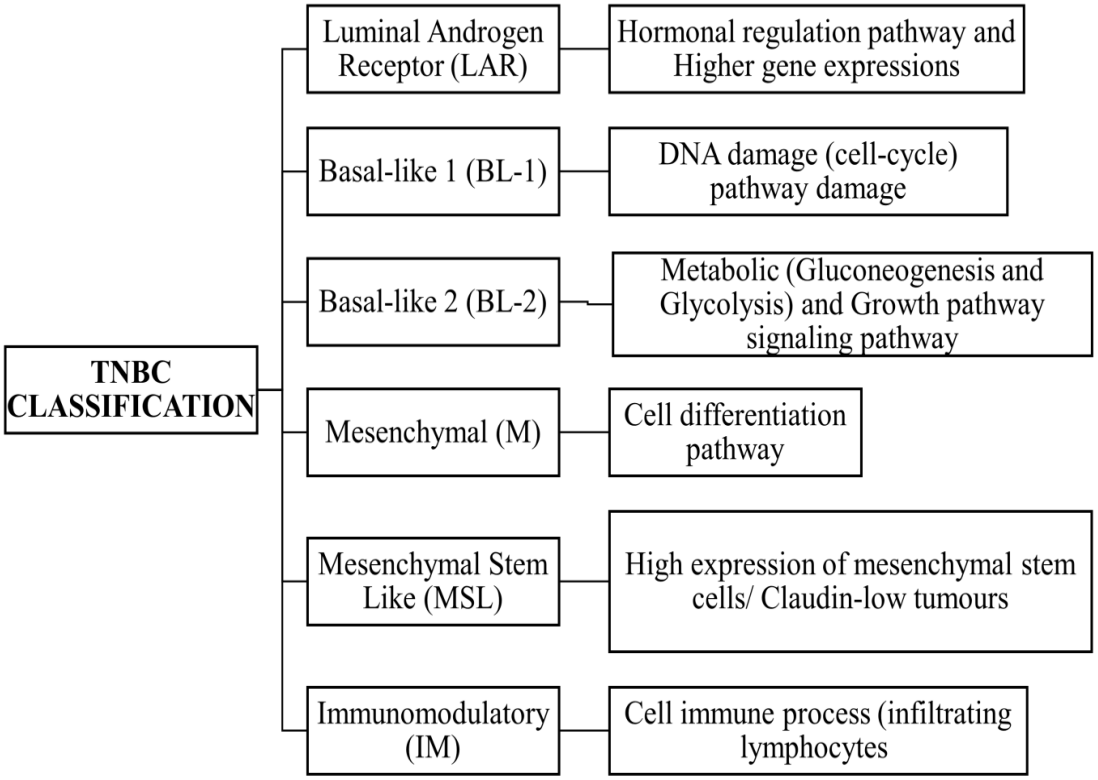


Figure 8- Molecular subtypes of TNBC

TNBC is frequently linked to unfavourable patient outcomes due to metastases to distant organ systems (skeletal/nervous/pulmonary).[87]

TNBC is a severely aggressive form of the disease that is not amenable to targeted therapeutic approaches like hormone therapy since it expresses none of major receptors (ER, PR, and HER2). Thus, novel markers that are accurate and which can help in treatment and prognosis of TNBC are required.[88]

ANTI-TUMOR IMMUNITY AND TNBC

Tumour microenvironment is increasingly acknowledged as a key factor in tumorigenesis. Most antigens are self-proteins that can activate T cells and initiate a regulatory immune response. The immune response needed to eliminate cancer is proinflammatory immunity, involving CD4 and CD8 T-helper cells.[89]

Immunological checkpoints maintaining immune tolerance and minimize tissue damage (such as from autoimmune reactions or infections), play a crucial role in immune resistance mechanisms.[90] Similar to melanoma, T cells that target neoantigens (novel peptides) created by mutations specific to cancer may drive the immune response against breast cancer.[91]

Moreover, it has been demonstrated that the tumour cell surface of 30% to 50% of all breast malignancies has elevated levels of the programmed cell death ligand 1 receptor (PD-L1).[92] There is mounting evidence that tumour-infiltrating T-cells have essential role in regulating basal subtype and that elevated levels of CD8+ T-cells may indicate improved prognosis with treatment sensitivity. However, tumour-associated macrophages indicate relatively poor prognosis.[93]

In TNBC, a higher quantity of TILs is correlated with a higher pathological complete response (pCR).[94] The extracellular matrix surrounding the tumour, fibroblasts, immune cells, signalling chemicals, and the surrounding blood arteries make up the tumour microenvironment.[95] TILs provide an endogenous anticancer immune response that slows the growth of tumours and increases the free survival rate of patients with TNBC.[96]

The International TILS Working Group has given few some guidelines to standardize evaluation of TILs (Fig 14).[97] Lymphocyte-predominant breast cancer (LPBC) is the term describing tumours with lymphocytic infiltration of greater than 50%.[89] However, definitions vary according to different studies and in practical terms, these are tumours with more lymphocytes than malignant cells.

Furthermore, TILs can be classified as- stromal, intra-tumoral and TILs at invasive margin. Increase in stromal TILs indicate immune response against tumor cells. Stromal TILs predict better responses to neoadjuvant chemotherapy and improved outcomes after adjuvant chemotherapy. Direct contact between TILs and carcinoma cells may indicate anti-tumor effects. Intra-tumoral TILs are harder to assess and provide less predictive value than stromal TILs. There is limited research on TILs at the invasive edge of breast cancer, particularly in neoadjuvant core biopsies. The invasive edge assessment may be difficult due to limited biopsy sampling and potential treatment-induced changes in tumor microenvironment, complicating reliable evaluation. Tumor-Lymphoid Structures (TLS) are typically located in normal tissue adjacent to the tumor, consisting of follicles with germinal centres. Although Tumor-Lymphoid Structures (TLS) are important for tumor-immune interactions, they are not yet suitable for routine clinical analysis. The main challenge is the spatial heterogeneity of TILs, which are typically localized around the

tumor and may not be captured in the plane of tissue sections, especially with core biopsies. Additionally, distinguishing lymphoid aggregates from true TILs can be difficult, particularly when germinal centres are not included in the section. [94,97]

In untreated triple-negative breast cancer (TNBC), TILs' presence is connected to enhanced overall & greater metastasis-free survival, and decreased likelihood of distant recurrence.[98-100] A 10% increase in intra-tumoral and stromal TILs showed that risk of relapse decreased by 17% and 15%, respectively, and the risk of death decreased by 27% and 17%, regardless of chemotherapy.[101] Few studies have examined outcome prediction of CD8+ TILs and FOXP3/ Forkhead Box P3 gene (IHC) as most TILs are CD8+ T cells.[102-106] Presence of TILs can signal an increased probability of achieving pCR.

Chemotherapy strengthens the immune system's fight against cancer by shrinking the tumor and changing the tumor's surroundings. Anthracyclines promote immunogenic death of tumor cells and decrease the number of myeloid-derived suppressor cells and regulatory T-cells, which typically dampen immune activity.[107] Neoadjuvant chemotherapy (NACT) increases TILs in residual TNBC, which is linked to better prognosis, including improved metastasis-free and overall survival, suggesting that a stronger immune response contributes to better long-term outcomes.[108-110]

IMMUNE CHECK POINT INHIBITORS AND TNBC

The necessity to create more efficacious treatment modalities for patients with TNBC prompts substantial research in this domain, leading to the evaluation of numerous novel therapeutic strategies in clinical trials, including immunotherapy utilizing immune checkpoint inhibitors (ICI).[111] PD-1 and CTLA-4 serve as negative

regulators of T cell immunological activity.[112] When a T-cell binds to PD-L1, it becomes inactivated. Blocking PD-1 or PD-L1 antibodies reactivates tumor-specific T-cells. Unlike most monoclonal antibodies for cancer, which target tumor cells directly, immune checkpoint inhibitors block lymphocyte receptors or their ligands. This enhances the immune system's ability to recognize and attack tumors by overcoming inhibitory pathways.[90] Pembrolizumab, an anti-PD-1 monoclonal antibody showed an 18.5% response rate in heavily pretreated TNBC.[113] PD-L1 may be a potential target for therapies directed at the PD-L1 axis, but its expression in tumor tissues may not serve as a consistent biomarker for selecting patients for anti-PD-1 or anti-PD-L1 treatments, as PD-L1 expression is variable and fluctuates over time.[114] There is lack of clinical data on the practical use of potential biomarkers to predict responses to immunotherapy. As new predictive biomarkers are developed, strict criteria must be followed, including verifying that the biomarker is linked to overall survival in multiple independent clinical trials.[115]

CD53 AND TNBC

CD53, also known as TSPAN30 (tetraspanin 30), is member of the tetraspanin family of proteins, which are characterized by the presence of four transmembrane domains. This glycoprotein is primarily expressed on immune cells such as T cells, B cells, NK cells, monocytes, and dendritic cells as well as hematopoietic stem cells (HSCs). CD53 is upregulated in HSCs in response to inflammatory and proliferative stressors.[116]

CD53 is involved in the regulation of immune cell signalling, adhesion, and the formation of the immune synapse, playing a crucial role in immune cell activation and response. As a member of the tetraspanin family, CD53 spans the plasma

membrane four times, and it interacts with variety of other membrane proteins to form complexes known as "tetraspanin-enriched microdomains" (TEMs). These complexes facilitate the coordination of signalling pathways, cell adhesion, and migration, and are integral to processes like antigen presentation and immune response modulation. CD53's functional properties are largely mediated through its ability to interact with integrins, signalling molecules, and other tetraspanins such as CD81, CD82, and CD9, thereby influencing immune cell behaviour.[117]

CD53 plays an essential role in immune cell signalling and adhesion.[118] (Fig.9) In T cells, for example, it is involved in T-cell receptor signalling and synapse formation, which is crucial for T-cell activation and antigen recognition.[119] CD53 is involved in activating and differentiating B cells and may also regulate innate immune responses in monocytes and NK cells. In T cells lacking CD53, altered CD45 phosphatase activity resulted in weakened immune function, impaired recall responses, lower interferon -γ, reduced proliferation and diminished anti-tumor activity in CD53−/− mice.[120] CD53 regulates early B cell growth, differentiation, and survival by associating with Interleukin 7 receptor-α and activating Phosphoinositide 3-Kinase pathway and Signal Transducer and Activator of Transcription pathway.[121] The protein's role in immune synapse formation with immune cells interacting with target cells is key for efficient immune surveillance. This interaction is essential for the elimination of malignancies and pathogens.

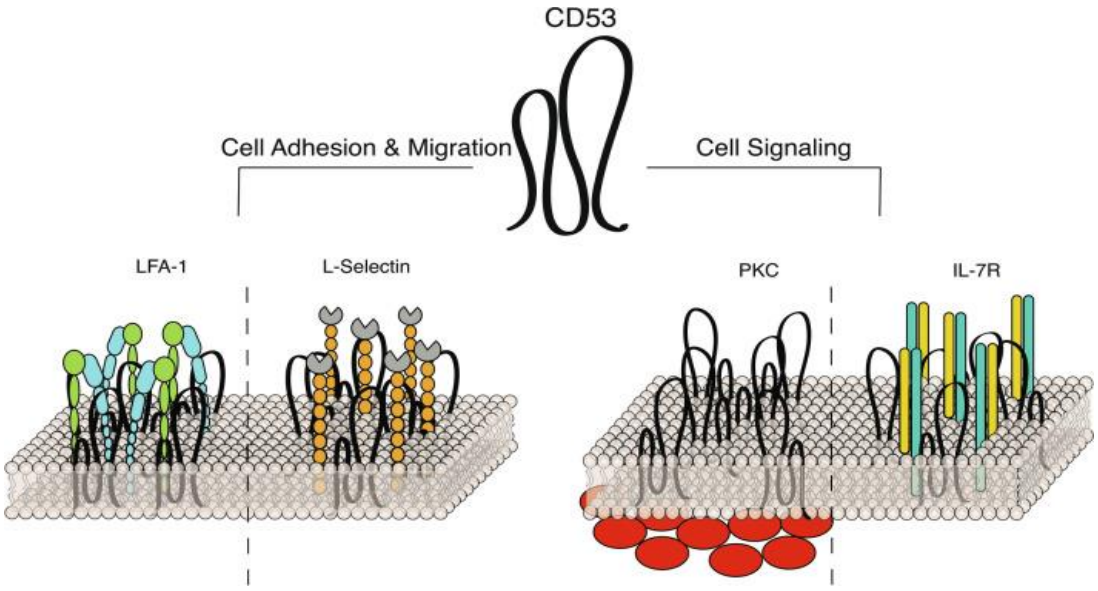


Figure 9- CD53 interactions with adhesion molecules and signalling molecules. [Taken from: Dunlock VE. Tetraspanin CD53: an overlooked regulator of immune cell function. Med Microbiol Immunol (Berl). 2020;209(4):545–52.]

Familial CD53 deficiency has been linked to recurrent infections and reduced serum Ig levels, indicating its role in immune function.[122] CD53 links inflammatory and metabolic signals to hepatocyte nutritional status, and its blockade mitigates pathophysiology in overnutrition-related diseases like non-alcoholic steatohepatitis and type 2 diabetes.[123]

The ability of CD53 to regulate cell signalling through its interactions with other molecules in TEMs highlights its potential to influence immune responses, particularly in cancer immunotherapy. Recent studies have shown that CD53 may participate in regulating immune cell infiltration into tumors, influencing the effectiveness of immune responses against tumors. In some cancers, aberrant expression of tetraspanins like CD53 could contribute to immune evasion, either by altering immune cell trafficking or by modifying the sensitivity of immune cells to activation signals. Ligation of CD53 on the cell surface may protect certain tumors

from programmed cell death, aiding their survival and enabling them to evade immune responses.[124]

Targeting or altering CD53 expression could enhance immune cell anti-tumor responses. CD53 also regulates tumor-associated macrophages, which contribute to tumor growth and metastasis, potentially affecting their pro-tumor behaviour.[125]

CD53 aids tumor antigen uptake by dendritic cells (DCs), crucial for T cell activation and tumor immune surveillance. Its high expression in DC subsets, especially plasmacytoid DCs, and interaction with Major Histocompatibility Class I molecules promote cross-presentation.[126,127] CD53−/− mice exhibited greater tumor growth than wild-type mice in syngeneic immunogenic tumor models, highlighting its predictive value.[12] The CD53 network predicts distant metastasis-free survival, particularly in ER− breast cancer.[128]

Although CD53 has potential as therapeutic target, its clinical relevance in cancer immunotherapy remains to be fully determined. Research is ongoing to understand how CD53 expression impacts immune cell function within the TME and its potential to serve as a biomarker for response to immunotherapies. Further investigations are needed to identify specific cancers or subsets of patients that might benefit from therapies targeting CD53.

Recent studies suggest that CD53 expression may be linked to patient prognosis in cancers like breast cancer and leukemia, making it a potential prognostic marker. With high expression in both intra- and peri-tumor TILs, CD53 shows promise as a prognostic marker for cancer patients.[129] Since CD53 expression is dynamic and may vary under different immunological conditions, it remains a challenge to establish it as a definitive biomarker for predicting immunotherapy outcomes.

MATERIALS AND METHODS

The study included 50 triple negative breast carcinoma (TNBC) cases. The operated cases of breast carcinoma specimens received from January 2023 to December 2024 in the Department of Pathology, KAHER's Dr. Prabhakar Kore Hospital and Medical Research Centre, a teaching hospital attached to Jawaharlal Nehru Medical College, Belagavi were included in this study.

Study Design- Hospital based cross sectional study

Study Period: January 2023 to December 2024

Study population: The operated cases of breast carcinoma specimens received from Jan 2023 to December 2024.

Inclusion criteria- Immunohistochemically proven TNBC cases

Exclusion Criteria: 1. Improperly fixed specimens

2. Inadequate biopsies

3. Cases where ER, PR and HER2 were not evaluated.

Sample size: The sample size was calculated with the help of Medcalc software by considering alpha = 0.01, beta = 0.05, AUC = 0.7931(from previous studies), null hypothesis = 0.5. Hence, sample size ≈30. However, we received **total 50** immunohistochemically proven TNBC cases which were included in this study.

Sampling technique: Fifty study samples obtained during study period were taken.

Ethical clearance: The present study was approved by Jawaharlal Nehru medical college's Institutional Ethics committee on Human Subjects research. (Ref No.: MDC/JNMCICE/1211)

Data collection: The breast carcinoma cases with modified radical mastectomy and breast conservative surgery or wide local excision specimens were included. These specimens were grossed according to the standard procedure, and bits were given from representative areas. Grossly, tumor location based on anatomical quadrants and tumor size were assessed and categorized into 2 groups as per the greatest dimension (Group 1: < 5 cm, Group 2:> 5cm).

All the formalin-fixed paraffin-embedded tissue blocks were screened and stained with haematoxylin and eosin (H and E), and detailed histopathological examination was done. All the tumors were classified in different histological subtypes and graded as per Modified Scarff-Bloom Richardson scoring system. Various parameters including lymphovascular invasion (LVI), perineural invasion (PNI), Paget's disease and axillary lymph node metastasis were evaluated.

Lymphocytic response was assessed and categorized as intra-tumoral (TILs having direct contact with neoplastic cells) and stromal or peri-tumoral lymphocytes (TILs seen in surrounding stroma, between cancer cells without direct contact).[97] The lymphocytic response was further graded as mild (<10%), moderate (10-50%) and high or LPBC (>50%) adopting to already reported cut-offs.[97,101,130]

Further, these cases were divided as per Nottingham Prognostic Index (NPI) using formula: tumor size (cm) x 0.2 + histological grade (1-3) + Axillary lymph node involvement (1: absent, 2: 1-3 nodes and 3: 4 or more nodes). Further, prognostic groups were divided as good (3.4), moderate (>3.4-5.4) and poor (>5.4).[131]

Tissues with the highest tumour areas were selected for ER, PR and Her2 IHC markers. Immunohistochemically proven TNBC cases were included in this study and were stained with CD53 marker.

Information regarding clinical data including age and clinical history were obtained from Medical Records Department (MRD) records.

IHC manual staining protocol: 2-3micron thin sections were taken from one representative formalin fixed paraffin embedded block from each case.

Steps for IHC staining for CD53 is as follows:

Materials Required-

- Formalin-fixed, paraffin-embedded tissue sections
- Anti-CD53 primary antibody (Polyclonal IgG type Antibody)
- Secondary antibody
- Antigen retrieval solution [Tris(hydroxymethyl)aminomethane Buffer/TRIS buffer-0.605 grams + Ethylenediaminetetraacetic Acid/ EDTA buffer solution-0.185 grams]
- Peroxidase-blocking solution (3% H_2O_2 or hydrogen peroxide)
- Chromogen substrate
- Mounting medium
- Poly L Lysine solution
- Staining dishes and appropriate coverslips

Preparation of sections: Prior to deparaffinization, slides were baked at 60 degrees centigrade for 1 hour.

Steps of deparaffinization

1. The paraffin-embedded tissue sections (4-5 µm thick) were placed on glass slides.
2. Xylene I- 10minutes
3. Xylene II-10minutes
4. Absolute alcohol I-10minutes
5. Absolute alcohol II-10minutes
6. Rinse in water for-5minutes
7. Rinse in distilled water for-1minute

Antigen Retrieval: Tris buffer +EDTA- Buffer solution -Heat-induced epitope retrieval was done by using Pressure Cooker (3 whistles). Then they were subjected for cooling at room temperature following which rinsing with wash buffer was done 3 times with gap of 2 minutes. Slides were placed in 3% hydrogen peroxide for 8-10 minutes and repeat buffer washing was done 3 times.

Primary antibody incubation: Primary CD53 antibody was incubated for 45-60 minutes in closed room and were washed with buffer 3 times with gap of 2 minutes each.

Secondary antibody incubation: The HRP polymer (Horse-radish peroxidase) was applied and incubated for 25-30 minutes at room temperature in closed chamber. And slides were again subject to wash buffer.

Detection: DAB (Diaminobenzidine) solution was applied for 10 minutes followed by tap-water washing for 2 minutes and distilled water washing for one minute

Counterstaining: Counterstaining with haematoxylin to stain the nuclei was done, followed by washing in running water for 2 minutes.

Mounting: Slides were blotted, cleared in xylene and mounted with Dibutylphthalate Polystyrene Xylene **(**DPX).

Antibody in study

Antibody: CD53 (Product code: CSB-PA004944ESR2HU)
Localization: Primarily plasma membrane
Clonality: Rabbit polyclonal clone (IgG)
Dilution: 1:100

Evaluation of immunoreactivity: Sections were examined to look for positivity in tumor cells and TILs. Intensity was graded a negative (No staining), +1 or weak (faint staining of CD53), +2 or moderate (clearly visible, but moderate staining intensity) and 3+ or strong (intense staining of CD53, indicating high expression levels).

Statistical analysis: Data was entered in Microsoft Excel and analyzed using SPSS software (version 20). The descriptive statistics including categorical variables were expressed as percentage and chi-square test was used to study association between the clinico-histopathological variables and CD53 expression. A p-value of lesser than 0.05 was considered as statistically significant.

RESULTS

Fifty immunohistochemically proven cases of triple negative breast carcinomas were taken into the study.

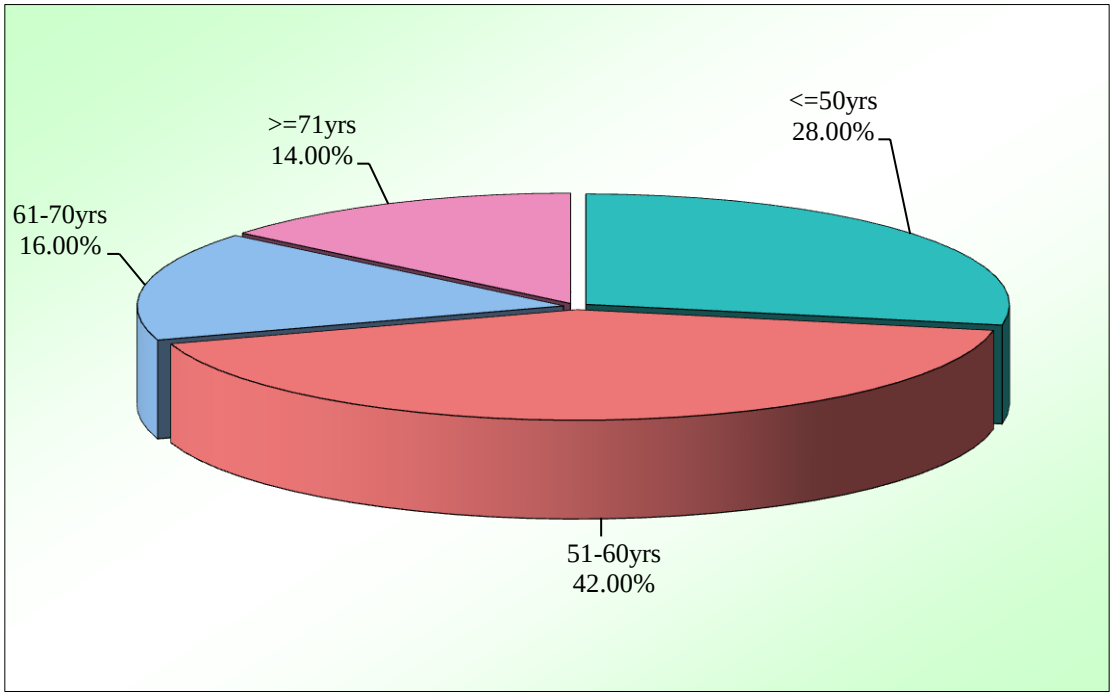


Figure 10- Age wise distribution of study subjects

Patients were divided into 4 age groups with majority (42%) belonging to 51-60 years. The mean age of participants was 57.34 years with standard deviation (SD) of 11.82. The youngest patient was 36 years old and oldest of 82 years.

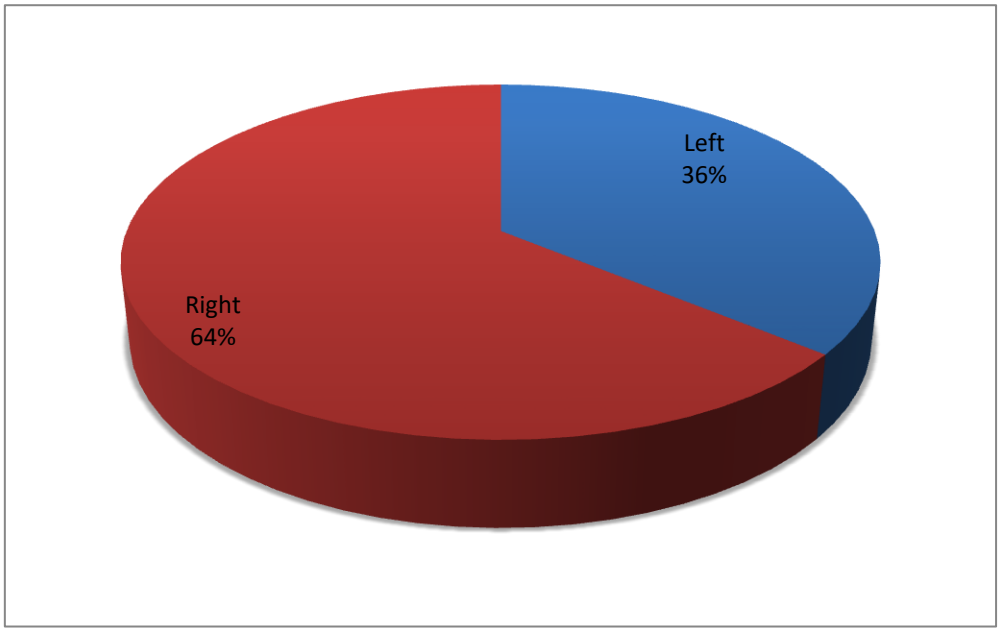


Figure 11- Distribution as per laterality

Most tumors were found in the right breast (64%) compared to the left (36%).

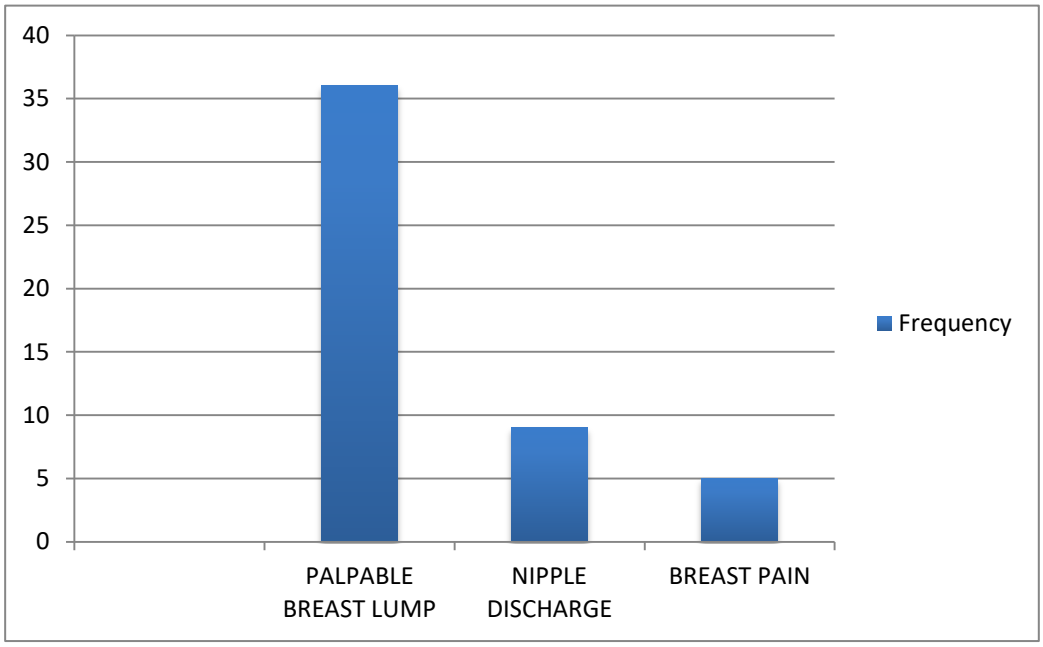


Figure 12- Distribution as per presenting clinical complaints

The most common symptom was palpable breast lump (highest frequency, 72%), followed by nipple discharge (18%) and breast pain (10%).

Table 5: Distribution as per tumor location -

The upper outer quadrant was commonest site (46%) followed by central (26%).

Tumor Site	Frequency	Percentage
Upper outer quadrant (UOQ)	23	46.00
Upper inner quadrant (UIQ)	5	10.00
Multicentric	2	4.00
Lower outer quadrant (LOQ)	6	12.00
Lower inner quadrant (LIQ)	1	2.00
Central	13	26.00

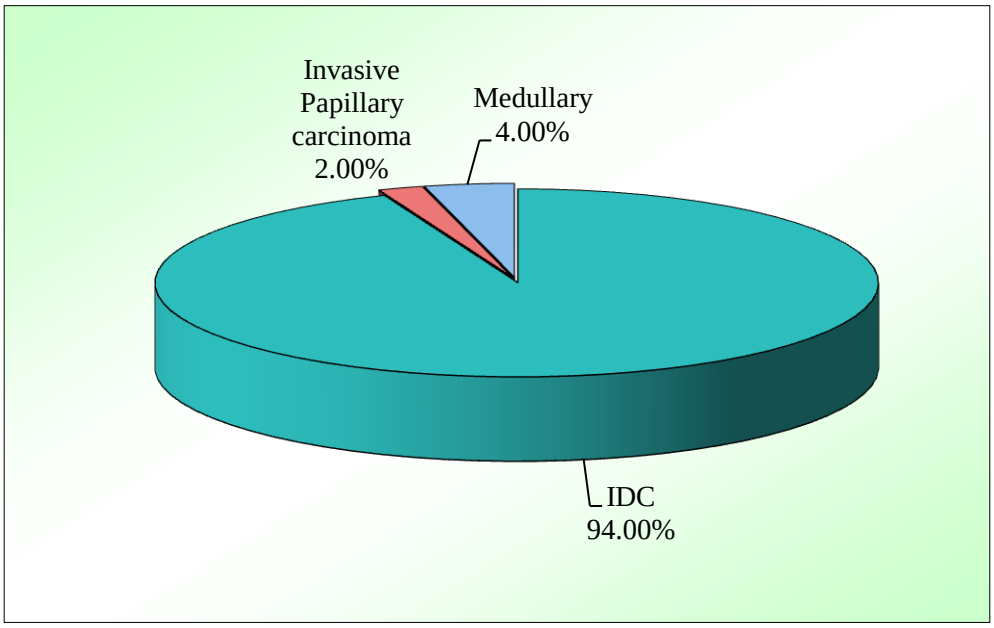


Figure 13- Distribution as per histopathological subtype

The most common type was invasive carcinoma of no special type or invasive ductal carcinoma (IDC) (94%).

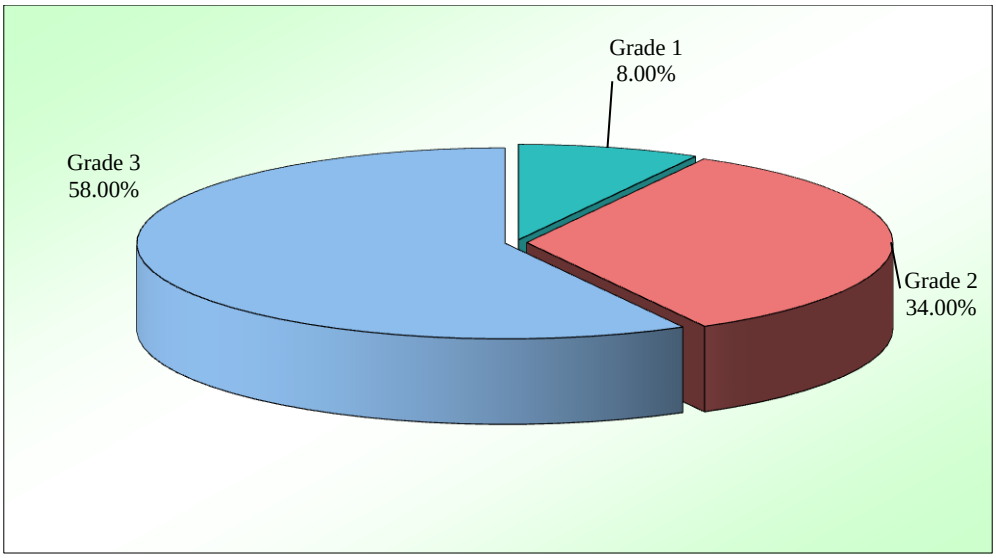


Figure 14- Distribution of study subjects as per histological grade

Significant portion of subjects (58%) had grade 3 tumors which indicates aggressiveness of TNBCs.

The study subjects were divided based on intra-tumoral and stromal lymphocytic response.

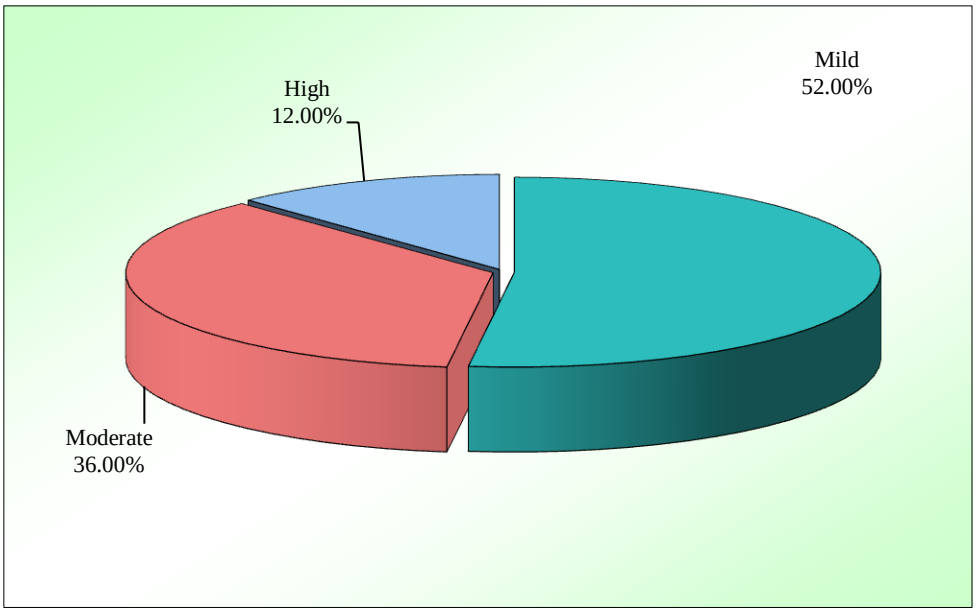


Figure 15- Distribution of study subjects as per Lymphocytic Response (Intra-tumoral lymphocytes)

The majority of subjects (52%) had mild intra-tumoral lymphocytic infiltration followed by moderate (36%) and high (12%).

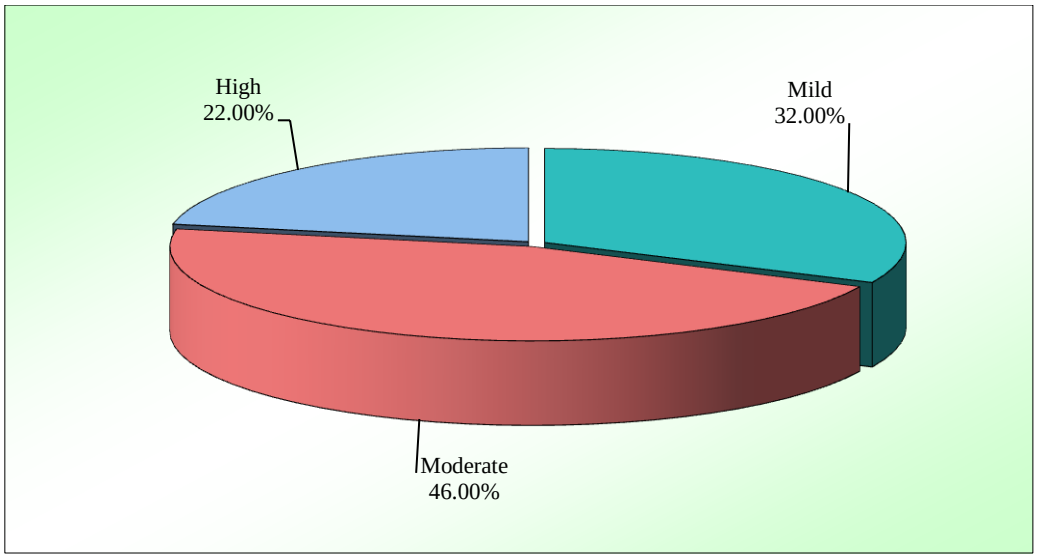


Figure 16- Distribution of study subjects as per Lymphocytic Response (Peri-tumoral lymphocytes)

The most common category was moderate stromal infiltration by lymphocytes (46%) followed by mild (32%) and high (22%).

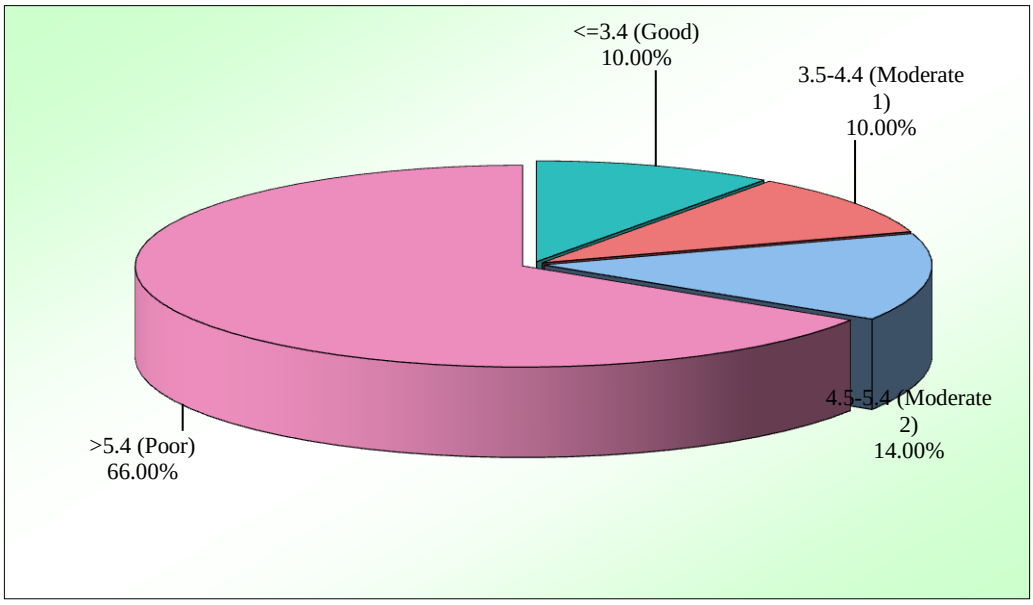


Figure 17- Distribution of study subjects as per Nottingham Prognostic Index (NPI)

NPI score >5.4 was seen in 66% of patients suggesting poor outcomes.

Table 6: Comparison between lymphocytic response and NPI

NPI		INTRA-TUMORAL LYMPHOCYTIC RESPONSE					
		Mild		Moderate		High	
		Freq	Percentage	Freq	Percentage	Freq	Percentage
<3.4	Good	0	0.00	3	6.00	2	4.00
3.5-5.4	Moderate	4	8.00	4	8.00	4	8.00
>5.4	Poor	22	44.00	11	22.00	0	0.00

(Chi Square: 17.43 and p-value 0.001*)

NPI		STROMAL/PERI-TUMORAL LYMPHOCYTIC RESPONSE					
		Mild		Moderate		High	
		Freq.	Percentage	Freq.	Percentage	Freq.	Percentage
<3.4	Good	0	0.00	0	0.00	4	8.00
3.5-5.4	Moderate	1	2.00	7	14.00	4	8.00
>5.4	Poor	15	30.00	16	32.00	2	4.00

(Chi square: 24.08 and p- VALUE 0.0005*)

- Patients with poor prognosis (NPI>5.4) had a higher proportion of mild lymphocytic response.
- Those with better prognosis (NPI<3.4) had moderate or high lymphocytic response.
- There was significant correlation seen with better prognostic index and higher lymphocytic response in both intra -tumoral and stromal TILs.

Table 7: Evaluation of CD 53 based on positivity range and localization

CD53	TUMOR CELLS	INTRA-TUMORAL LYMPHOCYTES	PERI-TUMORAL/ STROMAL LYMPHOCYTES	NORMAL DUCTS (ADJACENT TO TUMOR)
Positivity range	90-100%	90-100%	90-100%	90-100%
Localization	Membranous Cytoplasmic	Membranous Cytoplasmic	Membranous Cytoplasmic	Membranous Cytoplasmic

- CD53 marker showed membranous and cytoplasmic positivity in almost all tumor cells, TILs and normal ducts adjacent to tumor.

Table 8: Evaluation of CD53 expression in tumor cells/ intra- and stromal or peri- lymphocytes/ normal ducts based on intensity

Intensity	Number	%
CD53 Intra-tumoral lymphocytes		
2+	4	8.00
3+	46	92.00
CD53 Stromal lymphocytes		
2+	25	50.00
3+	25	50.00
CD53 tumor cells		
2+	8	16.00
3+	42	84.00
CD53 normal ducts		
NA*	6	12.00
2+	37	74.00
3+	7	14.00
Total	50	100.00

(NA: Not able to asses as normal breast ducts were absent adjacent to tumor)

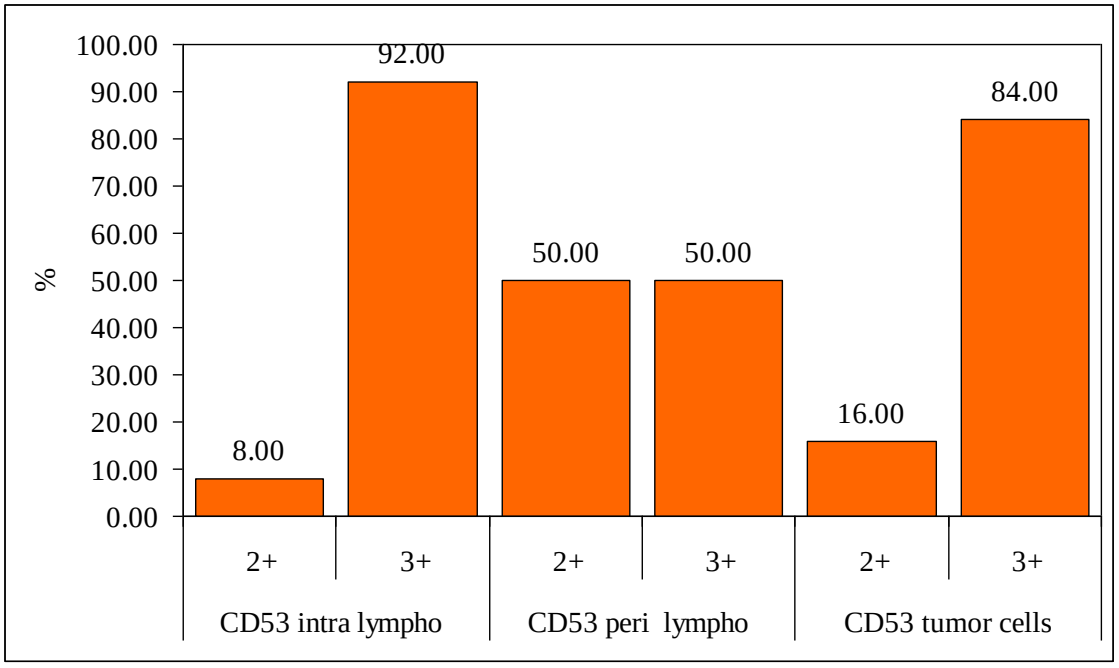


Figure 18- Evaluation of CD53 intensity in tumor cells/ intra- and stromal or peri- lymphocytes based on intensity

- CD53 expression was seen in 90%-100% tumor cells and lymphocytes.
- Most of the tumor cells (84%) showed higher intensity (3+).
- Majority of intra-tumoral (92%) lymphocytes had 3+ intensity.
- However, CD53 intensity (2+ and 3+) was evenly distributed in peri-tumoral lymphocytes.
- CD53 expression in normal breast ducts was studied in 44 cases which showed positivity.

Table 9: Comparison between age groups and CD53 intensity in intra-tumoral lymphocytes

Age groups	2+	%	3+	%	Total
</=50yrs	2	14.29	12	85.71	14
51-60yrs	1	4.76	20	95.24	21
61-70yrs	0	0.00	8	100.00	8
>/=71yrs	1	14.29	6	85.71	7
Total	4	8.00	46	92.00	50
Chi-square=2.1220, p=0.5470					

- Across all the age groups, intensity of 3+ was commonest.
- The chi-square value (2.1220) and p-value (0.5470) indicated no significant association between age and CD53 expression in intra-tumoral lymphocytes.

Table 10: Comparison between age groups and CD53 intensity in peri-tumoral/stromal lymphocytes

Age groups	2+	%	3+	%	Total
<=50yrs	8	57.14	6	42.86	14
51-60yrs	10	47.62	11	52.38	21
61-70yrs	2	25.00	6	75.00	8
>=71yrs	5	71.43	2	28.57	7
Total	25	50.00	25	50.00	50
Chi-square=3.6190, p=0.3060					

- The overall distribution was evenly split (50% with 2+ and 50% with 3+) in stromal lymphocytes.
- Chi-square value=3.6190 and p value=0.3060 suggested no significant correlation between age and peri-tumoral lymphocytes.

Table 11: Comparison between age groups and CD53 intensity in neoplastic cells

Age groups	2+	%	3+	%	Total
<=50yrs	1	7.14	13	92.86	14
51-60yrs	4	19.05	17	80.95	21
61-70yrs	1	12.50	7	87.50	8
>=71yrs	2	28.57	5	71.43	7
Total	8	16.00	42	84.00	50
Chi-square=1.8580, p=0.6020					

- CD53 intensity in tumor cells remained high (3+) regardless of age.
- Age did not significantly impact CD53 expression level in tumor cells.

Table 12: Comparison between tumor size and CD53 intensity in TILs/tumor cells

CD53 (intensity)	Tumor size (largest dimension in cm)	Grade	Frequency	Percentage	P VALUE
CD53 intra lymphocytes	<5	2+	2	4.00	<0.0001*
		3+	40	80.00	
	>5	2+	2	4.00	0.157
		3+	6	12.00	
CD53 peri/stromal lymphocytes	<5	2+	18	36.00	0.354
		3+	24	48.00	
	>5	2+	7	14.00	0.03*
		3+	1	2.00	
CD53 tumor cells	<5	2+	6	12.00	0.05*
		3+	36	72.00	
	>5	2+	2	4.00	0.157
		3+	6	12.00	

*p<0.05

- Higher CD53 intensity (3+) was more frequent in tumor size <5 cm compared to tumor size >5cm.

Table 13: Comparison between lymphovascular invasion (LVI) and CD53 intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

CD53	LVI				Total	%	p-value
	Absent	%	Present	%			
CD53 intra-tumoral lymphocytes							
2+	0	0.00	4	100.00	4	8.00	0.0890
3+	20	43.48	26	56.52	46	92.00	
CD53 peri – tumoral lymphocytes							
2+	0	0.00	25	100.00	25	50.00	.000*
3+	20	80.00	5	20.00	25	50.00	
CD53 tumor cells							
2+	3	37.50	5	62.50	8	16.00	0.8750
3+	17	40.48	25	59.52	42	84.00	
Total	20	40.00	30	60.00	50	100.00	

*$p<0.05$

- There was no significant association between LVI and CD53 intensity in tumor cells and intra-tumoral lymphocytes.
- Higher CD53 intensity (3+) in peri-lymphocytic cells was more associated with absence of LVI, while moderate expression (2+) was linked to LVI presence.
- Significant association was seen with higher intensity of CD53 in stromal lymphocytes and LVI absence.

Table 14: Comparison between perineural invasion (PNI) and CD53 expression intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

1CD53	PNI				Total	%	p-value
	Absent	%	Present	%			
CD53 intra-tumoral lymphocytes							
2+	0	0.00	4	100.00	4	8.00	0.0300*
3+	26	56.52	20	43.48	46	92.00	
CD53 peri-tumoral lymphocytes							
2+	3	12.00	22	88.00	25	50.00	0.0001*
3+	23	92.00	2	8.00	25	50.00	
CD53 tumor cells							
2+	4	50.00	4	50.00	8	16.00	0.9020
3+	22	52.38	20	47.62	42	84.00	
Total	26	52.00	24	48.00	50	100.00	

*$p<0.05$

- In case of intra-tumoral lymphocytes, 2+ intensity was observed in 4% cases where PNI was present and 3+ intensity was associated with 56.52% in PNI - absent and 43.48% in PNI present groups.
- There was 2+ intensity expression in peri-tumoral lymphocytes associated with PNI presence and 92% cases showed 3+ intensity with absence of PNI group.
- Hence, there was statistically significant association between PNI and CD53 intensity in TILs.
- However, no significant association (p-value= 0.9020) was seen between PNI and tumor cells CD53 intensity

Table 15: Comparison between histological grade and CD53 intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

CD53	Histological grade (I/II/III)						p-value
	I	%	II	%	III	%	
CD53 intra-tumoral lymphocytes							
2+	0	0.00	0	0.00	4	100.00	0.2070
3+	4	8.70	17	36.96	25	54.35	
CD53 peri-tumoral lymphocytes							
2+	0	0.00	2	8.00	23	92.00	0.0001*
3+	4	16.00	15	60.00	6	24.00	
CD53 tumor cells							
2+	1	12.50	1	12.50	6	75.00	0.3660
3+	3	7.14	16	38.10	23	54.76	
Total	4	8.00	17	34.00	29	58.00	

*$p<0.05$

- CD53 expression in intra-tumoral lymphocytes and tumor cells did not show a significant correlation with histological grade.
- However, 2+ expression in peri-tumoral lymphocytes was seen in 92% cases having histological grade 3 and 3+ intensity was seen in all the grades including grade 1.
- There was significant association (p-value=0.0001) between intensity of CD53 in peri-tumoral lymphocytes and histological grade.

Table 16: Comparison between Paget's Disease and CD53 intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

CD53	Paget's disease				Total	%	p-value
	Absent	%	Present	%			
CD53 intra- tumoral lymphocytes							
2+	0	0.00	4	100.00	4	8.00	0.0020*
3+	34	73.91	12	26.09	46	92.00	
CD53 peri-tumoral lymphocytes							
2+	10	40.00	15	60.00	25	50.00	0.0001*
3+	24	96.00	1	4.00	25	50.00	
CD53 tumor cells							
2+	4	50.00	4	50.00	8	16.00	0.2340
3+	30	71.43	12	28.57	42	84.00	
Total	34	68.00	16	32.00	50	100.00	

- In TILs, 3+ intensity was associated more with absence of Paget's disease showing significant association.
- CD53 intensity in tumor cells showed no significant association with Paget's diseases (p-value: 0.2340)

Table 17: Comparison between Axillary lymph node involvement and CD53 intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

CD53	Axillary lymph node involvement				Total	%	p-value
	Absent	%	Present	%			
CD53 intra-tumoral lymphocytes							
2+	0	0.00	4	100.00	4	8.00	0.1530
3+	16	34.78	30	65.22	46	92.00	
CD53 peri -tumoral lymphocytes							
2+	1	4.00	24	96.00	25	50.00	0.0001*
3+	15	60.00	10	40.00	25	50.00	
CD53 tumor cells							
2+	3	37.50	5	62.50	8	16.00	0.7160
3+	13	30.95	29	69.05	42	84.00	
Total	16	32.00	34	68.00	50	100.00	

*$p<0.05$

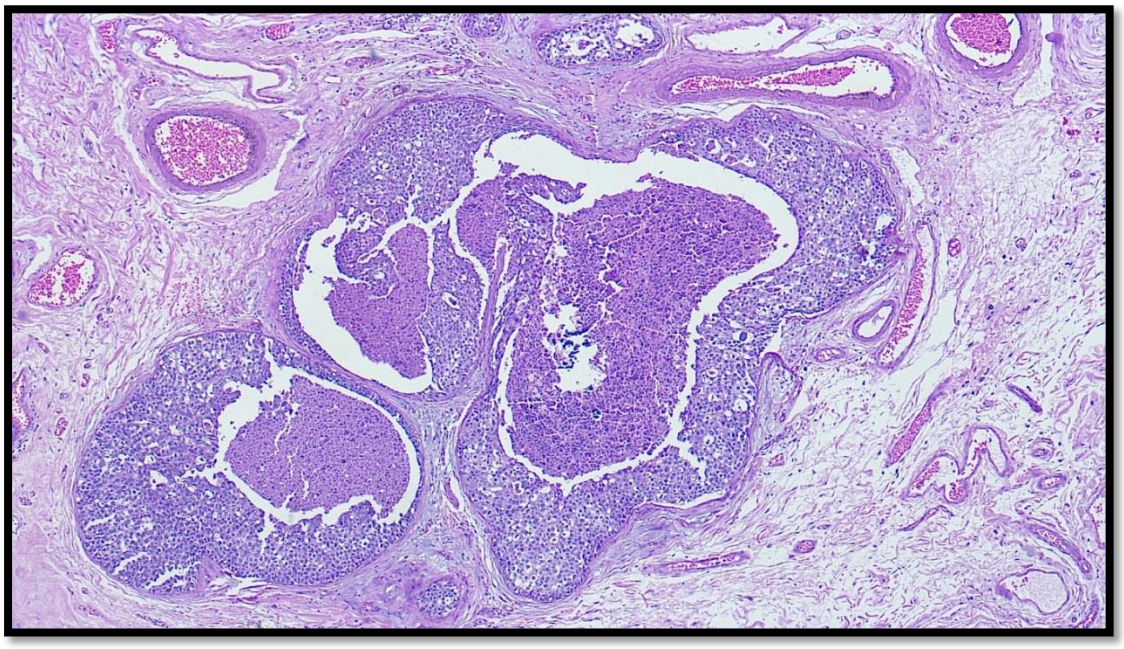
- Ninety six percent cases with 2+ expression had axillary lymph node involvement whereas 60% of cases with 3+ intensity did not show lymph node metastasis in stromal lymphocytes.
- Thus, there was significant association between intensity in stromal lymphocytes(p-value=0.0001) and axillary lymph node involvement.
- There was no such association in case of tumor cells and intra-tumoral lymphocytes.

Table 18: Comparison between Nottingham Prognostic Index/NPI (good/moderate/poor) and CD53 expression intensity in tumor cells, intra-tumoral lymphocytes and stromal lymphocytes

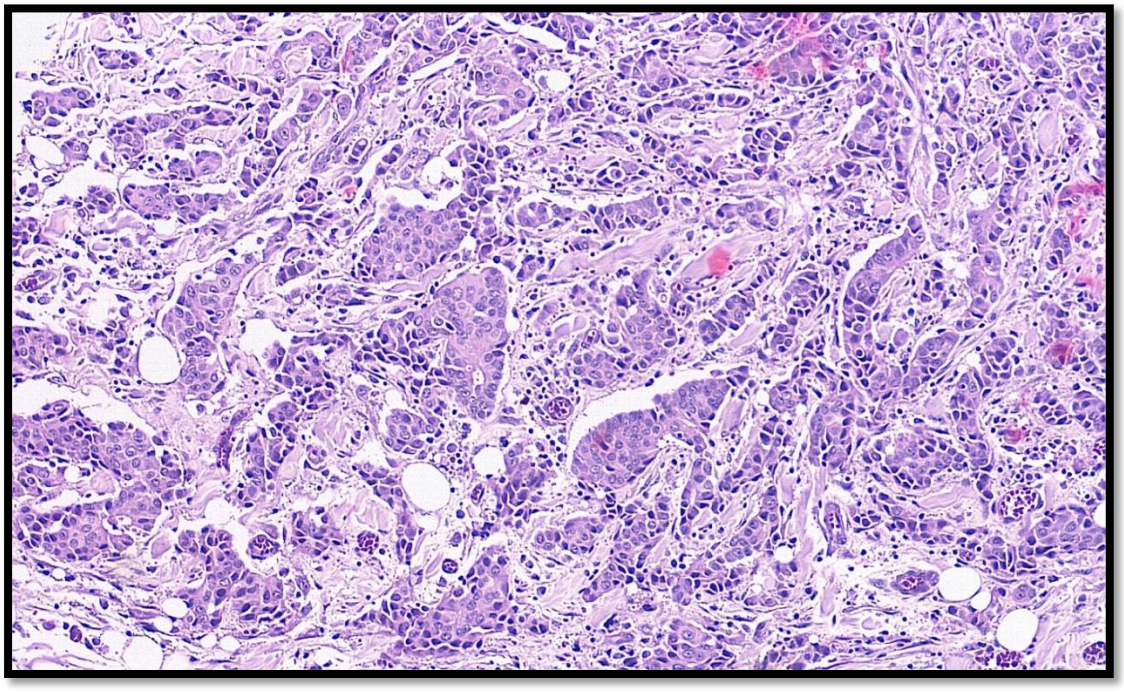
CD53	NPI						p-value
	Good	%	Moderate	%	Severe	%	
CD53 intra-tumoral lymphocytes							
2+	0	0.00	0	0.00	4	100.00	0.1530
3+	5	10.87	12	26.09	29	63.04	
CD53 peri-tumoral lymphocytes							
2+	0	0.00	0	0.00	25	100.00	0.0001*
3+	5	20.00	12	48.00	8	32.00	
CD53 tumor cells							
2+	0	0.00	3	37.50	5	62.50	0.7160
3+	5	11.90	9	21.43	28	66.67	
Total	5	10.00	12	24.00	33	66.00	

*$p<0.05$

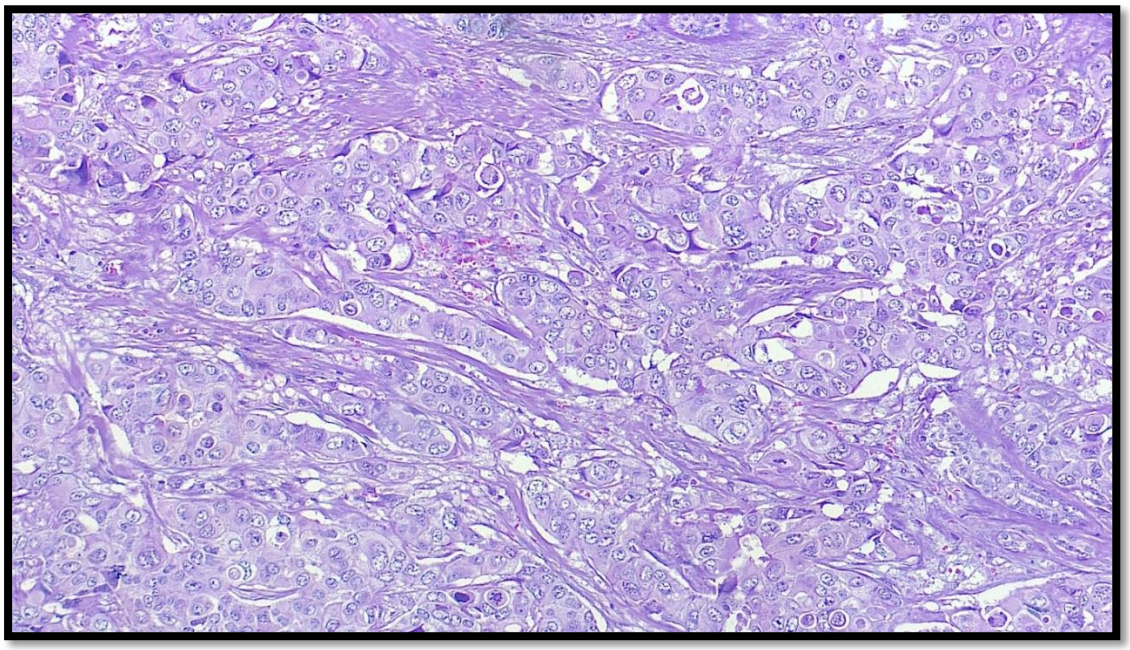
- CD53 marker intensity in intra-tumoral lymphocytes and neoplastic cells was not significantly correlated with NPI in this study.
- Around 62.50% of cases with 2+ expression were in severe prognostic category and 11.90% cases in good prognosis category had 3+ intensity, hence showing significant correlation of CD53 intensity in peri-tumoral lymphocytes with NPI.



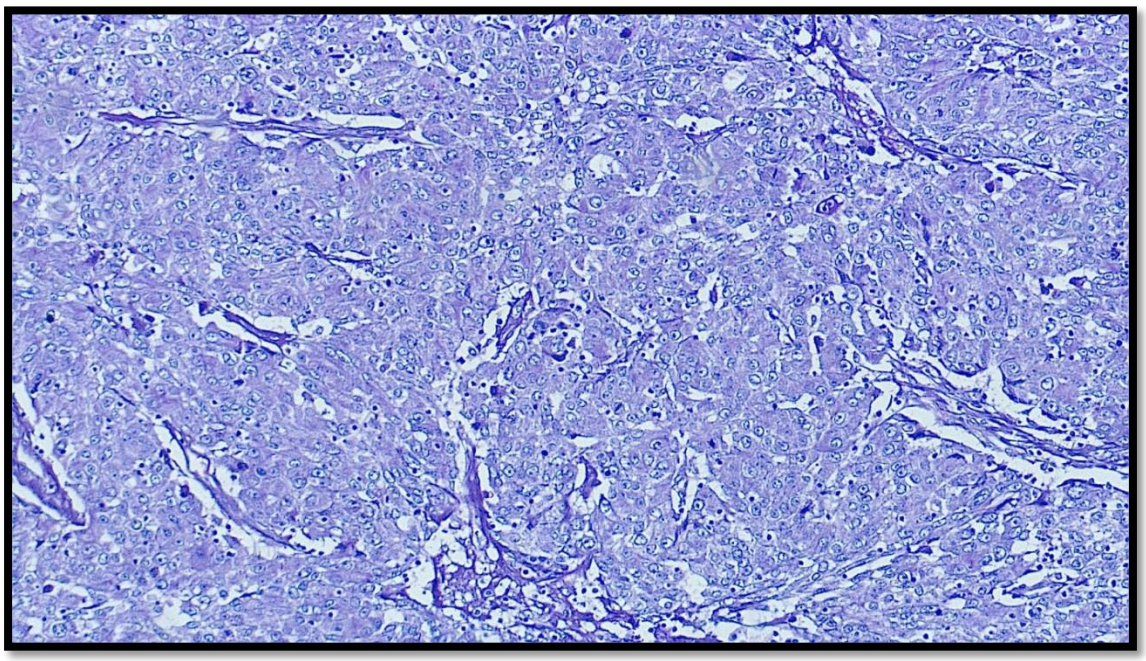
Photomicrograph 1: Ductal Carcinoma in Situ (H and E; X200)



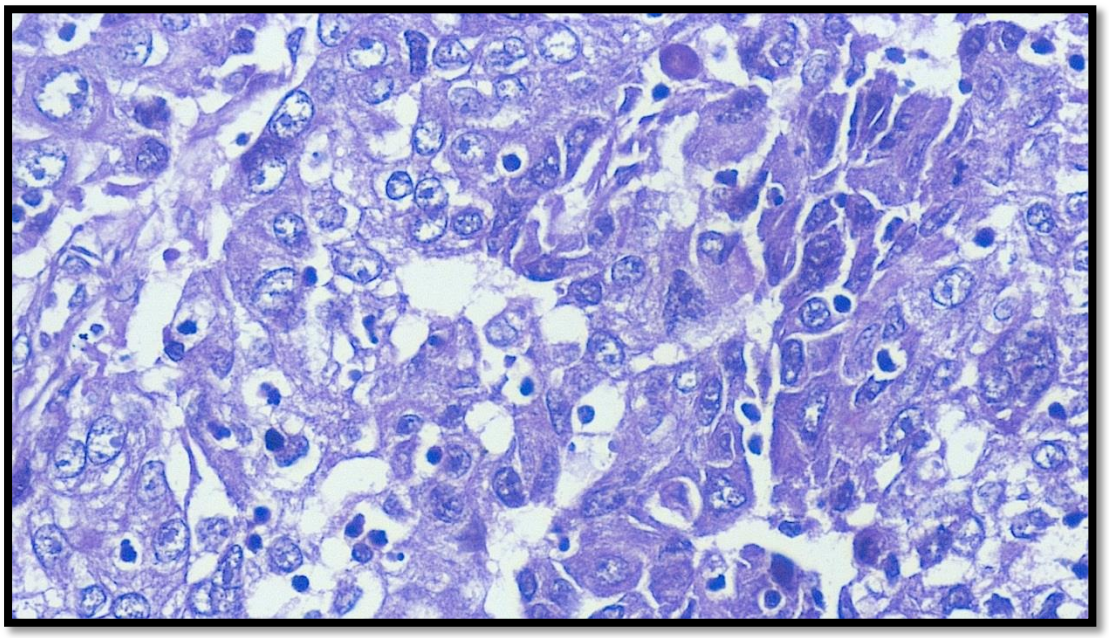
Photomicrograph 2: Invasive breast carcinoma NST Grade 1 (H and E; X100)



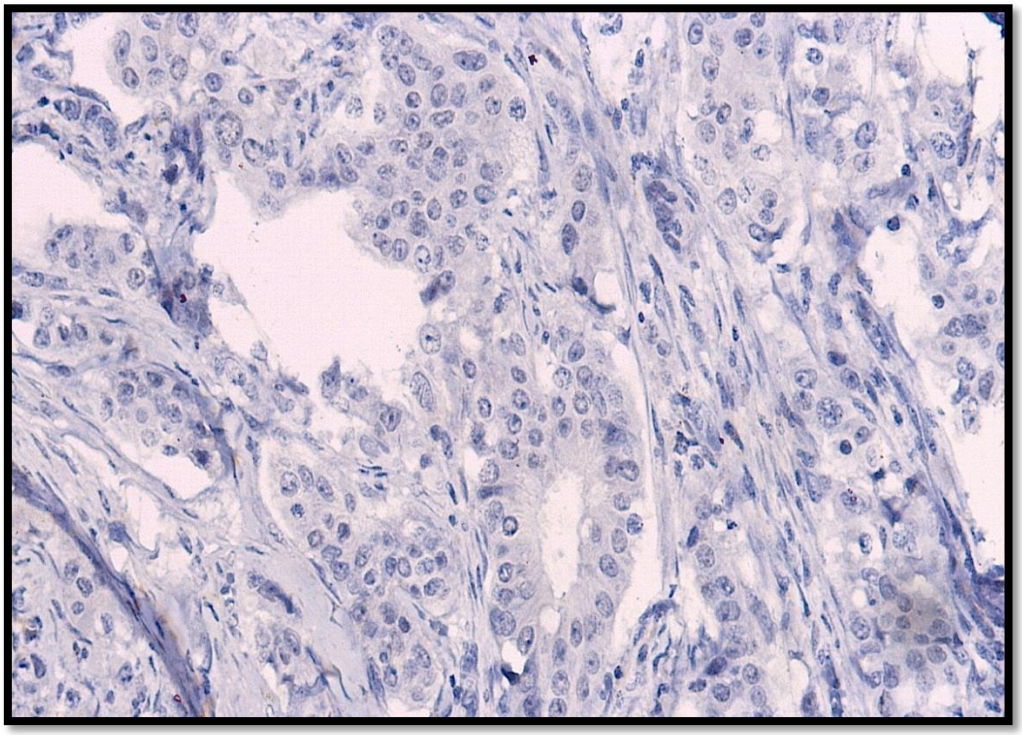
Photomicrograph 3: Invasive breast carcinoma NST Grade 2 (H and E; X100)

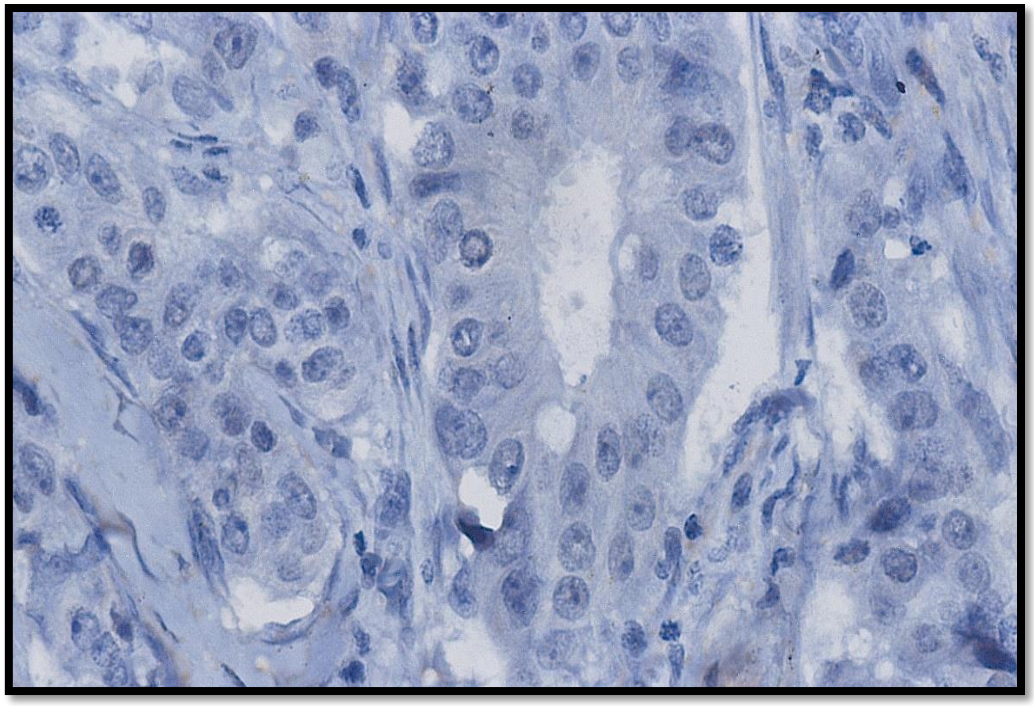


Photomicrograph 4: Invasive breast carcinoma NST Grade 3 (H and E; X100)

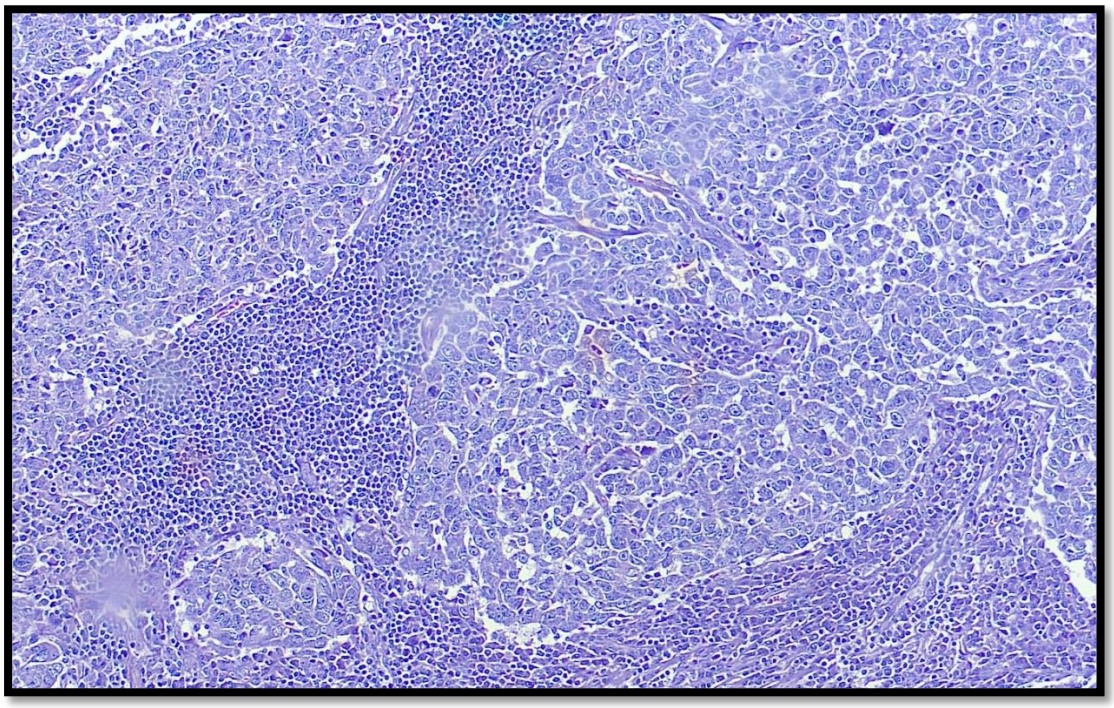


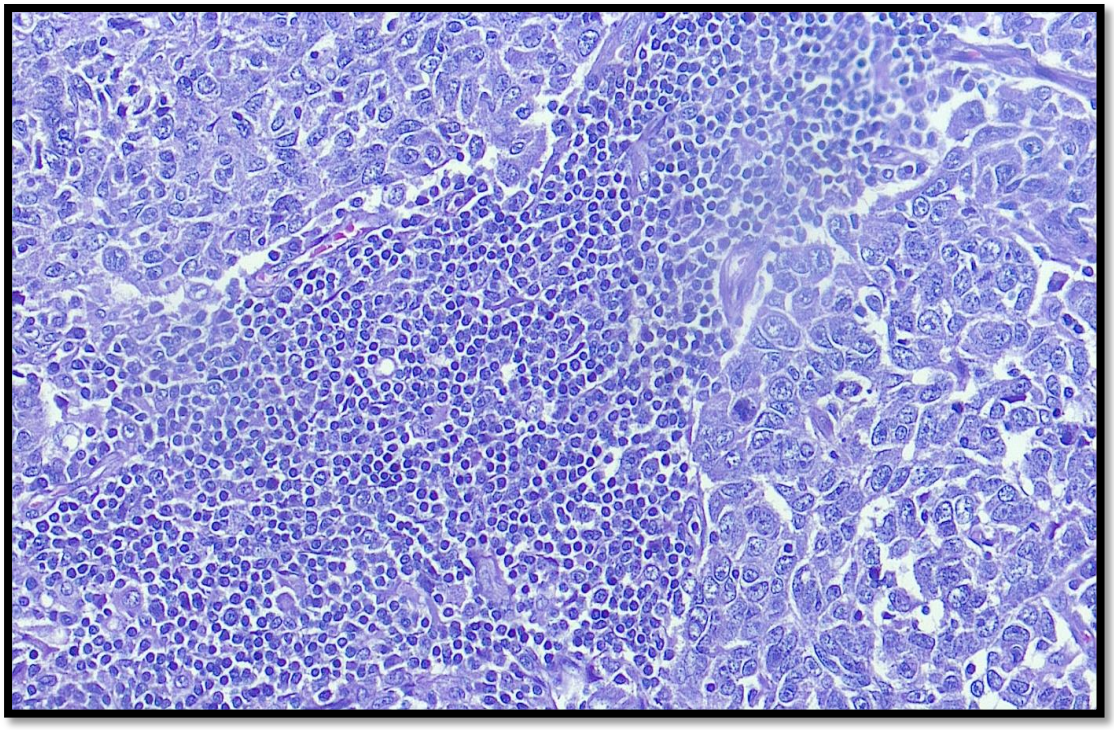
Photomicrograph 5: Invasive breast carcinoma NST Grade 3- nuclear features (H and E; X400)



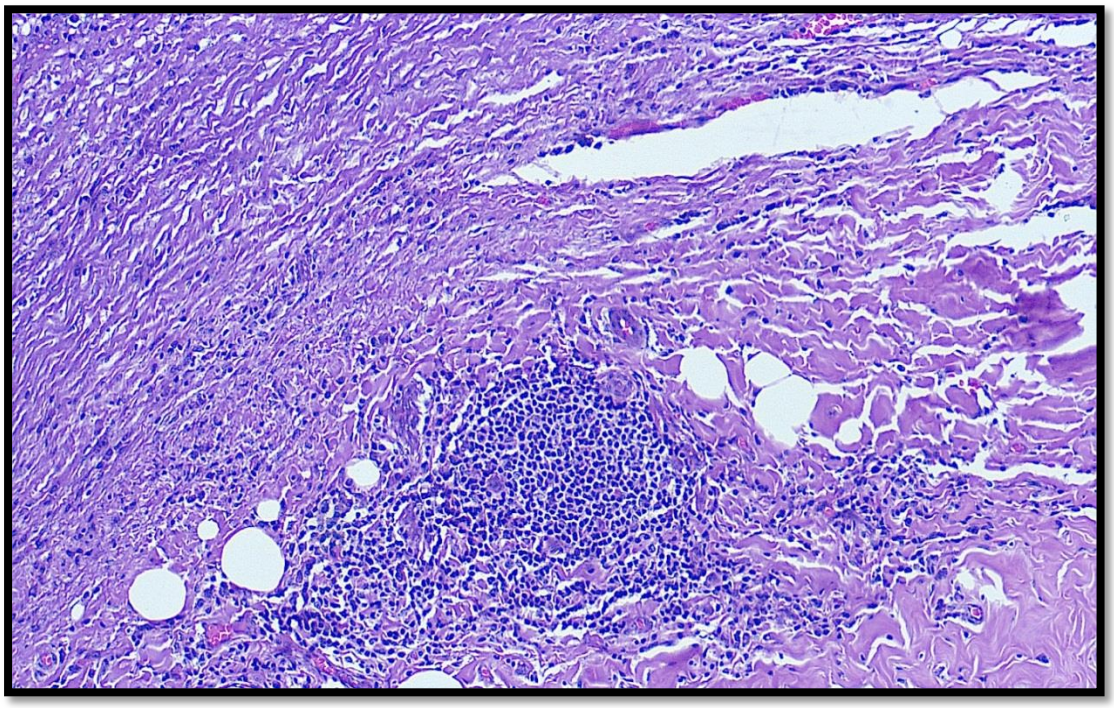


Photomicrograph 6 and 7: TNBC- No expression of ER/PR/HER2 (IHC: X100 and X200)

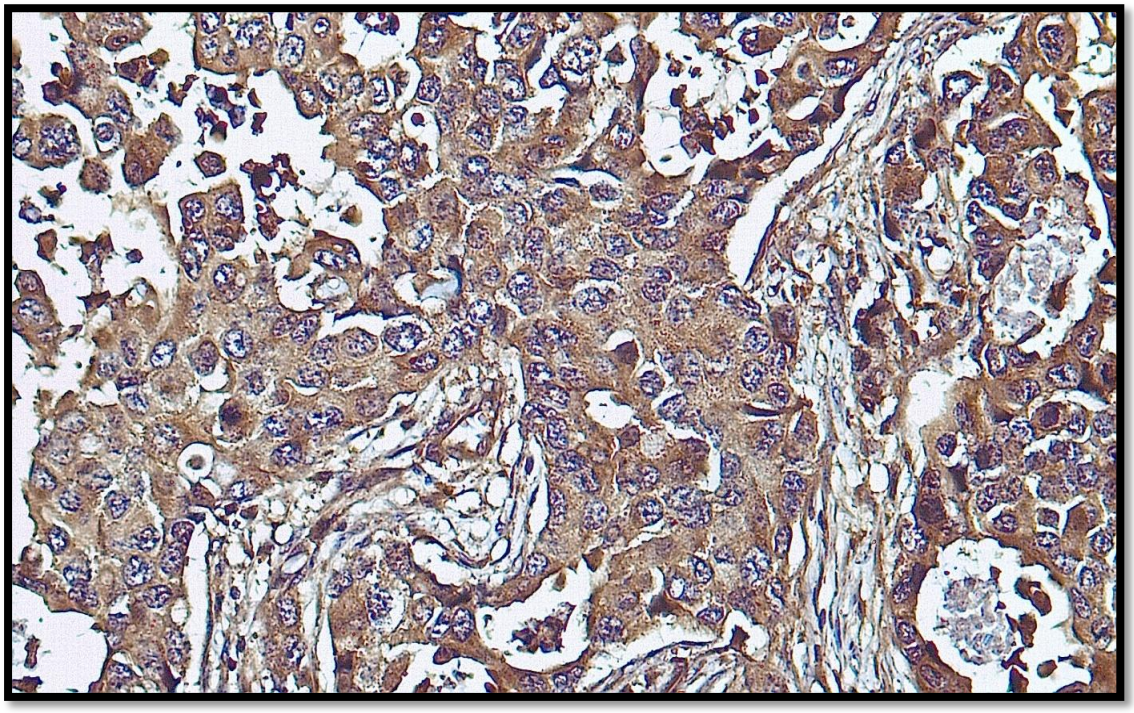




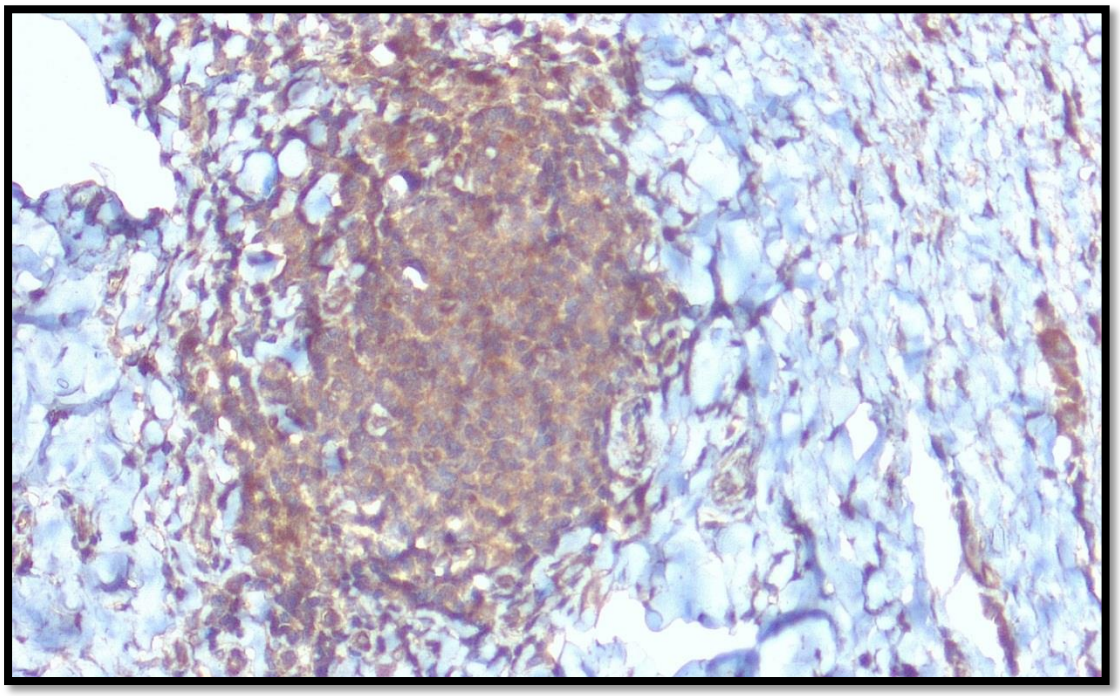
Photomicrograph 8 and 9: High grade peri-tumoral lymphocytic response (H and E; X100 and X400)



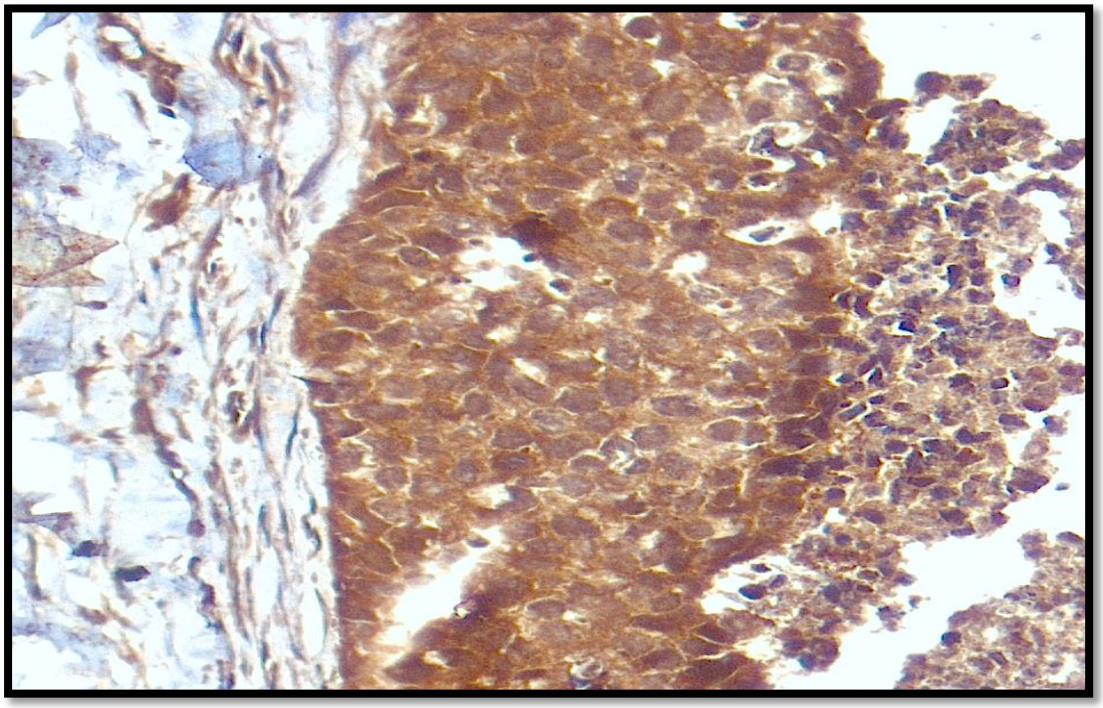
Photomicrograph 10: Peri-tumoral lymphocytic response (H and E; X100)



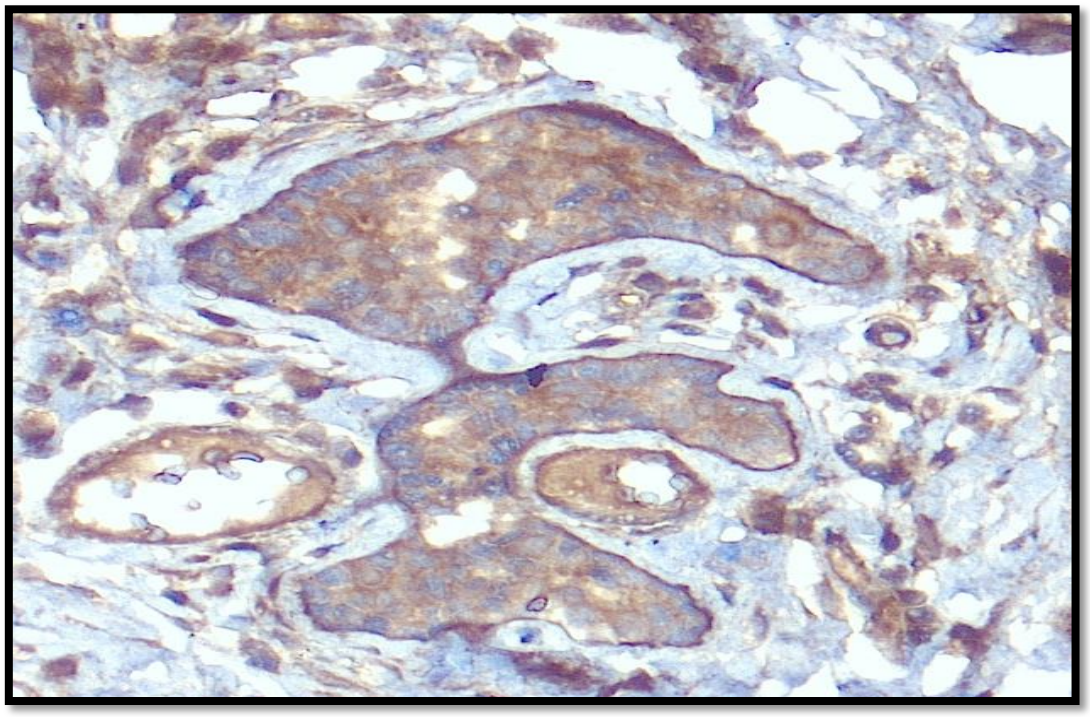
Photomicrograph 11: Cytoplasmic and Membranous Expression in Neoplastic Cells (CD53 IHC staining; X400)



Photomicrograph 12: Cytoplasmic and Membranous Expression in Stromal Lymphocytes (CD53 IHC staining; X200)



Photomicrograph 13: Cytoplasmic and Membranous Expression in Intra-Tumoral Lymphocytes (Right side) (CD53 IHC staining; X200)



Photomicrograph 14: Cytoplasmic and Membranous Expression in Normal Breast Ducts (CD53 IHC staining; X200)

DISCUSSION

Breast cancer molecular subtypes guide treatment decisions based on prognosis. Despite ongoing debates, this classification is widely used in clinical practice to personalize treatment and improve outcomes.[77]

The purpose of this study was to study CD53 expression in TNBC and association with various clinicopathological parameters. In the present study, 50 immunohistochemically proven TNBC cases were taken.

In present study, demographic data showed mean age of 57.34 +/-11.82 years. Majority of breast cancers, including TNBCs, are diagnosed in people with >50 years of age.[132] Around 28% patients belonged to less than 50 years age group. Younger women often have more aggressive tumors, like triple-negative breast cancer, and higher recurrence rates.[133] Research from India and around the world has shown that TNBC is more commonly found in younger populations, however present study showed no such significant age variation.[134]

Majority of TNBCs had tumor size more than 5 cm which was consistent with other studies.[135-137] Majority of cases had poorer prognosis as per NPI which was consistent with other literature.[137,138]

Studying tumor microenvironment is essential to develop effective treatment strategies.[139] The research done to understand variation of TILs in different breast carcinoma subtypes showed TNBCs having highest incidence of LPBCs.[140] Loi et al. conducted study which showed higher levels of stromal TILs were associated with improved overall survival.[141] In our study, poor category as per NPI was associated with mild lymphocytic response and higher response showed better prognosis. This

finding was seen in other studies evaluating TILs and prognosis. High level TILs demonstrated improved short and long term outcomes.[142] Increased immune infiltration was recognized as an independent factor for overall survival and a possible biomarker for pathological complete response.[143,144]

CD53, a transmembrane molecule has been proven to interact with protein kinases in living cells.[145] Protein kinase C isoenzymes activate multiple pathways and regulate genes involved in cell cycle progression, tumorigenesis, and metastasis and understanding these mechanisms are crucial for evaluating their potential as targets for cancer therapy.[146] Thus, CD53 has role in intricate tumor microenvironment.

We studied CD53 expression in TILs, tumor cells and normal ducts adjacent to tumor tissue with all the cases showing CD53 positivity in membrane and cytoplasm with slightly variable intensity. The findings were different from study done by Marchetti et al. in 2021 in 12 cases. [129] However, data from The Human Protein Atlas showed nuclear as well as membranous and cytoplasmic positivity in tumor cells where they used polyclonal antibody.[147] Monoclonal antibodies recognize a single epitope on the antigen, leading to higher specificity while polyclonal antibodies recognize multiple epitopes on the target antigen, which can increase sensitivity.

Table 19: Comparison based on positivity, intensity and localization

PARAMETER	Marchetti et al. [129]	Present Study
Antibody type	Monoclonal	**Polyclonal**
Localization of marker in tumor cells, TILs and normal ducts	Nuclear	**Membranous and cytoplasmic**
Positivity range in tumor cells	5-75%	90-100%
Positivity range in intra-tumoral lymphocytes	80%	90-100%
Positivity range in peri-tumoral lymphocytes	80-100%	90-100%
Positivity range in normal ducts	ABSENT	**PRESENT**
Intensity in tumor cells	1+ to 2+	2+ to 3+
Intensity in intra-tumoral lymphocytes	2+ to 3+	2+ to 3+
Intensity in peri-tumoral lymphocytes	2+ to 3+	2+ to 3+

CD53 was highly expressed in TILs which is in accordance with study done by Marchetti et al.; however intense nuclear staining was absent. Their study did not find any significant correlation between various newer immunological markers and

lymph node metastases and recurrence. The methodology differed from their study in context of clonality.

In present study, CD53 higher intensity specifically in stromal lymphocytes was seen significantly associated with absence of LVI, PNI, axillary lymph node involvement and Paget's disease along with good NPI cases. This finding can be attributed to higher stromal lymphocyte response which is usually associated with good prognostic outcome.

CD53 is a dynamic marker which fluctuates depending on various cell types and immunological conditions. Moreover, the presence of CD53 on multiple cell types, including both immune and non-immune cells, adds another layer of complexity, making its interpretation more challenging in tissue samples.

Immunotherapy is a game-changing treatment for TNBC, but overcoming challenges like biomarker identification, combination approaches, and resistance to treatment is crucial to maximize its clinical effectiveness. More studies are needed to provide insights about CD53 or other potential biomarkers in aggressive tumors like TNBCs. Further research, including larger clinical trials, preclinical models, and more refined techniques/detailed methodologies for measuring CD53 expression, is necessary to establish its significance in anti-tumor immunity. This would help determine whether CD53 could serve as a reliable biomarker for prognosis, treatment response, or a potential target for new therapies in TNBC patients.

SUMMARY

This study was hospital based cross-sectional study on 50 TNBC cases from 1st January 2023 to 31st December 2024. Study aimed at investigating CD53 expression in TNBCs and correlating it with different clinicopathological parameters.

Significant findings in study-

- Mean age of participants: 57.34 years.
- Most common site: UOQ
- Laterality: right side
- Most common clinical presentation: palpable breast lump
- Most common histological subtype: invasive carcinoma of no special type
- Most prevalent histological grade- Grade 3
- Significant correlation was seen with better prognostic index and higher lymphocytic response in both intra and stromal TILs.
- CD53 positivity was seen in almost all cells (TILs, tumor cells as well as normal ducts) in all 50 cases in cytoplasm and membrane with varying intensity.
- CD53 intensity (2+ and 3+) was evenly distributed in peri-tumoral lymphocytes.
- The chi-square value and p-value indicated no significant association between age and CD53 intensity in TILs and tumor cells.
- Statistically significant correlation was seen between tumor size and intensity in TILs as well as neoplastic cells.
- Significant association (p-value<0.05) was observed with higher intensity of CD53 in stromal lymphocytes and absence of LVI.

- Statistically significant correlation was seen between PNI and intensity in TILs.
- No significant association (p-value= 0.9020) was seen between PNI and CD53 intensity in tumor cells.
- There was significant association (p-value=0.0001) between intensity of CD53 in peri-tumoral lymphocytes and histological grade.
- Significant association was seen with CD53 intensity in TILs and absence of Paget's disease.
- There was significant association between CD53 intensity in stromal lymphocytes(p-value=0.0001) and axillary lymph node involvement.
- Around 11.90% cases in good prognosis category have 3+ intensity, hence showing significant correlation of CD53 intensity in peri-tumoral lymphocytes with NPI.

CONCLUSION

- Our study evaluated CD53 expression and correlation with various clinicopathological parameters. This study is one of the very few studies evaluating potential biomarker- CD53 in TNBCs.
- Higher intensity of CD53 in TILs was associated with parameters like absence of LVI, PNI, Paget's disease, axillary lymph node metastasis and better prognosis as per NPI.

LIMITATIONS

Clinical follow-up was not conducted due to time constraints, preventing assessment of actual clinical outcomes.

FUTURE PROSPECTS

- Further studies to assess prognostic clinical outcomes and correlation with CD53 expression, are necessary.
- Additionally, studies evaluating the efficacy of adjunctive immunotherapy could provide valuable insights for improving patient outcomes.

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ANNEXURES

ANNEXURE I

WHO HISTOLOGICAL CLASSIFICATION OF BREAST TUMOURS

- Invasive carcinoma of no special type (ductal)
- Micro-invasive carcinoma
- Invasive lobular carcinoma
- Invasive carcinoma with mixed ductal and lobular features
- Tubular carcinoma
- Invasive cribriform carcinoma
- Mucinous carcinoma
- Invasive micropapillary carcinoma
- Apocrine adenocarcinoma
- Metaplastic carcinoma
- Encapsulated papillary carcinoma with invasion
- Solid papillary carcinoma with invasion
- Intraductal papillary adenocarcinoma with invasion
- Adenoid cystic carcinoma
- Neuroendocrine tumor
- Neuroendocrine carcinoma
- Invasive carcinoma, type cannot be determined
- Other histologic type

ANNEXURE II

PATHOLOGIC STAGE CLASSIFICATION (PTNM, AJCC 8TH EDITION)

Primary Tumor (pT)

pTX: Primary tumor cannot be assessed

pT0: No evidence of primary tumor

pTis (DCIS): Ductal carcinoma in situ

pT1 is further divided into 4 groups:

- T1mi means the cancer is 0.1 cm across or less
- T1a means the cancer is more than 0.1 cm but not more than 0.5 cm
- T1b means the cancer is more than 0.5 cm but not more than 1 cm
- T1c means the cancer is more than 1 cm but not more than 2 cm

pT2: Tumor >0.2 but ≤0.5 mm in greatest dimension

pT3: Tumor >0.5 mm in greatest dimension

pT4 is divided into 4 groups:

- T4a means the cancer has spread into the chest wall
- T4b means the cancer has spread into the skin and the breast might be swollen
- T4c means the cancer has spread to both the skin and the chest wall
- T4d means inflammatory carcinoma.

Regional Lymph Nodes (pN)

pN0: No tumor in any nearby nodes or only small clusters of cancer cells less than 0.2 mm across (isolated tumour cells).

pN1 is divided into 4 groups:

- pN1mi-Micrometastases. They are larger than 0.2 mm but are less than 2 mm.
- pN1a -Metastases in 1 to 3 axillary lymph nodes, at least 1 metastasis larger than 2.0 mm
- pN1b -- Metastases in ipsilateral internal mammary sentinel nodes
- pN1c - pN1a and pN1b combined

pN2 is divided into 2 groups:

- pN2a – Metastasis 4 to 9 axillary lymph nodes
- pN2b - Positive internal mammary nodes

pN3 is divided into 3 groups:

- pN3a – Metastases in 10 or more axillary lymph nodes and at least one is larger than 2 mm, or infra-clavicular nodes
- pN3b –positive internal mammary lymph nodes
- pN3c – supraclavicular lymph nodes

ANNEXURE III- CONSENT FORM

"EVALUATION OF CD53 MARKER EXPRESSION IN TRIPLE NEGATIVE BREAST CARCINOMA: A HOSPITAL BASED CROSS-SECTIONAL STUDY"

Student/Principal Investigator: Reg. No. BN0122003

Guide/Co Investigators: Dr._____

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer the best of your knowledge. If you agree to enrol yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations. The blocks will be taken from representative areas of carcinoma. These blocks will be used to study expression of CD53 marker.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study – You will not get any benefits by participating in this study.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data

collected from you will be kept confidential and only processed or aggregated data will be used for publication. No information about you or information provided by you during research will be disclosed to others without your written permission except _

1.In emergency to protect your rights and welfare.

2.If required by law.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

1**.** Reg No. BN0122003, Department of Pathology, J.N. Medical College.

2. Dr. _____, Department of Pathology, J.N. Medical College

3. If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waiving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"EVALUATION OF CD53 MARKER EXPRESSION IN TRIPLE NEGATIVE BREAST CARCINOMA: A HOSPITAL BASED CROSS SECTIONAL STUDY"**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:**BN0122003**

Signature of the investigator:

ANNEXURE IV

PROFORMA

- NAME:
- AGE:
- BRIEF CLINICAL HISTORY
- DATE OF COLLECTION
- PAST HISTORY –

 H/O Carcinoma Breast

 H/O Carcinoma Endometrium

 H/O Carcinoma Ovary

 Others

- FAMILY HISTORY (Ca Breast) -

o Mother

o Sister

o Daughter

- LOCAL EXAMINATION (Breast)-

o Site

o Side

o Size

o Skin changes

o Axillary lymph node involvement

o Nipple, Areola

o Others

- INVESTIGATIONS –

GROSS FINDINGS -

- Size
- Quadrant involved
- Margins
- Nipple and areola
- Skin involvement
- H and E findings
- Histological type
- Grade
- Necrosis
- Fibrosis
- In situ component
- Lympho-vascular involvement
- Lymph node metastasis

IHC -

- ER
- PR
- HER2

- **CD53-**

CD53	EXPRESSION	POSITIVITY RANGE	INTENSITY GRADE
Tumor cells			
Non- tumor ducts			
Intra – tumor TILs			
Peri – tumor TILs			

- Others – Nottingham prognostic index (NPI)

ANNEXURE V

KEY TO MASTERCHART

S no.- Serial number

P- Present

A -Absent

Age (yrs)- Age in years

Past H/O- Past history of breast/ovary/endometrium malignancy

Family H/O- Family history of breast carcinoma

Laterality- Breast tumor location- Right/Left

MRM – Modified radical mastectomy

WLE- Wide local excision

ALND- Axillary lymph node dissection

IDC- Invasive duct carcinoma

DCIS- Ductal carcinoma in situ

LVI- Lymphovascular invasion

PNI- Perineural invasion

LNs- Lymph nodes

Intra-tumo-lympho- Intra tumoral lymphocytes

Peri-tumo-lympho- Peri tumoral lymphocytes

TILs- Tumor infiltrating lymphocytes

NPI- Nottingham prognostic index

ANNEXURE VI MASTER CHART

Sl. NO.	AGE (yrs)	BRIEF CLINICAL HISTORY	PAST H/O	FAMILY H/O	LATERALITY	TUMOR SIZE (cm)	SURGERY DONE	HISTOLOGICAL TYPE	HISTOLOGICAL GRADE	DCIS	DCIS NUCLEAR GRADE	PAGETS	LVI	PNI	AXILLARY LN INVOLVEMENT	NECROSIS	REGIONAL LNs	Metastatic pos LNs	INTRA-TUMO-LYMPHO RESPONSE	STROMAL/PERI TUMO LYMPHO RESPONSE	NPI	CD53 INTRA TILs(INTENSITY)	CD53 PERI TILs(INTENSITY)	CD53 TUMOR CELLS	CD53 NORMAL DUCTS
1	66	PALPABLE BREAST LUMP	A	A	Right	5X3.5X1	MRM with ALND	IDC	1	P	High	A	A	A	A	A	20	0	MODERATE	HIGH	3	3+	3+	3+	2+
2	59	PALPABLE BREAST LUMP	A	A	LEFT	8X4.5X4	WLE WITH ALND	IDC	3	A	NA	A	P	A	A	A	15	0	MODERATE	MODERATE	5.6	3+	2+	2+	2+
3	54	PALPABLE BREAST LUMP	A	A	Right	5X5X3.5	MRM with ALND	Invasive Papillary carcinoma	2	A	NA	A	P	A	A	A	11	0	MILD	MODERATE	5	3+	3+	3+	2+
4	46	PALPABLE BREAST LUMP	A	A	Right	2.5X2X2	WLE WITH ALND	IDC	2	A	NA	A	A	A	A	A	15	0	MODERATE	HIGH	3.25	3+	3+	3+	3+
5	56	NIPPLE DISCHARGE	A	A	Right	3.5X3X3	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	28	8	MILD	MILD	6.75	3+	2+	2+	2+
6	65	PALPABLE BREAST LUMP	A	A	Right	3X2X2	MRM with ALND	IDC	1	P	High	A	A	A	A	A	13	0	HIGH	HIGH	3.5	3+	3+	3+	2+
7	50	PALPABLE BREAST LUMP	A	A	Right	4X2.5X2	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	35	10	MILD	MILD	8	3+	2+	2+	3+
8	58	PALPABLE BREAST LUMP	A	A	Right	3.5X3X2.5	MRM with ALND	IDC	2	A	NA	A	P	A	P	A	21	3	MODERATE	MILD	5.75	3+	3+	3+	2+
9	35	NIPPLDISCHARGE	P	P	Right	7.5X6.5X4	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	26	6	MILD	MILD	9.75	2+	2+	3+	2+
10	66	BREAST PAIN	A	A	LEFT	2.5X2X2	MRM with ALND	IDC	2	P	High	A	A	A	P	A	11	2	MILD	MODERATE	5.25	3+	3+	3+	3+
11	52	PALPABLE BREAST LUMP	A	A	LEFT	3x2x1.5	MRM with ALND	IDC	2	p	High	A	A	A	A	A	12	0	HIGH	HIGH	3.5	3+	3+	3+	2+
12	60	PALPABLE BREAST LUMP	A	A	Right	3.8X2.8X2	MRM with ALND	IDC	1	A	NA	A	A	A	A	A	13	0	MODERATE	HIGH	3.9	3+	3+	2+	NA
13	50	PALPABLE BREAST LUMP	A	P	Right	2.8x2x2	MRM with ALND	IDC	2	A	NA	A	A	A	P	A	18	2	MODERATE	MODERATE	5.4	3+	3+	3+	3+
14	52	PALPABLE BREAST LUMP	A	A	LEFT	4X3.8X2.6	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	30	8	MILD	MILD	8	3+	2+	2+	3+
15	44	PALPABLE BREAST LUMP	A	A	LEFT	2.5X1.5X1.5	MRM with ALND	IDC	3	P	High	A	A	A	P	A	19	2	MILD	MILD	5.25	3+	3+	3+	2+
16	38	BREAST PAIN	A	A	LEFT	4x2.5x2	MRM with ALND	IDC	3	P	High	A	P	P	P	A	12	1	MODERATE	MODERATE	6	3+	2+	3+	2+
17	36	PALPABLE BREAST LUMP	A	A	Right	5X4.5X3.5	MRM with ALND	IDC	2	A	A	A	A	A	A	A	26	0	MILD	HIGH	5.5	3+	3+	3+	3+

18	58	BREAST PAIN	A	A	LEFT	2.5x2x1	MRM with ALND	IDC	2	A	NA	A	A	A	A	A	20	0	HIGH	HIGH	3.25	3+	3+	3+	2+
19	55	PALPABLE BREAST LUMP	A	A	Right	2X2X2	MRM with ALND	IDC	3	A	NA	A	P	P	P	A	11	1	MODERATE	HIGH	6	3+	3+	3+	2+
20	43	PALPABLE BREAST LUMP	A	A	Right	3.5x3x1.5	MRM with ALND	IDC	3	A	NA	P	P	P	P	A	33	5	MODERATE	MODERATE	6.75	3+	2+	3+	2+
21	82	NIPPLE DISCHARGE	P	P	LEFT	4X3X3	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	24	6	MILD	MILD	8	3+	2+	3+	3+
22	60	BREAST PAIN	A	A	Right	2.5X2.5X1	MRM with ALND	IDC	2	A	NA	A	P	A	P	A	35	15	MILD	MODERATE	6.25	3+	2+	3+	2+
23	70	PALPABLE BREAST LUMP	P	P	Right	6.4X4.5X4	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	22	4	MILD	MILD	9.2	3+	2+	3+	2+
24	55	PALPABLE BREAST LUMP	A	A	Right	2.4X2X1	WLE WITH ALND	IDC	2	A	NA	A	A	A	A	A	10	0	MODERATE	HIGH	3.2	3+	3+	3+	2+
25	45	NIPPLE DISCHARGE	A	A	LEFT	4X2X2	MRM with ALND	IDC	3	P	High	P	P	A	P	A	19	1	MODERATE	MODERATE	6	3+	2+	3+	2+
26	69	PALPABLE BREAST LUMP	A	A	LEFT	3X2.5X2.3	MRM with ALND	IDC	3	A	NA	A	A	A	A	A	7	0	HIGH	MODERATE	4.5	3+	3+	3+	2+
27	65	PALPABLE BREAST LUMP	A	A	Right	2X1.5X1	MRM with ALND	IDC	3	P	HIGH	A	A	A	P	A	15	2	MILD	MODERATE	5	3+	3+	2+	2+
28	79	NIPPLE DISCHARGE	A	P	LEFT	6X5.5X5.5	Toilet Mastectomy with ALND	IDC	3	A	NA	P	P	P	P	P	23	5	MILD	MILD	9	3+	2+	2+	2+
29	66	PALPABLE BREAST LUMP	A	A	LEFT	4X4X2	MRM with ALND	IDC	2	A	NA	A	A	A	A	A	12	0	MODERATE	MODERATE	5	3+	3+	3+	NA
30	52	PALPABLE BREAST LUMP	A	A	Right	4.1X2.5X2.5	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	14	3	MODERATE	MILD	7	3+	2+	3+	2+
31	80	PALPABLE BREAST LUMP	P	P	Right	5.7x4x3	WLE WITH ALND	IDC	3	A	NA	P	P	P	P	P	21	6	MILD	MILD	8.85	2+	2+	3+	2+
32	71	PALPABLE BREAST LUMP	A	A	Right	2X2X1.5	MRM with ALND	MEDULLARY	1	A	NA	A	A	A	A	P	11	0	HIGH	HIGH	3	3+	3+	3+	NA
33	60	PALPABLE BREAST LUMP	A	A	LEFT	5x4x3	MRM with ALND	IDC	2	A	NA	A	P	P	P	A	31	3	MILD	MODERATE	6.5	3+	2+	3+	NA
34	54	NIPPLE DISCHARGE	A	A	Right	3X2.5X2.5	WLE WITH ALND	IDC	3	A	NA	A	P	P	P	P	12	4	MILD	MODERATE	7.5	3+	2+	3+	2+
35	55	PALPABLE BREAST LUMP	A	A	LEFT	3.5X2.5X2	MRM with ALND	IDC	3	A	NA	A	A	A	P	A	17	1	MILD	MODERATE	5.75	3+	3+	3+	2+
36	40	PALPABLE BREAST LUMP	A	A	Right	3X3X2	MRM with ALND	IDC	2	A	NA	A	A	A	A	A	15	0	MILD	MODERATE	5.5	3+	3+	3+	2+
37	74	PALPABLE BREAST LUMP	A	A	Right	5.5X5X4	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	8	1	MILD	MILD	7.75	3+	2+	3+	2+
38	60	PALPABLE BREAST LUMP	A	A	Right	4X2X2	WLE WITH ALND	IDC	2	A	NA	A	A	A	A	A	11	0	MODERATE	MODERATE	5	3+	3+	3+	2+
39	57	PALPABLE BREAST LUMP	A	A	Right	5x3x3	MRM with ALND	MEDULLARY	3	A	NA	P	P	P	P	P	14	3	MILD	MILD	7.5	3+	2+	3+	2+
40	60	BREAST PAIN	A	P	LEFT	3X2.5X2	MRM with ALND	IDC	3	A	NA	A	P	P	P	A	20	2	MILD	MODERATE	6.5	3+	2+	3+	2+
41	57	PALPABLE BREAST LUMP	A	A	Right	3X2.5X2	MRM with ALND	IDC	2	A	NA	P	P	A	P	A	16	4	MODERATE	MILD	5.5	3+	3+	3+	NA
42	38	NIPPLE DISCHARGE	A	P	Right	6.4X5X4.5	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	22	9	MILD	MILD	9.2	2+	2+	3+	2+
43	44	PALPABLE BREAST LUMP	P	A	LEFT	7X3X3	MRM with ALND	IDC	3	A	NA	A	P	P	P	P	12	2	MODERATE	MODERATE	8.5	3+	2+	3+	2+

44	82	PALPABLE BREAST LUMP	A	A	RT	2X1.5X1	MRM with ALND	IDC	2	A	NA	A	A	A	A	A	10	0	HIGH	HIGH	4	3+	3+	2+	2+
45	74	PALPABLE BREAST LUMP	A	A	Right	4.5X4.5X3.5	MRM with ALND	IDC	3	A	NA	A	P	P	P	P	26	6	MILD	MODERATE	6.25	3+	2+	3+	2+
46	49	PALPABLE BREAST LUMP	A	A	LEFT	6X5X5	MRM with ALND	IDC	3	A	NA	A	P	P	P	P	14	4	MILD	MILD	8	3+	2+	3+	2+
47	55	PALPABLE BREAST LUMP	A	A	Right	3.5X3X3	MRM with ALND	IDC	2	A	NA	A	A	A	P	A	19	1	MODERATE	MODERATE	5.5	3+	3+	3+	2+
48	61	NIPPLE DISCHARGE	A	A	LEFT	2X1.5X1	WLE WITH ALND	IDC	3	A	NA	A	P	P	P	P	37	6	MILD	MODERATE	7	3+	2+	3+	NA
49	60	NIPPLE DISCHARGE	A	A	Right	5X4X4	MRM with ALND	IDC	3	A	NA	P	P	P	P	P	21	9	MILD	MODERATE	8.5	2+	2+	3+	2+
50	50	PALPABLE BREAST LUMP	A	A	Right	6X3X3	MRM with ALND	IDC	3	A	NA	A	P	P	P	A	7	1	MODERATE	MODERATE	7	3+	3+	3+	2+