"EXPRESSION OF NESTIN IN BREAST CANCER AND CORRELATION WITH HISTOPATHOLOGICAL GRADING: HOSPITAL BASED CROSS-SECTIONAL STUDY"

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LIST OF ABBREVIATIONS USED

Sr.No	Abbreviation	Expansion
1.	AJCC	American Joint Committee on Cancers system
2.	BRCA	Breast cancer gene
3.	IDC	Invasive Ductal carcinoma
4.	SBR	Scarff Bloom Richardson
5.	GLOBOCAN	Global cancer observatory
6.	HER2	Human epidermal growth factor receptor
7.	ER	Estrogen receptor
8.	H&E	Hematoxylin and Eosin
9.	IHC	Immunohistochemistry
10.	MRM	Modified radical mastectomy
11.	TNM	Tumor, nodes and metastases
12.	TNBC	Triple Negative Breast cancer
13.	WHO	World Health Organization
14.	DCIS	Ductal carcinoma in situ
15.	IARC	International Agency for Research on Cancer

ABSTRACT

Title: "EXPRESSION OF NESTIN IN BREAST CANCER AND CORRELATION WITH HISTOPATHOLOGICAL GRADING: HOSPITAL BASED CROSS-SECTIONAL STUDY "

BACKGROUND: Breast cancer stands as the leading and most fatal form of cancer among women worldwide, with invasion and metastasis being the primary culprits behind the majority of fatalities. Many studies have been done to understand cancer progression and metastasis in breast cancers. Nestin is type VI intermediate filament protein expressed in myoepithelial cells, endothelial cells and cytoplasm of tumor cells in breast carcinoma. Its expression is seen in multiple carcinomas and recent studies have shown that it plays a significant role in higher tumor grade, TNBC and as prognostic marker in breast cancers.

OBJECTIVES: This study aimed to evaluate Nestin expression in Infiltrating ductal carcinoma of breast and investigate possible association between Nestin expression and clinicopathological parameters including histological grading and immunohistochemical expression of ER, PR, Her2 and Ki67.

METHODS: A total of 52 breast carcinoma cases diagnosed during January 2023 to December 2024 were included in this study. Slides stained with H&E, and immunohistochemically for Nestin were evaluated for histopathological examination. Results were subjected to appropriate statistical analysis.

RESULTS: The study showed higher Nestin expression in moderately positive stained tumor cells. (46.20%)

Significant association was found between positive Nestin expression with ER and PR receptors, TNBC, Luminal A and B, high Ki67%, menopausal status and DCIS; while no significant association was found between Nestin expression with tumor grade, tumor size and lymph node status. Additionally, lymphovascular invasion and perineural invasion also showed increased prevalence for Nestin positivity.

CONCLUSION: In conclusion, the study shows increased expression of Nestin is associated with more aggressive nature of tumor and TNBC molecular subtype. The findings of this study may give certain directions towards exploring Nestin as a target for potential anticancer therapy.

KEYWORDS: IDC Breast carcinoma; Nestin; Triple negative breast Carcinoma; prognosis

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INTRODUCTION

Breast cancer is a major health concern worldwide, with a high incidence rate among women, particularly those aged 35-70 years. It ranks as the most common malignancy in women and is the second leading cause of cancer-related deaths globally.[1] According to the WHO Global Cancer Observatory 2020, breast cancer accounts for approximately 13.5% of all cancer cases and 10.6% of all cancer-related deaths in India, highlighting the urgent need for improved diagnostics and prognostic markers.[2]

The histopathological classification of breast cancer into subtypes, such as Luminal A, Luminal B, HER2-positive, and Triple-Negative Breast Cancer (TNBC), is crucial for determining treatment protocols and predicting patient outcomes.[3] Triple-negative breast cancer, which lacks expression of estrogen receptors (ER), progesterone receptors (PR), and HER2, is particularly aggressive and associated with poor prognosis due to the lack of targeted therapies.[4]

The expression of Nestin, a type VI intermediate filament protein, has been increasingly recognized in the context of various cancers, including breast cancer. Traditionally known as a marker for neural stem cells, Nestin plays a significant role in cell proliferation, survival, and angiogenesis. In breast cancer, Nestin is primarily expressed in basal-like tumors, particularly those within the TNBC subgroup, and is thought to contribute to tumor aggressiveness.[5]

Nestin is typically found in the basal/myoepithelial layers of normal breast tissue, but its expression becomes more pronounced in breast carcinoma, particularly in the basal-like subtype. This has been linked to a more aggressive tumor behavior, shorter survival times, and poor clinical outcomes. The protein's role in cell migration, invasion, and angiogenesis suggests its potential as a therapeutic target for TNBC.[6]

In terms of clinical implications, Nestin expression has been correlated with poor histological grading in several studies. It has been suggested that higher levels of Nestin positivity in tumor cells are associated with larger tumor size, lymph node metastasis, and higher tumor grade. However, the correlation between Nestin expression and other clinicopathological parameters remains unclear and warrants further investigation.[7]

Various studies have attempted to evaluate the prognostic significance of Nestin in different cancers. For instance, studies on glioblastoma and prostate cancer have highlighted the role of Nestin in tumor angiogenesis and its potential as a therapeutic target. A similar pattern has been

observed in breast cancer, where Nestin expression is linked with poorer survival outcomes, particularly in lymph node-positive patients.[8]

A prospective study conducted by Yoko Matsuda in 2009 investigated Nestin's role in tumor angiogenesis in breast cancer and other malignancies, suggesting its potential use as a prognostic marker and therapeutic target. Furthermore, several studies have explored Nestin's relationship with established biomarkers such as ER, PR, and HER2, with mixed results. [9]

Recent studies have also explored the molecular mechanisms underlying Nestin expression in cancer cells. The interaction of Nestin with signalling pathways such as Wnt/β-catenin, Notch, and Hedgehog may contribute to tumor progression and resistance to therapy.[10]

This study aims to investigate the expression of Nestin in invasive ductal carcinoma (IDC) and its correlation with histopathological grading. Additionally, the study will evaluate the potential association between Nestin expression and clinicopathological parameters such as ER, PR, Her2 Neu, and lymph node metastasis. Understanding these relationships could provide valuable insights into the biology of breast cancer and help identify new therapeutic targets for TNBC.

AIMS & OBJECTIVES

- Primary,

1)To evaluate expression of Nestin in invasive ductal carcinoma and its correlation with histological grading.

- Secondary,

1)To correlate expression of Nestin with ER, PR and Her2 Neu status wherever available.

2)To correlate Nestin positivity score with clinicopathological parameters.

REVIEW OF LITERATURE

Development of the Breast

Breast development is one of the first significant somatic changes in females, beginning at puberty and establishing its foundation between 4 and 6 weeks of gestation. During early fetal development, ectodermal cells on the anterior body wall thicken to form a ridge known as the "milk line" or "milk ridge." This ridge extends from the axilla to the groin, but the thickening regresses, except in the pectoral region, where the mammary gland develops in relation to the second to sixth ribs. At birth, a newborn's breast can be palpated, showing variable amounts of tissue without obvious gender differences. From birth to puberty, the breast remains dormant, and growth is stimulated by sex hormones, primarily estrogen. It is during puberty that sexually dimorphic breast development occurs.[11]

Anatomy of the Breast

The breast is a highly modified sebaceous gland, found in both sexes; however, in males, it remains rudimentary and is located in the superficial fascia of the pectoral region. The mammary primordium, which is the origin of breast tissue, differentiates into lactiferous ducts and mammary glands (parenchyma). Surrounding mesenchyme develops into the fibrous and fatty components of the breast (stroma). The nipple begins to form around the 8th month of gestation. During thelarche, estrogen promotes the growth of adipose and ductal tissues, while progesterone encourages lobular development and alveolar budding. Ductal elongation and branching initiate at the terminal end bud, specifically in the cap cell layer of the mammary stem cells. From these primary ducts, segmental and subsegmental ducts form. The subsegmental ducts contribute to the formation of the terminal duct, which then differentiates into multiple terminal ductules or acini.[12]

Each breast consists of 15-20 lobes, and each lobe is drained by a lactiferous duct. These ducts converge and open at the nipple, with each duct having a lactiferous sinus near its terminus. The tubulo-alveolar glands of the breast secrete milk, and each lobule contains clusters of alveoli. The stroma serves as the supporting structure for the gland and is composed of both fatty and fibrous tissues. The fibrous septa, known as Cooper's ligaments, hold the skin and breast tissue to the pectoral fascia. The bulk of the breast is made up of fatty stroma.[12]

Vascular Supply

The arterial supply to the breast is primarily from branches of the axillary, internal thoracic, and intercostal arteries. These vessels provide oxygenated blood to the breast tissue, ensuring its metabolic needs are met. The venous drainage is organized as follows: the internal thoracic vein collects blood from the superficial veins, while the deep veins drain into the axillary and posterior intercostal veins. [12,13]

Nerve Supply

The breast is innervated by the anterior and lateral cutaneous branches of the fourth to sixth intercostal nerves. These nerves are responsible for both sensory and motor functions in the breast, contributing to sensations such as touch and pain.[12]

Lymphatic Supply

Lymph from the breast drains into several groups of lymph nodes, including the axillary, internal mammary, supraclavicular, cephalic, and posterior intercostal nodes. The skin over the breast, except for the nipple and areola, is drained by the superficial lymphatics. The parenchymal tissue is drained by deep lymphatics, which also collect lymph from the nipple and areola. Given the central role of the lymphatic system in the spread of metastatic disease, the lymphatic drainage of the breast holds significant clinical importance. [12,13]

Physiology of the Breast

The breast is a specialized organ primarily involved in lactation, a process that includes the synthesis, secretion, and ejection of milk. The ability of the secretory units within the breast to produce milk is regulated by a complex network of hormones and growth factors. [14]

Hormonal Regulation:

- **Estrogen** is the primary female hormone responsible for the growth and maintenance of the breast. It promotes the development of the ductal system, maturation of the nipples, and the proliferation of ductal epithelium, myoepithelial cells, and surrounding stroma. [14]
- **Progesterone** plays a key role in the development of terminal ducts and lobuloalveolar units. [14]

- **Prolactin**, working in conjunction with estrogen and progesterone, contributes to the development of ducts and lobuloalveolar structures. Additionally, prolactin, along with **cortisol** and **insulin**, is involved in the differentiation of alveolar cells into milk-secreting cells. [14]
- The release of **oxytocin** is triggered by the sucking reflex. Oxytocin stimulates myoepithelial cells to contract, which pushes milk out of the lobules and into the lactiferous ducts for ejection. [14]

This intricate hormonal interplay ensures the proper development and function of the breast during lactation.

Histology of the Breast

The histology of breast tissue is influenced by factors such as age, the stage of the menstrual cycle, pregnancy, and lactation. The breast is primarily composed of the ductal-lobular system, the stroma, and the nipple-areolar complex. [13,14]

Ducts and Acini:

The ducts and acini are lined by two layers of cells:

- The **inner luminal layer**, which is lined by epithelial cells.
- The **outer basal layer**, which consists of flattened myoepithelial cells.

In smaller ducts, there is a single layer of columnar or cuboidal cells, while larger ducts feature two layers. The **myoepithelial cells** are discontinuous and stellate in shape, originating from ectodermal tissue, and are closely associated with the basal aspects of the epithelial cells. [14,15]

Lobular Structure: Each breast lobe is drained by **lactiferous ducts**, which are connected to a network of ducts and lobules. These ducts and lobules are encircled by **connective tissue stroma** to form a breast lobule. The glandular components of the lobule are responsible for secretion and undergo structural changes based on hormonal influence. [14,15]

In the **resting adult breast**, each lobule consists of a collection of blind-ended, branched ductules, but these lack the mature terminal alveoli (acini) found in the lactating breast, which are crucial for milk secretion. **Collagenous interlobular tissue** surrounds the glandular lobules, providing structural support. [14,15]

Breast cancer has been recognized as a disease since ancient times, with its presence documented throughout recorded history.

Table 1: History of Breast Cancer [16]

Year	Event/Discovery
400 B.C.	Hippocrates described breast cancer as a **humoral disease**, attributed to an imbalance of the four humors (blood, phlegm, yellow bile, and black bile). For over 2000 years, breast cancer was considered a **systemic disease**.
1750s	**Johanes de Gorter** proposed that tumors originated from **pus-filled inflammations** in the breast, which solidified into tumors after mixing with blood.
1757	**Henri Le Dran**, a French physician, suggested **surgical treatment** for breast cancer, removing the infected **axillary lymph nodes**, which laid the foundation for the **radical mastectomy**.
Mid-19th Century	**William Halstead** refined **radical breast surgery**, establishing it as the gold standard for breast cancer treatment for the next 100 years.
1895	**George Beatson**, a Scottish surgeon, discovered that **removal of ovaries** caused **tumor shrinkage**, sparking interest in **hormonal influences** on breast cancer.
1920s	**Oophorectomy** (ovary removal) was used as a **last-resort treatment** in advanced breast cancer, as it was found to sometimes help with tumor reduction.
1976	**Fisher's study** showed that **breast-conserving surgery** followed by **radiation** or **chemotherapy** was as effective as radical mastectomy, shifting toward **less invasive treatments**.
1990s	With the decline of **radical mastectomy**, new hypotheses on the origins of breast cancer were explored, including **diet**, **chemical pollution**, **race**, **delayed childbirth**, and **breastfeeding**.
Post-1995	After an initial increase in breast cancer rates, **deaths began to decline**, due to **advancements in early detection** and **improved treatments**.

Epidemiology of Breast Cancer:

Breast cancer has become the most commonly diagnosed cancer globally, surpassing lung cancer, with 2.3 million women diagnosed in 2020, leading to 685,000 deaths worldwide. This represents 11.7% of all cancer cases globally. According to the International Agency for Research on Cancer (IARC), predictions indicate that the incidence of invasive breast cancer will rise by more than 40% by 2040, reaching nearly 3 million cases annually. In India, breast cancer accounted for 13.5% of all cancer cases in 2020, with 178,361 diagnosed cases, and 10.6% of cancer-related deaths, totalling 90,408. The survival rate for breast cancer is notably higher in developed countries compared to those still evolving. In transitioned countries, the age-standardized rate is 55.9 per 100,000, while in transitioning countries, it is 29.7. Survival rates vary by stage, with a 95% 5-year survival rate for Stage I, 92% for Stage II, 70% for Stage III, and only 21% for Stage IV patients. The survival rate in India is lower than in Western countries, primarily due to factors such as earlier onset, delayed diagnosis, later-stage presentation, delayed initiation of treatment, and fragmented care.[17]

Prognostic Factors in Breast Cancer:

Breast cancer, once considered a homogeneous disease, is now recognized as a heterogeneous entity, exhibiting varying morphological and biological characteristics. This diversity contributes to different clinical behaviors, responses to treatment, and molecular features, which influence prognosis and therapeutic decisions. Traditionally, breast cancer prognosis was based on broad assumptions, but advancements in molecular biology and personalized medicine have transformed the approach to its treatment and prognosis.[18]

The primary prognostic factors in breast cancer include clinical and pathological variables that help predict disease progression and the likelihood of benefiting from specific treatments. These factors should be included in every breast cancer pathology report to assist clinical teams in making personalized treatment decisions. [19,20] The key prognostic factors include:

1. **Tumor Biomarkers**:
    - **Estrogen Receptor (ER)** and **HER2** are the principal biomarkers that predict prognosis in early-stage breast cancer. Tumors that are ER-positive tend to have a better prognosis and are more responsive to hormonal therapies, while HER2-positive tumors are more aggressive but respond well to targeted therapies like trastuzumab.[18,19]

- **Progesterone Receptor (PR)** expression is also considered alongside ER to gauge tumor responsiveness to hormone therapy. [18,19]
- **Germline BRCA mutations** and **PI3KCA mutations** are predictive of response to certain therapies, with testing for these markers providing insights into treatment options, especially for stage 4 patients. [18,19]
- **PD-L1 expression** is another important factor, especially in assessing response to immune checkpoint inhibitors. [18,19]

2. **Tumor Size and Grade**:
   - Larger tumors and higher-grade tumors are typically associated with worse prognosis. Tumor grade refers to how abnormal the cancer cells appear under the microscope, with higher grades being more aggressive. [20]
3. **Histological Type**:
   - The histological subtype of breast cancer, such as invasive ductal carcinoma (IDC) or invasive lobular carcinoma (ILC), plays a critical role in prognosis. Certain subtypes are associated with a more favourable prognosis, while others, like inflammatory breast cancer, are more aggressive. [20]
4. **Staging**:
   - Tumor staging, commonly done through the TNM (Tumor, Node, Metastasis) classification, assesses the extent of the tumor and its spread to lymph nodes or distant organs. This is a critical factor in determining prognosis and treatment plans. [20]
5. **Molecular Subtyping**:
   - The molecular classification of breast cancer helps predict the tumor's behavior and treatment response. Subtypes such as **Luminal A**, **Luminal B**, **HER2-enriched**, and **Basal-like** (triple-negative) each have distinct biological behaviors and prognostic outcomes. For example, Luminal A tumors tend to have a better prognosis and are more likely to respond to hormone therapy, while triple-negative breast cancer is more aggressive and lacks targeted therapy options. [20,21]

Diagnosis of Breast Cancer:

The diagnosis of breast cancer involves a combination of clinical and imaging investigations to assess the presence and extent of the disease. The process begins with a physical examination, followed by imaging techniques to stage the cancer clinically. After definitive surgical treatment, pathological evaluation of the primary tumor and regional lymph nodes is conducted for a more precise staging.[21]

A. Imaging:

1. **Mammography:**
    - Mammography is often the first-line imaging modality for breast cancer detection. It is particularly effective in identifying **microcalcifications** and can sometimes reveal a lesion even before it becomes palpable. Mammography is widely used for **screening** and **diagnosis**, especially in asymptomatic women or those with early-stage disease. [21]
2. **Ultrasonography:**
    - **Ultrasound** is commonly used to assist in the clinical examination of suspicious lesions identified on mammography or physical examination. It helps distinguish between **solid tumors** and **cystic lesions** and provides valuable information on the tumor's **size**, **shape**, and **margins**. It is often used in combination with other imaging methods. [21]
3. **Magnetic Resonance Imaging (MRI):**
    - MRI, particularly with a combination of **T1**, **T2**, and **3-D contrast-enhanced techniques**, is highly sensitive in detecting breast malignancies. It is especially useful in **dense breasts** or in evaluating the **extent of disease**. MRI can be used for pre-surgical planning, assessing tumor size, and detecting multifocal or **multicentric** disease. It is also beneficial for detecting **recurrences**. [21]
4. **Nuclear Imaging:**
    - Nuclear imaging techniques are often employed to evaluate the **axillary lymph nodes** and to predict potential **drug resistance**. This can help guide treatment decisions and determine whether **neoadjuvant therapy** is necessary. [21]

5. **Positron Emission Tomography (PET) Scanning:**
    - PET scans are the **most sensitive** and **specific** imaging modality for detecting breast cancer. [21] It is particularly useful in:
        - **Detecting recurrences** in scarred breasts.
        - Identifying **multifocal disease**.
        - Assessing **axillary involvement**.
        - Evaluating **systemic metastases** when results from other modalities are **equivocal**.

B. Breast Biopsy:

1. Core Biopsy:

- The **core biopsy** is the recommended diagnostic approach for **newly diagnosed breast cancers**. This technique involves a **percutaneous vacuum-assisted** biopsy with image guidance, such as ultrasound or mammography. [21] It has several advantages:
    - It **spares operative intervention** and avoids the scarring associated with surgery.
    - It provides **pathologic results** more quickly than **surgical excision**.
    - It avoids the risk of **positive margins**, which can require further surgeries.
- Core biopsies allow for **assessment of tumor histology**, **hormone receptor status**, and **HER2 expression**, all critical for treatment planning.

2. Excisional Biopsy:

- An **excisional biopsy** involves the surgical removal of the lesion, typically performed under **general anaesthesia**. [21] While this is considered the **gold standard** for diagnosing breast cancer, it has limitations:
    - It may increase the rate of **positive margins** when performed as the initial diagnostic procedure, leading to the need for further surgeries.
    - It can be associated with more **morbidity** due to the surgical procedure.

- Excisional biopsies are sometimes performed when core biopsy results are inconclusive or when a lesion is too difficult to target for a core biopsy.

WHO CLASSIFICATION OF INVASIVE BREAST CANCER:

The **World Health Organization (WHO)** provides a standard classification for invasive breast cancers, which is most widely used by clinicians and pathologists. [22]

- The latest **5th edition of the WHO classification** includes updates focusing on:
    - **Morphologic classification**: Changes to how mitotic count is expressed.
    - **Molecular classification**: Incorporates genetic and molecular data to categorize tumors more precisely.
    - **Expression profile**: Changes in the expression of certain biomarkers like **HER2** and **estrogen receptors** are now included in the classification.

Key Factors for Classification:

- **Mitotic Count**: The number of cells undergoing mitosis (cell division) is an important factor in determining the aggressiveness of the tumor. The mitotic count is now expressed per unit area (mm^2) in the latest WHO classification. [22]
- **Molecular Profile**: The molecular subtype, which includes hormone receptor status (ER, PR) and HER2 expression, plays a crucial role in determining prognosis and guiding treatment. [22]
- **Expression Profile**: This includes the expression of various biomarkers, such as hormone receptors and HER2, which are important for treatment decisions. [22]

WHO 5th Edition Classification of Breast Tumours: [20,22]

1. **Epithelial Tumours of the Breast**
    - Benign epithelial proliferations and precursors
    - Adenosis and benign sclerosing lesions
    - Adenomas
    - Epithelial-myoepithelial tumours
    - Papillary neoplasms

- Non-invasive lobular neoplasia
- Ductal carcinoma in situ (DCIS)
- Invasive Breast Carcinoma
    - Invasive breast carcinoma – No special type (IDC-NST)
    - Invasive lobular carcinoma (ILC)
    - Tubular carcinoma (TC)
    - Cribriform carcinoma
    - Mucinous carcinoma
    - Mucinous cystadenocarcinoma
    - Invasive micropapillary carcinoma
    - Carcinoma with apocrine differentiation
    - Metaplastic carcinoma
- Rare and Salivary Gland-Type Tumours
    - Neuroendocrine neoplasms

2. **Fibroepithelial Tumours and Hamartomas of the Breast**
    - Hamartoma
    - Fibroadenoma
    - Phyllodes tumour
3. **Tumours of the Nipple**
    - Syringomatous tumour
    - Nipple adenoma
    - Paget disease of the breast
4. **Mesenchymal Lesions**
    - Vascular tumours

- Fibroblastic and myofibroblastic tumours
- Peripheral nerve sheath tumours
- Smooth muscle tumours
- Adipocytic tumours

5. **Haematolymphoid Tumours of the Breast**
    - Lymphoma
6. **Tumours of the Male Breast**
    - Gynaecomastia
    - Carcinoma in situ
    - Invasive carcinoma
7. **Metastases to the Breast**
8. **Genetic Tumour Syndromes of the Breast**
    - BRCA1/2 associated hereditary breast and ovarian cancer syndrome
    - Cowden syndrome
    - Ataxia–telangiectasia
    - Li-Fraumeni syndrome, TP53 associated
    - Li-Fraumeni syndrome, CHEK2 associated
    - CDH1 associated breast cancer
    - PALB2 associated cancers
    - Peutz-Jeghers syndrome
    - Neurofibromatosis type 1

IDC-NST (Invasive Ductal Carcinoma) and its Subtypes:

- **IDC-NST**, or **Invasive Ductal Carcinoma**, is the most prevalent histologic form of invasive breast cancer, accounting for 70–80% of all cases.

- According to the current **WHO (5th edition)** classification, **invasive breast carcinoma** is subdivided into the following histologic subtypes:
    - Infiltrating duct carcinoma (NOS) (Not Otherwise Specified)
    - Oncocytic carcinoma
    - Lipid-rich carcinoma
    - Glycogen-rich carcinoma
    - Sebaceous carcinoma
    - Lobular carcinoma NOS
    - Tubular carcinoma (TC)
    - Cribriform carcinoma NOS
    - Mucinous adenocarcinoma (MC)
    - Mucinous cystadenocarcinoma NOS
    - Invasive micropapillary carcinoma
    - Metaplastic carcinoma NOS

Favourable Histologic Subtypes:

- Tubular carcinoma (TC)
- Mucinous carcinoma (MC)
- Cribriform carcinoma
- Adenoid cystic carcinoma

These histologic subtypes are recognized clinically as having **favorable prognosis**, with better overall outcomes compared to other invasive types.

Histological Type of Invasive Ductal Carcinoma (IDC)

A. Invasive Ductal Carcinoma, NOS (IDC-NST)

- IDC-NST is the most common form of breast cancer, representing **90%** of all malignancies. It is rare in women below the age of 40 years.[23]

- **Gross Examination**:
    - Tumors exhibit **marked variation in size**, ranging from **10mm to over 10cm**.
    - Typical tumors are **firm**, **poorly circumscribed**, gritty, and exhibit a **yellowish-grey colour** on cut sections.
    - **Necrosis**, **haemorrhage**, and **cystic degeneration** may be present in the tumor.
    - Tumors with a significant amount of stroma are referred to as **'scirrhous carcinoma'** and feel particularly hard.
- **Microscopic Examination**:
    - IDC can grow in **diffuse sheets**, **well-defined cords**, or as **individual cells**.
    - The nuclei of the tumor cells are **highly pleomorphic**, with **prominent nucleoli** and **frequent mitoses**.
    - Foci of **squamous metaplasia**, **apocrine metaplasia**, or **clear cell change** may be observed.
    - The **stroma** can range from none to abundant, with appearances ranging from **densely fibrotic** to **cellular** (desmoplastic).
    - A **mononuclear inflammatory infiltrate** is usually present at the interface between the tumor and stroma.

B. Invasive Lobular Carcinoma (ILC)

- Invasive Lobular Carcinoma (ILC) is the **second most reported type** of breast cancer and accounts for **5-15%** of all invasive breast tumors. [24]
- The **mean age** of patients diagnosed with ILC is between **40 and 49 years**. [25]
- **Gross Examination**:
    - ILC typically presents as a **firm to hard tumor** with **irregular borders**.
    - The tumor appears **gray or white** with a **scirrhous** (hard and fibrous) appearance.
    - **Numerous fine, hard nodules** may form, which feel like **tiny pebbles** or **grains of sand** in the breast parenchyma.

- **Microscopic Features**:
    - ILC is characterized by **proliferation of small clefts** that lack cohesion.
    - The tumor often grows in **single-file linear cords** that invade the **desmoplastic stroma**.[26]
    - The neoplastic cells typically have **round or notched ovoid nuclei**, with a **thin rim of cytoplasm** and occasional **intracytoplasmic mucin**.
    - **Mitosis** is usually **infrequent**, and **lymphovascular invasion** is uncommon.
- **Hormone Receptor Status**: [23]
    - **Estrogen Receptor (ER) positivity** is observed in **80-95%** of cases.
    - **Progesterone Receptor (PR) positivity** is observed in **60-70%** of cases.

C. Tubular Carcinoma

- Tubular carcinoma accounts for **1-5%** of all invasive breast cancers. It is more commonly found in **postmenopausal women**, with the **median age** of presentation being **63 years**. [27]
- **Gross Examination**:
    - The tumor size typically ranges from **0.2 cm to 2.5 cm**.
    - On gross examination, it presents as an **ill-defined, firm to hard** tumor with a **pale grey** colour.
- **Microscopic Features**:
    - At **low power**, tubular carcinoma (TC) often exhibits a **satellite outline** and invades the surrounding normal breast parenchyma.
    - It is characterized by **small, round to ovoid or angular glands and tubules** with **open lumina**.
    - These tubules are lined by a **single layer** of **cuboidal to slightly elongated cells**, with a moderate amount of cytoplasm.
    - The cells have **uniform round to oval nuclei**, **homogeneous fine granular chromatin**, and **punctate nucleoli**.

- **Apical snouts** (extensions of the luminal aspect) may be seen in the tubules.
- The ducts are separated by **abundant desmoplastic fibrous stroma**.

- **Hormone Receptor Status**:
  - Tubular carcinoma is **nearly always ER (Estrogen Receptor)** and **PR (Progesterone Receptor)** positive.
  - It has a **low growth fraction** and is **HER2/neu and EGFR negative**.

D. Cribriform Carcinoma

- Cribriform carcinoma accounts for **0.8-3.5%** of all breast carcinomas.[28]
- The **median age** of patients is **46.5 years**. It may present as a **mass lesion**, but **is frequently clinically occult**, meaning it may not be easily detectable through physical examination.
- **Microscopic Features**:
  - **Histologically**, pure invasive cribriform carcinoma is characterized by a **cribriform growth pattern**, where the tumor cells form a sieve-like arrangement, often with **round or oval spaces** within the tumor mass.
- **Hormone Receptor Status**:
  - **Estrogen Receptor (ER)** is **positive in 100%** of cases. [29]
  - **Progesterone Receptor (PR)** is positive in **69%** of cases.
  - **HER2/neu** is **negative** in this subtype.

E. Mucinous Carcinoma

- Mucinous carcinoma accounts for **1-7%** of all breast cancers and is known for presenting at one of the **oldest median ages** for diagnosis, typically around **71 years**.[30]
- **Gross Examination**:
  - The tumor usually has a **glistening gelatinous cut surface** with a **bosselated pushing margin** and a **soft consistency**.
  - The tumor size can range from **less than 1 cm to over 20 cm**, with an average size of **2.8 cm**. [31]

- **Microscopic Features**:
    - Mucinous carcinoma is **well-differentiated** and characterized by **clusters of uniform round cells** with **minimal eosinophilic cytoplasm**, which float in **lakes of mucus**.
    - For the tumor to be classified as **pure mucinous carcinoma**, **more than 90%** of the tumor must consist of **mucinous components**.
- **Hormone Receptor Status**:
    - Mucinous carcinoma is **ER (Estrogen Receptor) positive** and **PR (Progesterone Receptor) positive**.
    - **HER2/neu negative**. [32]

F. Adenoid Cystic Carcinoma (AdCC)

- **Gross Examination**:
    - AdCC range from **2 mm to 250 mm** and presents as a **well-circumscribed nodule** with **pushing borders**.
    - The tumor typically exhibits **grey, pale yellow, tan, and pink** colours. [33]
- **Microscopic Features**:
    - Microscopically, AdCC consists of a **mixture of proliferating glands** (the **adenoid component**) and **basaloid/myoepithelial cells**.
    - These cells produce **abundant basement membrane material**, resulting in the formation of a **"pseudoglandular" or cylindromatous component**.

G. Apocrine Carcinoma

- Apocrine carcinoma represents **less than 1%** of all breast cancers.[34] The **mean age** at diagnosis is **58.5 years**.[35]
- **Microscopic Features**:
    - The term **"apocrine pattern"** refers to a distinctive pathological finding characterized by the presence of **enlarged cells** with a **cuboidal or columnar shape**, **eosinophilic cytoplasm**, and **apical snouts or blebs**. [36]

- The tumor cells typically have **round nuclei**. Apocrine changes have been observed in benign, atypical, and malignant breast lesions.
- **Essential criteria** for apocrine carcinoma include the presence of **>90%** of these characteristic cell features in the tumor cells.
- Apocrine carcinoma is typically **ER-negative**, **androgen-receptor positive**. [37]

H. Invasive Micropapillary Carcinoma

- Invasive micropapillary carcinoma accounts for **0.9-2%** of all breast carcinomas.[38]
- **Microscopic Features**:
  - More than **90% of the tumor** consists of **hollow** or **morula-like aggregates** of **cuboidal to columnar neoplastic cells**.
  - These cell aggregates are **devoid of a fibrovascular core** and are **immersed in spongy stroma**.
  - The stroma is characterized by **clear and empty spaces** around the epithelial clusters.
  - **Reverse polarity** is a key feature, where the **apical pole** of the tumor cell membrane faces outward, towards the **clear stromal space**, instead of towards the center of the clusters.

I. Metaplastic Carcinoma

- Metaplastic carcinoma accounts for **0.2-1%** of all breast carcinomas. [39]
- The **mean age** at diagnosis ranges from **51.6 to 58.5 years**.
- **Gross Examination**:
  - The tumor may present as a **well-circumscribed mass** or have **indistinct irregular borders**.
- **Microscopic Features**:
  - Metaplastic carcinoma can be **monophasic** (composed of a single cell type) or **biphasic** (composed of two distinct cell types).

- The cells are **elongated**, **plump**, and have **enlarged pleomorphic vesicular nuclei** with **distinct nucleoli**.
- **Admixed inflammatory cells** may be seen within the tumor.

Breast Cancer Staging and Prognosis

Staging plays a crucial role in classifying individuals into risk groups, which helps define their prognosis and guides treatment options for patients with similar conditions. The most commonly used system for staging breast cancer is the **TNM classification** developed by the **American Joint Committee on Cancer (AJCC)**, specifically the **8th edition**.[40] This system divides breast cancer patients into different stage groups based on three key factors:

1. **T (Tumor Size)**: Refers to the size of the largest and most invasive component of the tumor in its largest dimension.
    - **T1**:
        - **T1mi**: Microinvasive tumor (1mm to 5mm).
        - **T1a**: Tumor larger than 1mm but not more than 5mm.
        - **T1b**: Tumor larger than 5mm but not more than 10mm.
        - **T1c**: Tumor larger than 10mm but not more than 20mm.
    - **T2**: Tumor size greater than 20mm but less than or equal to 50mm.
    - **T3**: Tumor size greater than 50mm.
    - **T4**: Tumors involving the chest wall or showing macroscopic skin changes:
        - **T4a**: Any size involving the chest wall.
        - **T4b**: Macroscopic skin changes (ulceration, edema, satellite nodules, but not meeting inflammatory breast cancer criteria).
        - **T4c**: Both T4a and T4b characteristics.
        - **T4d**: Diffuse edema and erythema (Peau d'orange, inflammatory breast cancer, at least involving 1/3 of the breast).
2. **N (Node Involvement)**: Refers to the status of regional lymph nodes.

- **cNx**: Lymph nodes removed but cannot be examined by imaging or physical examination.
- **cN0**: No regional lymph nodes examined by imaging or physical examination.
- **cN1**: Movable Level 1 and 2 ipsilateral lymph nodes.
- **cN2**: Metastasis to fixed or matted ipsilateral level 1 and 2 lymph nodes, or metastasis to ipsilateral internal mammary lymph nodes without axillary involvement.
- **cN3**: Ipsilateral level 3 lymph node involvement, supraclavicular lymph node involvement, or internal mammary lymph node involvement with level 1 and 2 lymph node involvement.

3. **M (Metastasis)**: Refers to the presence or absence of distant metastasis.
   - **cM0**: No distant metastasis (clinically/imaging).
   - **cM1**: Distant metastasis observed (clinically/imaging).
   - **pM1**: Distant metastasis confirmed based on pathological findings.

Prognosis by Stage: [22]

- **Stage 0 and Stage I**: Both stages have a **5-year survival rate of 100%**.
- **Stage II and Stage III**: The **5-year survival rates** are approximately **93%** and **72%**, respectively.
- **Stage IV**: The prognosis worsens significantly with distant metastasis, and the **5-year survival rate** drops to **22%**.

Early detection and treatment contribute to a favorable prognosis, especially in early-stage breast cancer. [41]

Breast Cancer Grading

Breast cancer grading is a crucial process that helps in assessing the tumor's aggressiveness. [21] The grade is determined based on three main features:

1. **Tubule Formation**: The extent to which the cancer cells form tubular structures, which is a characteristic of well-differentiated tumors.

1 point: > 75% of tumor

2 points: 10 - 75% of tumor

3 points: < 10% of tumor

2. **Nuclear Pleomorphism**: The variation in size and shape of the cancer cell nuclei. Greater pleomorphism often indicates a more aggressive tumor.

   1 point: minimal nuclear variation in size and shape (< 1.5 times the size of benign epithelial cell nuclei), even chromatin, inapparent to inconspicuous nucleoli

   2 points: moderate nuclear variation in size and shape (1.5 - 2 times the size of benign epithelial cell nuclei), typically visible but small nucleoli

   3 points: marked nuclear variation in size and shape (> 2 times the size of benign epithelial cell nuclei), often prominent nucleoli

3. **Proliferation (Mitotic Index)**: The number of cancer cells dividing, which indicates the tumor's growth rate. Based on Nikon or Labophot 40x objective or comparable with field diameter of 0.44 mm,

   1 point: 0 – 5 mitosis/10 hpf

   2 points: 6-11 mitosis/ 10 hpf

   3 points: >11 mitosis/ 10 hpf

The **Nottingham modification of the Scarff Bloom Richardson (SBR) grading system** is commonly used to assess these parameters. [20] Each of the three factors is scored on a scale from **1 to 3**, with **1 being the least aggressive** and **3 being the most aggressive**. The sum of these scores produces a **grade** for the cancer, helping clinicians determine the treatment approach.

- **Grade 1 (Well-differentiated)**: 3-5 points, tumor cells resemble normal cells and grow slowly.
- **Grade 2 (Moderately differentiated)**: 6-7 points, tumor cells look less like normal cells and grow at a moderate rate.
- **Grade 3 (Poorly differentiated)**: 8-9 points, tumor cells look very abnormal and tend to grow quickly.
- This grading is crucial for prognosis, particularly before treatment, as neoadjuvant therapies may alter the cancer's presentation. [20,21]

Hormonal Receptors:

Hormonal receptors, namely the **Estrogen Receptor (ER)** and **Progesterone Receptor (PR)**, play a critical role in the prognosis and treatment response of breast cancer.[41]

1. **Estrogen Receptor (ER)**:
    - There are two subtypes of ER: **ERα** (most relevant in breast cancer diagnosis and treatment) and **ERβ**.
    - **ER** expression is present in **70-80%** of breast tumors and is associated with **slower growth** and **better prognosis**.
    - The **Allred scoring system** combines the **proportion** and **intensity** of ER-positive tumor cells. Scores of **3-8** are interpreted as **positive**.
2. **Progesterone Receptor (PR)**:
    - **PR** is induced by **ER** and modifies ER's behavior.
    - PR expression is linked to **better clinical outcomes** and can influence **tumor differentiation** and **chemotherapy resistance**.
    - **PR expression** is typically evaluated using the **Allred scoring system**.
3. **Human Epidermal Growth Factor 2 (HER2)**:
    - **HER2** is a **prognostic** and **predictive** marker, with **15-20%** of breast tumors exhibiting **HER2 expression**.
    - **HER2-positive tumors** are often more **aggressive** but respond to **anti-HER2 therapy**.
    - HER2 is assessed by **ASCO/CAP guidelines**, with interpretation based on membrane staining intensity.

Molecular Subtyping of Breast Cancer:

Molecular subtyping divides breast cancer into distinct groups based on the expression of **ER**, **PR**, **HER2**, and **Ki67**. [41] This classification helps in personalized treatment planning and prognostic predictions. The four primary subtypes are:

1. **Luminal A**:

- ER-positive, HER2-negative, low Ki67 i.e. <14%.
- Best prognosis and most responsive to hormonal therapy.

2. **Luminal B**:
   - ER-positive, HER2-negative or HER2-positive, high Ki67 i.e. >14%.
   - More aggressive than Luminal A, but still responsive to hormonal therapy.
3. **HER2 Enriched**:
   - ER-negative, HER2-positive, high Ki67.
   - Aggressive, but responds to HER2-targeted therapies.
4. **Basal-like** (Triple Negative):
   - ER-negative, PR-negative, HER2-negative, often high Ki67.
   - Most aggressive subtype, typically treated with chemotherapy.

Aetiological Factors of Breast Cancer :[42]

Relative Risk Factors:

1. **Relative Risk > 4.0**
   - **Non-modifiable**: Female gender, increasing age, germline BRCA1 or BRCA2 mutations of high penetrance, strong family history, high breast density.
2. **Relative Risk 2.1–4.0**
   - **Non-modifiable**: Germline mutations of moderate penetrance, family history.
   - **Modifiable**: High-dose chest radiation at a young age.
3. **Relative Risk 1.1–2.0**
   - **Non-modifiable**: Early menarche (<12 years), late menopause (>55 years), late first pregnancy (>35 years), nulliparity.
   - **Modifiable**: Absence of breastfeeding, exogenous hormone therapy, postmenopausal obesity, physical inactivity, high alcohol consumption, smoking, diet.

NESTIN: Overview

Nestin is a protein primarily found in **progenitor cells** during early development and plays an essential role in cell movement through developmental and healing stages. Its expression is temporary and is mostly seen in **embryonic** and **fetal tissues** during the cell differentiation and regeneration process. While nestin is generally absent in mature tissues, its expression can reappear in certain **adult stem cells**, particularly during tissue damage or in cancerous cells. High nestin expression is notably observed in various tumors, including **brain**, **central nervous system**, **skin**, **digestive system**, **prostate**, and **breast cancer**. In breast cancer, nestin is especially prominent in **basal-like** tumors, and its presence in the **basal/myoepithelial cells** of the mammary gland makes it a useful **marker**.[49]

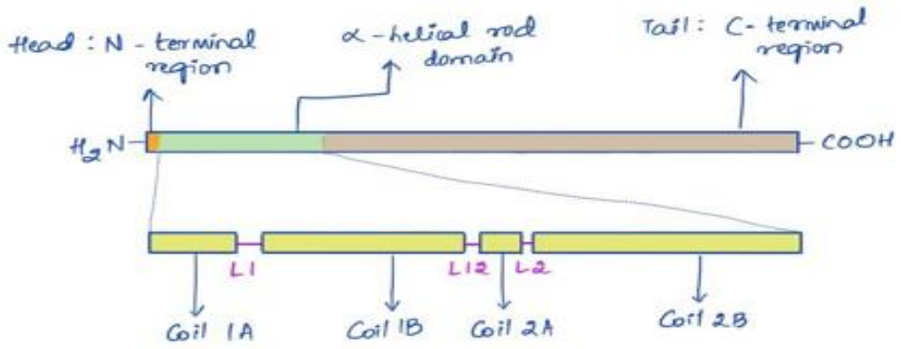
Domain Structure of Nestin

The human nestin protein consists of **1621 amino acids**, divided into three distinct regions: the **N-terminal head**, the **central α-helical rod domain**, and the **C-terminal tail**. Each domain plays a unique role in the formation and function of nestin as a key structural component in cells. [50]

1. **N-terminal head (1-7 amino acids)**:
    - The **N-terminal region** of nestin, composed of approximately **8 amino acids**, is crucial for **filament formation**. However, this short N-terminus prevents nestin from self-assembling into higher-order structures on its own. To form these structures, nestin requires assistance from other intermediate filaments, such as **vimentin**, **desmin**, and **α-internexin**.
2. **α-helical rod (8-313 amino acids)**:
    - The **central α-helical rod domain** forms the core of nestin. This domain consists of three segments, **coli 1A**, **coli 1B**, and **coli 2A**, separated by **linkers** (L1, L2, L12). These segments play a key role in **dimerization** and the formation of **coiled coils**. [50]
    - The **rod domain** is essential for nestin's function as it mediates interactions with other proteins and structures, facilitating **filament formation**. This region contains **heptad repeats**, hydrophobic sequences that allow two **α-helices** to

wrap around each other and form a **'coiled coil'**, a structure necessary for filament formation. [50]

3. **C-terminal tail (314-1621 amino acids)**:
    - The **C-terminal tail** of nestin is the longest domain, stretching from **amino acid 314 to 1621**. This region is exposed outside the filament structure and is **accessible to post-translational modifications** and interactions with various **proteins**. The C-tail contributes to nestin's role in **cell signalling**, **regulation**, and interactions with other cellular components.



DOMAIN STRUCTURE OF NESTIN

Gene and Evolutionary Background

The **nestin gene** is located on **chromosome 1**, specifically at **1q23.1**, and consists of **four exons** separated by three introns. Notably, two of these introns are shared with the **neurofilament** gene, suggesting that nestin and neurofilaments may have a common evolutionary origin. [49,50]

Role of Nestin in Cell Cycle Regulation and Survival

Nestin's role in breast cancer development and other cellular processes is still under investigation. However, recent studies suggest that nestin is involved in several key aspects of cell regulation, particularly the cell cycle, cell growth, and cell survival. One notable mechanism is the phosphorylation of nestin, which significantly alters its behavior in cells. [51]

Nestin contains several phosphorylation sites, and its modification by cyclin-dependent kinases (CDKs), such as Cdk1 (also known as cdc2) and Cdk5, plays a role in regulating the cell cycle.

Recent studies have shown that the phosphorylation of nestin affects the growth of cancer cells. Moreover, nestin has been found to influence the activity of Cdk5, a kinase that is associated with tumor formation and various processes like cell growth, invasion, and angiogenesis (formation of new blood vessels). [52]

In breast cancer, Cdk5/p35 complex overexpression correlates with poor prognosis indicators, including triple-negative breast cancer, Her2+ status, and higher malignancy grades. Nestin regulates this process by sequestering the Cdk5/p35 complex in the cytoplasm, preventing it from entering the nucleus, where it would otherwise trigger cell death due to oxidative stress. By stabilizing the Cdk5/p35 complex, nestin protects cells from apoptosis (programmed cell death). [53]

Additionally, nestin interacts with the transcriptional activator Gli3, a zinc finger transcription factor that inhibits Hedgehog signalling, further driving tumor growth. While it is clear that nestin plays a role in tumor growth and spread, more research is needed to fully understand its mechanisms in cancer progression. [54]

Physiological Functions of Nestin

Nestin is primarily expressed in progenitor cells during embryonic development, where it contributes to the structure and organization of various tissues. It is notably found in developing tissues such as the muscle, testis, kidney, and central nervous system (CNS), as well as in new blood vessels. [55]

In the adult body, nestin is typically replaced by other intermediate filaments like GFAP, vimentin, and desmin as tissues mature. However, nestin can still be found in adult stem cells, particularly in areas like the pancreas, pituitary gland, and adrenal glands, where it aids in maintaining cell shape and function. [56]

Nestin is essential for the structural integrity of specialized cells, such as the podocyte foot processes in the adult kidney. Its expression is regulated by various growth factors and ligands, which affect its levels during differentiation and regeneration. For instance, EGF, neurotrophin-3, and thrombin can induce nestin expression, although their effects vary depending on the tissue type. [57]

Nestin in Regeneration

Nestin plays a critical role in tissue repair and regeneration, often reappearing in response to injury. It is particularly involved in the regrowth of cells in various tissues, including blood vessels and muscle fibres, after damage. [58]

In the context of muscular dystrophy myositis, nestin expression was observed in muscle fibers that were regenerating, indicating its role in tissue repair. This was further confirmed in injured skeletal muscles, where nestin levels increased in myoblasts near the site of injury. [59]

Nestin also plays a pivotal role in the heart during healing. After a heart attack, nestin-positive neural-like stem cells and myofibroblasts migrate to the damaged area, supporting angiogenesis and nerve regeneration. Similarly, nestin is involved in the healing process following spinal cord and brain injuries, where it is expressed in scar tissue formed by glial cells. [59]

In the kidney, nestin is upregulated in response to stress signals such as hypoxia, where it helps immature kidney cells migrate to areas in need of repair. Nestin expression also increases in other organs, such as the pancreas, liver, skin, retina, and teeth, during injury, though its precise role in these tissues remains under investigation. [59]

Nestin in Angiogenesis During Development

Nestin also plays a crucial role in the development of blood vessels. During embryogenesis and in structures such as the corpus luteum, nestin is expressed in endothelial cells of early capillaries. However, mature endothelial cells do not express nestin. In adults, nestin expression is largely absent from mature endothelial cells, further suggesting that nestin is a marker of immature endothelial cells and is important in early blood vessel development. [66]

Nestin in Pathology

Initially considered merely a marker for progenitor cells, nestin’s true role in cell function and pathology is becoming clearer. Its involvement in the central nervous system (CNS) ties nestin to neurological disorders such as Alzheimer's Disease (AD), where its expression increases, especially in the disease's later stages, possibly linked to cell proliferation. Nestin has also been associated with diabetes and Alzheimer’s Disease (AD) due to its interactions with the insulin-degrading enzyme (IDE). Furthermore, nestin has been found in smooth muscle cells in coronary artery plaques, suggesting its involvement in cardiovascular diseases and possibly angina. [60]

Nestin's role in cancer is complex, with varying correlations between its expression and patient outcomes. It has been found in a wide variety of cancers, including osteosarcoma, prostate, breast, testicular, ovarian, skin, gastrointestinal, lung, pancreatic, glioma, and other CNS tumors. The increase in nestin expression is linked to tumor progression, tumor grade, and recurrence, although this does not always correlate with patient survival. Studies have also shown that nestin-positive progenitor cells can be attracted to tumors and their edges, where they may influence tumor behavior by promoting angiogenesis or tumor spread. However, distinguishing whether nestin-positive cells are part of the tumor or a response from the surrounding tissue remains a challenge. [60]

Nestin has emerged as a key marker for newly formed blood vessels in tumors, with its expression largely limited to immature blood vessels rather than mature ones. In various cancers, including colorectal, prostate, and glioblastoma, nestin-positive vessels are associated with poor prognosis. For example, in colorectal cancer, nestin is predominantly expressed in blood vessel cells, which are smaller and more proliferative compared to CD34-positive vessels. In glioblastomas, nestin expression correlates with tumor severity, and in melanoma, it is linked to advanced stages. Interestingly, in gastric cancer, nestin-positive blood vessels do not correlate with patient outcomes, although in larger tumors, higher nestin levels are associated with longer survival compared to CD34. [68]

Role of Nestin in Breast Cancer Angiogenesis

Angiogenesis, the process of new blood vessel formation, plays a critical role in tumor progression and metastasis. Recent research highlights the involvement of nestin, an intermediate filament protein, in angiogenesis within breast cancer (BC). Nestin is expressed not only in tumor cells but also in tumor-associated blood vessels. A study by Kruger et al. (2019) investigated angiogenesis through the evaluation of immature (nestin+) and proliferating (Ki-67+) endothelial cells in breast tumor tissue. The findings demonstrated a significant correlation between the co-expression of nestin and Ki-67 in tumor vessels. Additionally, tumors with higher nestin+ microvessel density (MVD) exhibited a basal-like phenotype and were negative for estrogen and progesterone receptors. The study also introduced the vascular proliferation index (VPI), which represents the percentage of nestin+Ki-67+ microvessels relative to all nestin+ microvessels. VPI was notably higher in invasive ductal carcinoma (IDC) compared to invasive lobular carcinoma (ILC), and was linked to poorer overall survival. [61]

Our recent findings confirm these observations, showing that nestin+ microvessels are significantly more abundant in invasive tumors than in pre-invasive lesions. Furthermore, a high nestin+ MVD correlates with disease progression, advanced tumor stage, lymph node metastasis, high tumor grade, and triple-negative (TN) phenotype. Notably, nestin-expressing vessels were strongly associated with immature CD34+ endothelial cells, rather than mature CD31+ endothelial cells, suggesting that nestin is a marker of endothelial progenitor cells in developing blood vessels. In vitro analyses of various endothelial cell lines revealed that nestin expression varies with the maturity of the endothelial cells, with the highest levels found in early progenitor cell lines derived from human umbilical cord blood, compared to dermal microvascular and umbilical vein endothelial cells. This further supports the idea that nestin expression is indicative of undifferentiated or immature blood vessels. [62]

Another significant study observed that nestin expression in breast cancer cells was positively associated with both the area and number of vessels expressing endothelial markers, such as CD31, CD34, and SOX-18, a transcription factor crucial for blood vessel formation. Additionally, nestin expression in tumor cells correlated with higher VPI and increased blood vessel invasion, but not with lymphatic invasion. These results reinforce the hypothesis that nestin might be involved in vascular mimicry, where cancer stem cells transform into endothelial-like cells. [63]

Nestin in Breast Cancer Targeted Therapy

Nestin's role in both tumor cells and tumor-associated vasculature makes it an appealing target for breast cancer therapy. In several cancer models, the blockade of nestin expression has been shown to reduce tumor cell proliferation, motility, and invasion. What makes nestin a promising therapeutic target is its limited expression in normal adult tissues, while it is highly expressed in tumor cells, newly formed blood vessels, and cancer stem cells. Targeting nestin could, therefore, offer a more specific therapeutic approach with fewer off-target effects compared to conventional treatments. [64]

Nestin's dual role as a marker of angiogenesis and cancer stem cells positions it as a novel biomarker for breast cancer diagnosis, prognosis, and treatment. Further studies are required to explore the potential of nestin-targeted therapies in clinical settings. [65]

Nestin in Angiogenesis in Non-Cancerous Diseases

In non-cancerous diseases, nestin is similarly involved in angiogenesis. For example, nestin-positive endothelial cells play a significant role in wound healing and the success of skin transplants. In cardiac repair following reduced blood flow (reparative fibrosis), nestin-positive cells, likely originating from the neural crest, assist in forming new blood vessels and nerve structures. Studies have also shown that nestin is expressed in endothelial precursors, important for the formation of new endothelial cells during the angiogenic process. Moreover, vascular endothelial growth factor (VEGF), a key pro-angiogenic factor, has been shown to induce nestin expression in endothelial cells. In placentas affected by pre-eclampsia, nestin levels were found to be elevated, indicating that hypoxia may stimulate nestin expression during pathological angiogenesis.

METHODOLOGY

A total of 52 specimens of breast carcinoma cases from patients diagnosed clinically and histopathologically at KLEs Dr. Prabhakar Kore Hospital and Medical Research Center, Belagavi from January 2023 to December 2024 were included in this study. Detailed clinicopathological information was taken from patient records. The specimens included modified radical mastectomy specimens.

1. Study Design and Duration

A hospital-based two-year prospective cross-sectional study

2. Study Setting

The study was conducted at KAHER's Jawaharlal Nehru Medical College and KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, a tertiary care hospital with a well-established histopathology laboratory.

3.Inclusion Criteria:

- Histopathologically confirmed female patients with more than 18 years of age diagnosed with Invasive Ductal Carcinoma (IDC) of the breast.

4.Exclusion Criteria:

- Inadequate biopsy samples that could not be evaluated histopathologically.
- Improperly preserved or improperly fixed specimens, which could lead to unreliable results.
- Patients diagnosed with premalignant lesions without presence of malignant counterpart.
- Other subtypes of breast carcinoma (e.g., invasive lobular carcinoma, inflammatory carcinoma) that did not fit the study criteria.

5. Study Sampling

Universal sampling

6. Study Sample Size

The sample size was calculated based on the formula for estimating the minimum sample required for a cross-sectional study. The formula used was:

$$n = \left[\left(Z_{\alpha/2} + Z_{\beta}\right)/c\right]^2 + 3$$

where:

- $Z_{\alpha/2} = 1.645$ (for a significance level of 10%),
- $Z_{\beta} = 0.8416$ (for a power of 80%),
- $r = 0.34$ (correlation coefficient between nestin and SOX18 vessel expression),
- $c = 0.5 \times \ln\left[\frac{1+r}{1-r}\right]$.

Substituting values into the formula provided an estimated sample size of **52**.

7. Ethical Clearance

The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics committee on Human Subjects research. (Ref.: MDC/JNMCIEC/142).

8. Study Parameters

- **Nestin Expression:** Measured by immunohistochemistry (IHC) in breast carcinoma tissue specimens, categorized into weak, moderate and strong expression.
- **Histopathological Features:** Grading of tumors based on the Modified Scarff Bloom Richardson classification, which includes tumor differentiation, mitotic index, and extent of tubule formation.
- **Clinicopathological Parameters:** Age, tumor size, lymph node metastasis, hormone receptor status (estrogen and progesterone receptors), HER2 status, and molecular subtypes (IDC).

9. Study Procedure

The breast carcinoma cases that were operated on in KLE's Dr. Prabhakar Kore Hospital and Research Centre were taken for this study. These specimens were grossed according to the standard procedure, and bits were given. Formalin-fixed, paraffin-embedded tissue blocks were sectioned and slides were prepared for both haematoxylin and eosin (H&E)

staining and immunohistochemistry (IHC) to assess Nestin expression. Slides were stained with H&E, and slides were screened. Suitable blocks were selected for Nestin IHC staining. The IHC staining was conducted using primary Nestin antibody and appropriate controls. All slides were evaluated under an Olympus CH20i microscope, and images were captured using a JENOPTIK SUBRA digital camera for further analysis. The clinical parameters including patient demographics, tumor size, stage, lymph node involvement, and hormone receptor status, were extracted from the hospital's Medical Records Department (MRD). Histopathological features and immunohistochemical results were collected from the pathology department (*as stated in Annexure I and II)*. The data was recorded in a structured format and entered into a database for further analysis.

Histopathological evaluation: Tissue specimens of breast carcinoma were collected and immediately fixed in 10% formalin and processed. Paraffin-embedded blocks were prepared. About 3– 4 micron thin sections were taken from each block and H & E-stained slides were examined by pathologist and reporting was done according to Modified Scarff Bloom Richardson Classification.

- Immunohistochemical analysis of Nestin: About 3 – 4 micron thick sections were taken from the formalin fixed paraffin embedded block from each case. Nestin Ab immunohistochemical staining was performed on slides with both positive and negative controls (*as stated in Annexure III*) and was further evaluated by pathologist. Slides were evaluated for intensity and percentage of positivity of neoplastic cells. The nature of non-neoplastic cells was noted too. Each case was assigned a score based on degree of expression.
- Immunohistochemical staining evaluation: The slides were examined under Olympus CH20i microscope and selected pictures were captured with the attached JENOPTIK SUBRA digital camera using GRYPHAX programme.
- Staining Intensity score will be:

  Score 0: No tumour cells stained

  Score 1: Weakly stained

  Score 2: Moderately stained

  Score 3: Strongly stained.

- Percentage of Nestin expression will be:

  0 - < 1% of neoplastic cells discretely expressed Nestin in their cytoplasm

  1+ - 1 ≥ and <10% of discrete cytoplasmic expression in morphological unequivocal neoplastic cells

  2+ - ≥ 10% of discrete cytoplasmic expression in morphological unequivocal neoplastic cells

  Cases graded as 1+ and 2+ will be considered positive

- Immunoreactivity score for Nestin expression will be calculated by multiplying the number representing the percentage of immunoreactive cells by the number representing staining intensity. The cases will be categorized as:

  1-3 = weak

  4-6 = moderate

  7-9 = strong

10. Data Analysis

The data was analysed using SPSS software (version 27). Descriptive statistics were used to summarize the clinicopathological parameters and the expression levels of Nestin. The correlation between Nestin expression and clinicopathological parameters including histological grading and immunohistochemical expression of ER, PR Her2 and Ki67 was evaluated using chi-square tests, with a significance level set at $p \leq 0.05$ Additionally, immunoreactivity scores for Nestin expression were calculated based on the intensity and percentage of positivity in neoplastic cells. Statistical methods were applied to determine any significant associations or correlations.

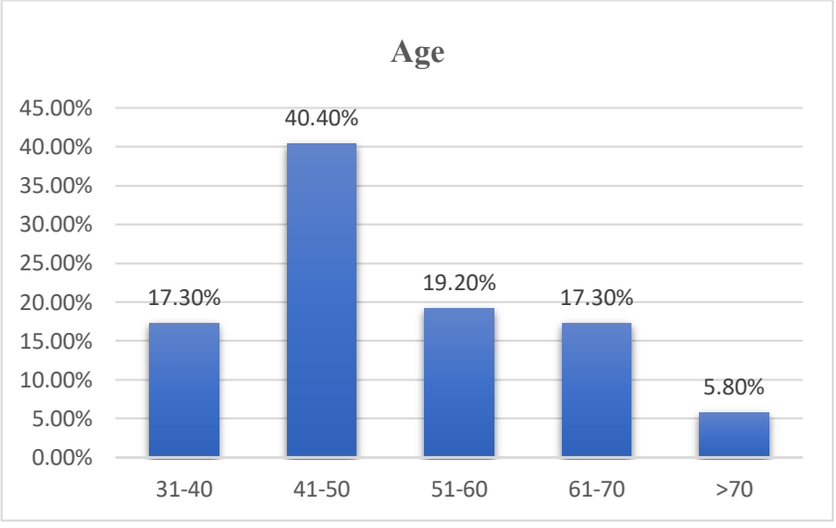
RESULT AND ANALYSIS

{A} SOCIODEMOGRAPHICS AND CLINICODEMOGRAPHICS

1. Age Distribution

Graph 1 represents the age distribution in the study and shows a varied patient demographic. The **mean age** is approximately **46.5 years**. The majority of cases occur in the 41–50 age group (40.40%), followed by 51–60 (19.20%), and both 31–40 and 61–70 age groups (each 17.30%). Only 5.80% of patients are older than 70 years.

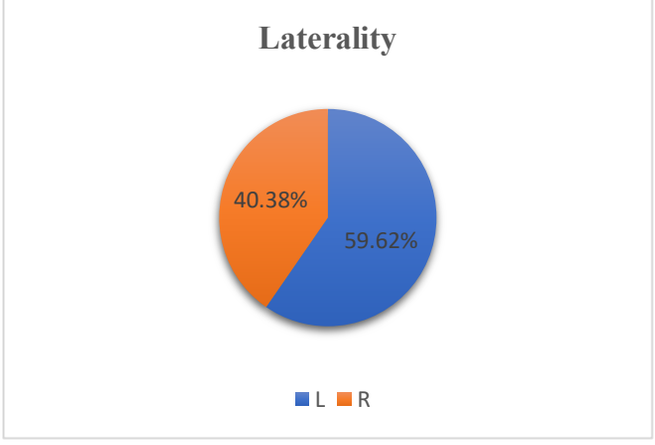
Graph 1: Age distribution among the study population.



2. Laterality

Graph 2 shows the laterality data and indicates that 59.62% of tumors occur on the left side, while 40.38% appear on the right.

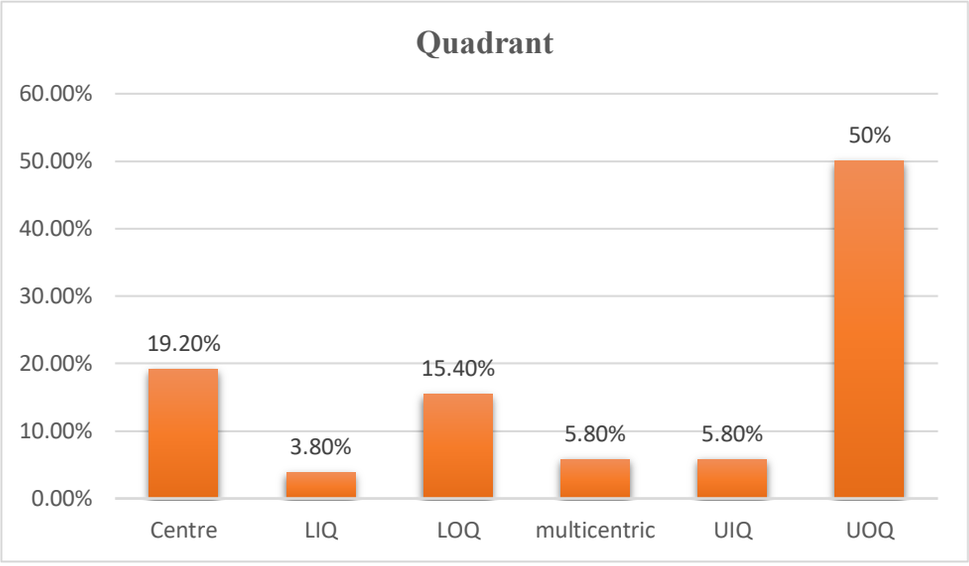
Graph 2: Laterality distribution among the study population.



3. Tumor Quadrant Distribution

Graph 3 illustrates tumor localization by quadrant, reveals that the upper outer quadrant (UOQ) is most frequently affected (50%). The central quadrant follows at 19.20%, with the lateral inferior (LIQ) at 3.80%, lateral outer (LOQ) at 15.40%, and both upper inner (UIQ) and multicentric lesions at 5.80% each.

Graph 3: Tumor Quadrant distribution among the study population



4. Menopausal Status

Table 1 represents the menopausal status and shows that 61.54% of patients are postmenopausal while 38.46% are premenopausal**.**

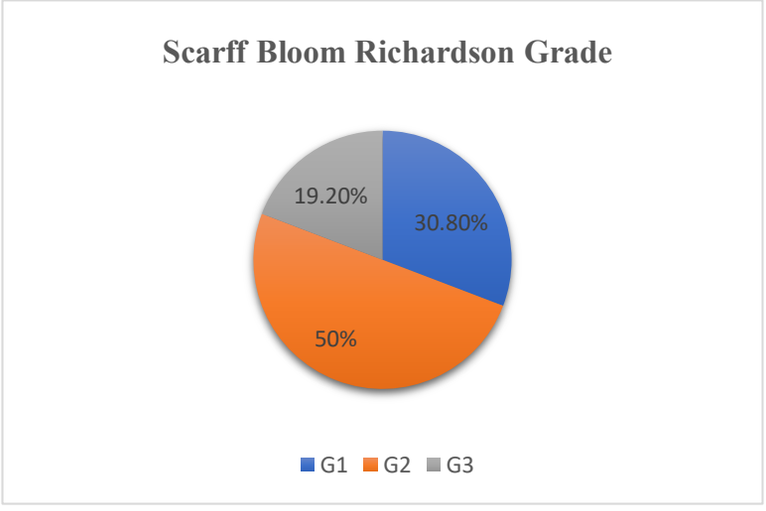
Table 1: Distribution of Menopausal status amongst the study group:

Menopause Status	**n (Number of Cases)**	**Percentage (%)**
Postmenopausal	32	61.54%
Premenopausal	20	38.46%
Total	**52**	**100%**

5. Scarff Bloom Richardson (SBR) Grade Distribution

Figure 4 illustrates Histopathological grading using the SBR grading system, indicates 30.80% of tumors are Grade 1 (16 out of 52 cases), 50% are Grade 2(26 out of 52 cases) and 19.20% are Grade 3 (10 out of 52 cases). This distribution shows a predominance of Grade 2 tumors.

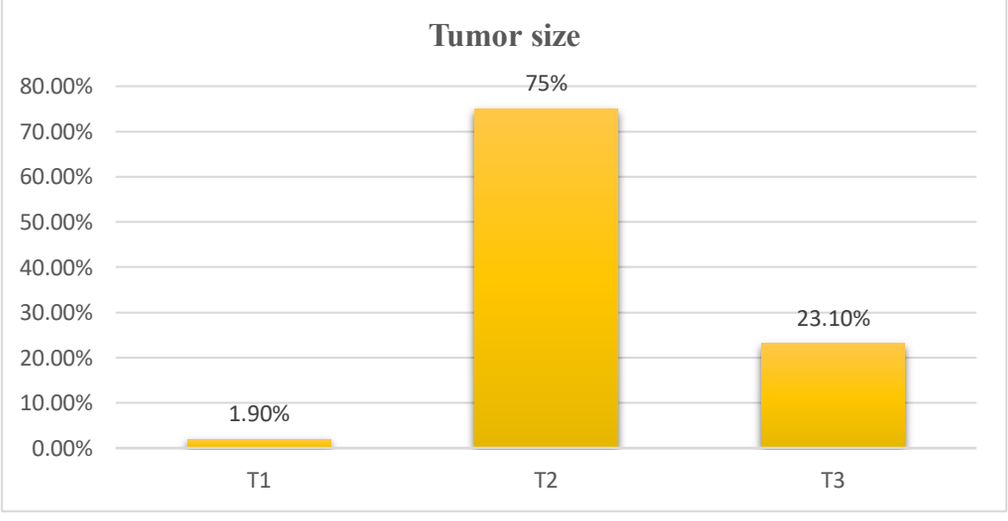
Figure 4: SBR Grade Distribution distribution among the study population.



.6. Tumor Size (T Stage Distribution)

Figure 5 illustrates tumor size classification, shows that the majority of tumors (75%) are T2 (39 out of 52 cases), with T1 tumors representing only 1.90% and T3 tumors 23.10%. This indicates that most patients present with intermediate-sized tumors.

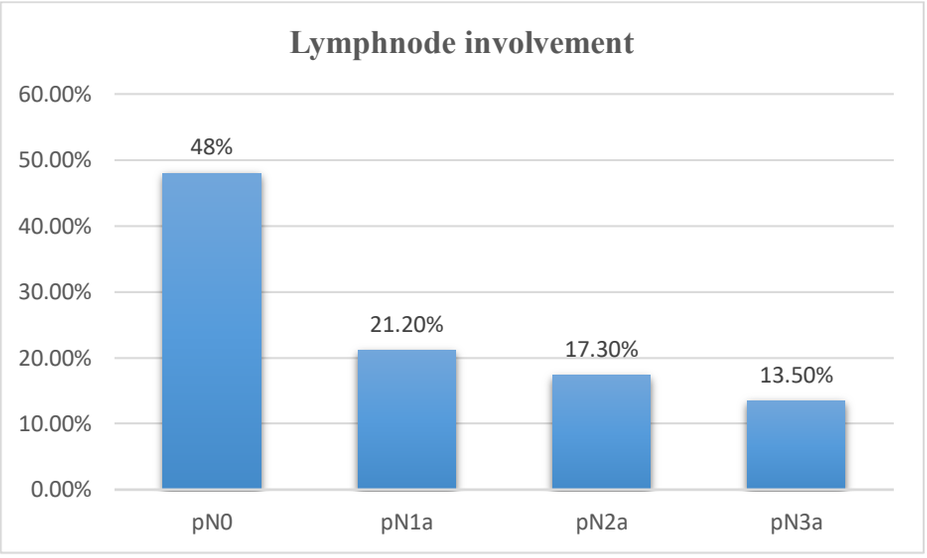
Figure 5: Tumor size distribution among the study population



7. Lymph Node involvement

Figure 6 illustrates Lymph node involvement distribution as follows: 48.00% of patients are pN0, 21.20% are pN1, 17.30% are pN2, and 13.50% are pN3.

Figure 6: Lymph node involvement distribution among the study population.



8. Perineural Invasion (PNI) Status

Table 2 shows Perineural invasion (PNI) is present in 40 (77%) cases compared to 12 without PNI. A high rate of PNI correlates with aggressive tumor behavior.

Table 2: Distribution of PNI status amongst the study group

PNI Status	**n (Number of Cases)**	**Percentage (%)**
No	12	23.00%
Yes	40	77.00%
Total	**52**	**100%**

9. Lymphovascular Invasion (LVI) Status

Table 3 shows Lymphovascular invasion in 45 (86.50%) of cases, while only 7 of tumors lack LVI.

Table 3: Distribution of LVI status amongst the study group

LVI Status	**n (Number of Cases)**	**Percentage (%)**
No (N)	7	13.50%
Yes (Y)	45	86.50%
Total	**52**	**100%**

{B} Expression of NESTIN

Table 5 illustrates expression of nestin and shows heterogeneous expression: 23 cases exhibit moderate expression, 17 cases exhibit weak expression, 6 cases show negative and strong nestin expression.

Table 5: Distribution of Nestin expression amongst the study group:

Nestin expression	**Frequency (n)**	**Percentage (%)**
Negative	6	11.54%
Weak	17	32.69%
Moderate	23	44.23%
Strong	6	11.54%
Total	**52**	**100%**

{C} DISTRIBUTION OF INTENSITY, PERCENTAGE AND IMMUNOREACTIVE SCORES IN NESTIN EXPRESSING CELLS.

Table 6 illustrates the Distribution of **Intensity score** in Nestin expressing cells**.** The results show that: A majority of Grade 1 and 2 tumors mostly exhibit score 1 to score 2, while Grade 3 tumors have stronger staining intensity exhibiting score 3 for nestin expression. The chi-square test shows that the correlation between Nestin intensity score and SBR grade is not statistically significant (p-value = 0.277).

Table 6: Demonstration of Intensity in Nestin expressing cells.

	IHC Intensity Score 0	IHC Intensity Score 1	IHC Intensity Score 2	IHC Intensity Score 3	Total (n and %)	p- value
SBR Grade 1	3	2	12	1	18 (34.62%)	0.277
SBR Grade 2	2	5	15	1	23 (44.23%)	
SBR Grade 3	1	3	3	4	11 (21.15%)	
Total	6	10	30	6	52	

Table 7 illustrates the Distribution of **Percentage score** in Nestin expressing cells**.** The results show that: A majority of tumors, regardless of grade, show IHC Percentage Score 2. The chi-square test shows that the correlation between Nestin percentage score and SBR grade is not statistically significant (p-value = 0.593).

Table 7: Demonstration of Percentage in Nestin expressing cells.

	IHC Percentage score (0)	IHC Percentage score (1)	IHC Percentage score (2)	Total (n and %)	p-value
SBR Grade 1	3 (0.67)	3 (0.67)	10 (0.43)	16 (30.77%)	0.593
SBR Grade 2	2 (0.33)	2 (0.33)	22 (0.20)	26 (50.00%)	
SBR Grade 3	1 (o.02)	1 (0.02)	8 (0.01)	10 (19.23%)	
Total	6	6	40	52	

Table 8 illustrates the Distribution of **Immunoreactivity score** in Nestin expressing cells**.** The results show that: A majority of Grade 1 and 2 tumours exhibit weak to moderate positive staining, while Grade 3 tumors show moderate to strong positive immunoreactivity score for nestin expression.

The chi-square test shows that the correlation between Nestin immunoreactivity score and SBR grade is statistically significant (p-value = 0.0025).

Table 8: Demonstration of Immunoreactivity score in Nestin expressing cells.

	IRS Negative	IRS Weakly positive	IRS Moderately positive	IRS Strongly positive	Total	p- value
SBR Grade 1	3 (28.57%)	6 (42.86%)	5 (28.57%)	0 (0.00%)	14	0.0025*
SBR Grade 2	3 (8.70%)	10 (43.48%)	10 (43.48%)	1 (4.35%)	23	
SBR Grade 3	0 (0.00%)	1 (6.67%)	9 (60.00%)	5 (33.33%)	15	
Total	6	17	23	6	52	

(* indicates $p \leq 0.05$ was considered as statistically significant)

{D} CLINICAL VARIABLES AND TUMOR CHARACTERISTICS: ASSOCIATION WITH NESTIN EXPRESSION

1. NESTIN expression with Menopausal status:

Table 9 illustrates the Expression of Nestin with Menopausal status amongst the study population. The results show that: Premenopausal patients are more likely to have Nestin IRS negative scores. Postmenopausal patients tend to have Nestin IRS weak and moderate scores. The chi-square test shows that the correlation between Nestin expression and menopausal status is statistically significant (p-value = 0.0031).

Table 9: Expression of Nestin with Menopausal status amongst the study population.

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
Premenopausal status	6	4	10	0	20 (38.46%)	13.83	0.0031*
Postmenopausal status	0	16	14	2	32 (61.54%)		
Total	6	20	24	2	52		

(* indicates $p \leq 0.05$ was considered as statistically significant)

2. NESTIN expression with Lymphovascular invasion (LVI) status:

Table 10 illustrates the Expression of Nestin with lymphovascular invasion (LVI) status amongst the study population. The results show that: Nestin expression was majorly present in moderately stained cases irrespective of presence or absence of LVI status and strong IRS was observed in all LVI present tumors. The chi-square test shows that the correlation between Nestin expression and LVI status is not statistically significant (p-value = 0.935).

Table 10: Expression of Nestin with LVI status amongst the study population.

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	P-value
LVI Present	5	15	21	6	45	0.42	0.935
LVI Absent	1	2	2	0	7		
Total	6	17	23	6	52		

3. NESTIN expression with Perineural invasion (PNI) status:

Table 11 illustrates the Expression of Nestin with Perineural invasion (PNI) status amongst the study population. The results show that: Nestin expression was present in moderately stained cases irrespective of presence or absence of PNI status and strong IRS was observed in all PNI present tumors. The chi-square test shows that the correlation between Nestin expression and PNI status is not statistically significant (p-value = 0.2448).

Table 11: Expression of Nestin with PNI status amongst the study population.

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
PNI Present	4	16	20	6	46	1.11	0.2448
PNI Absent	2	1	3	0	6		
Total	6	17	23	6	52		

4. NESTIN expression with DCIS status:

Table 12 illustrates the Expression of Nestin with DCIS component status amongst the study population. The results show that: DCIS-positive cases show moderate Nestin expression whereas, the majority of DCIS-negative cases have weak Nestin expression.

The chi-square test shows that the correlation between Nestin expression and DCIS status is statistically significant (p-value = 0.0012).

Table 12: Expression of Nestin with DCIS status amongst the study population.

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
DCIS Present	2	3	16	2	24 (48%)	15.8	0.0012*
DCIS Negative	4	17	8	0	28 (52%)		
Total	6	20	24	2	52		

(* indicates $p \leq 0.05$ was considered as statistically significant)

5. NESTIN expression with SBR Grade:

Table 13 A, B illustrate the Correlation between Nestin expression and SBR grade. The results show that: SBR Grade 1 is significantly linked to IRS Negative to weak staining, SBR Grade 2 is associated with weak to moderate IRS staining and SBR Grade 3 is significantly associated with moderate to strong IRS nestin staining.

The chi-square test shows that the correlation between Nestin IRS score and SBR grade is not statistically significant (p-value = 0.638).

Table 13: A Correlation between Nestin positivity/negativity and SBR grade

Tumor Grade	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
SBR G1	5	11	16 (30.80%)	0.0042*
SBR G2	1	26	27 (49.10%)	
SBR G3	0	9	9 (19.10%)	

(* indicates $p \leq 0.05$ was considered as statistically significant)

Table 13: B Correlation between Nestin IRS expression and SBR grade

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
SBR Grade 1	5	5	4	0	16	14.28	0.0042*
SBR Grade 2	1	10	13	2	27		
SBR Grade 3	0	2	6	4	9		
Total	6	17	23	6	52		

(* indicates $p \leq 0.05$ was considered as statistically significant)

6. NESTIN expression with Tumor size:

Table 14 A, B illustrates the Correlation between Nestin expression and Tumor size. The results show that: Moderate IRS staining is the most common across all tumor stages.

The chi-square test shows that the correlation between Nestin IRS score and Tumor size is not statistically significant (p-value = 0.786).

Table 14: A Correlation between Nestin positivity/negativity and Tumor size

Tumor Size	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
pT1	0 (0%)	1 (1.90%)	1 (1.90%)	0.786
pT2	5 (6.92%)	34 (68.08%)	39 (75.00%)	
pT3	1 (1.95%)	11 (21.15%)	12 (23.10%)	
pT4	0	0	0	

Table 14: B Correlation between Nestin IRS expression and Tumor size

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p- value
pT1	0	1	0	0	1	3.18	0.786
pT2	5	13	19	2	39		
pT3	1	3	4	4	12		
Total	6	17	23	6	52		

7. NESTIN expression with Lymph node involvement status:

Table 15 A, B illustrates the Correlation between Nestin expression and lymph node involvement. The results show that: As lymph node involvement increases (pN2 and pN3) moderate and strong Nestin IRS staining increases.

The chi-square test shows that the correlation between Nestin IRS score and lymph node involvement is statistically significant (p-value = 0.0131).

Table 15: A Correlation between Nestin positivity/negativity and lymph node involvement.

Lymph Nodes	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Negative	4 (7.70%)	21 (40.30%)	25 (48.00%)	0.0131
Positive	2 (3.80%)	25 (48.20%)	27 (52.00%)	

Table 15: B Correlation between Nestin IRS expression and lymph node involvement

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
pN0	4	11	10	0	25	10.97	0.0131*
pN1	2	5	7	0	14		
pN2	0	1	4	2	7		
pN3	0	0	2	4	6		
Total	6	17	23	6	52		

(* indicates $p \leq 0.05$ was considered as statistically significant)

8. NESTIN expression with Estrogen receptor (ER) status:

Table 16 A, B illustrates the Correlation between Nestin expression and ER status. The results show that: A higher number of ER-positive cases are observed in the IRS Moderate category whereas IRS Weak category is most common in ER-negative cases.

The chi-square test shows that the correlation between Nestin expression and Estrogen receptor status is statistically significant (p-value = 0.0244).

Table 16 A: Correlation between Nestin positivity/negativity and ER status

ER Status	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Negative	1	18	19 (40.43%)	0.0244
Positive	3	25	28 (59.57%)	

Table 16 B: Correlation between Nestin IRS expression and ER status

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
ER positive	3	9	15	1	28 (59.57%)	10.49	0.0244*
ER negative	1	12	06	0	19 (40.43%)		
Total	4	21	21	1	47		

(* indicates $p \leq 0.05$ was considered as statistically significant)

9. NESTIN expression with Progesterone receptor (PR) status:

Table 17 A, B illustrates the Correlation between Nestin expression and PR status. The results show that: A higher number of PR-positive cases are observed in the IRS Moderate category.

The chi-square test shows that the correlation between Nestin expression and Progesterone receptor status is statistically significant (p-value = 0.0144).

Table 17 A: Correlation between Nestin positivity/negativity and Progesterone receptor status

PR Status	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Negative	1	22	21 (59.57%)	0.0144*
Positive	1	25	26 (40.43%)	

Table 17 B: Correlation between Nestin IRS expression and Progesterone receptor status

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
PR positive	1	5	18	2	26	10.55	0.0144*
PR negative	1	9	11	0	21		
Total	2	14	29	2	47		

(* indicates $p \leq 0.05$ was considered as statistically significant)

10. NESTIN expression with Her2 neu receptor status:

Table 18 A, B illustrates the Correlation between Nestin expression and Her2 neu receptor status. The results show that: A majority of Her2neu positive and negative cases are observed in the IRS Weak to Moderate category.

The chi-square test shows that the correlation between Nestin expression and Her2 neu receptor status is not statistically significant (p-value = 0.142).

Table 18 A: Correlation between Nestin positivity/negativity and Her2 neu receptor status

HER2 Status	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Negative	2	32	33	0.142
Positive	4	10	14	

Table 18 B: Correlation between Nestin IRS expression and Her2 neu receptor status.

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
Her 2 Neu positive	4	4	6	0	14	3.93	0.142
Her 2 Neu negative	2	10	18	3	33		
Total	6	14	24	3	47		

11. NESTIN expression with Ki67 expression status:

Table 19 A, B illustrates the Correlation between Nestin expression and Ki67 expression status. The results show that: Ki67-low tumors tend to have weak IRS staining, whereas Ki67-high tumors are more likely to have moderate IRS staining.

The chi-square test shows that correlation between Nestin expression and Ki67 expression status is statistically significant (p-value = 0.0043).

Table 19 A: Correlation between Nestin positivity/negativity and Ki67 expression status

Ki67 Level	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Low	5	12	17 (36.17%)	0.0043*
Borderline	1	3	4 (8.51%)	
High	1	25	26 (55.32%)	

Table 19 B: Correlation between Nestin IRS expression and Ki67 expression status

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi – square value	p-value
Ki67 low	5	9	3	0	17 (36.17%)	18.92	0.0043*
Ki67 borderline	1	1	1	1	4 (8.51%)		
Ki67 high	1	5	19	1	26 (55.32%)		
Total	7	15	23	2	47		

(* indicates $p \leq 0.05$ was considered as statistically significant)

12. NESTIN expression with Luminal A / B status

Table 20 A, B illustrates the Correlation between Nestin expression and Luminal A / B status. The results show that: Luminal A tumors have significantly weak to moderate IRS scores, with most cases showing moderate nestin expression whereas, Luminal B tumors exhibit moderate to strong nestin expression.

The chi-square test shows that the correlation between Nestin expression and Luminal A / B is statistically significant (p-value = 0.0043).

Table 20 A: Correlation between Nestin positivity/negativity and Luminal A / B

Subtype	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Luminal A	2	13	15 (68.18%)	0.0125*
Luminal B	0	7	7 (31.82%)	

Table 20 B: Correlation between Nestin IRS expression and Luminal A / B

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square value	p-value
Luminal A	2	3	10	0	15 (68.18%)	9.60	0.0125*
Luminal B	0	1	2	4	7 (31.82%)		
Total	2	4	12	4	22		

(* indicates $p \leq 0.05$ was considered as statistically significant)

13. NESTIN expression with Triple Negative Breast Carcinoma (TNBC) status

Table 21 A, B illustrates the Correlation between Nestin expression and Triple Negative Breast Carcinoma status. The results show that: Most TNBC cases are in the IRS Strong category (14 out of 19 cases). Among non-TNBC cases, most are in moderate IRS category. TNBC cases are almost absent in lower IRS scores (Negative & Weak).

The chi-square test shows that the correlation between Nestin expression and Triple Negative Breast Carcinoma status is statistically significant (p-value = 0.0020).

Table 21 A: Correlation between Nestin positivity/negativity and Triple Negative Breast Carcinoma status

TNBC Status	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Positive	0	17	19 (40.43%)	0.0020*
Negative	2	26	28 (59.57%)	

Table 21 B: Correlation between Nestin IRS expression and Triple Negative Breast Carcinoma status

	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square	p- value
TNBC	0	1	4	14	19 (40.43%)	14.26	0.0020*
Non-TNBC	2	2	16	8	28 (59.57%)		
Total	2	3	20	22	47		

(* indicates $p \leq 0.05$ was considered as statistically significant)

14. NESTIN expression with Her2 neu enrichment status

Table 22 A, B illustrates the Correlation between Nestin expression and Her2 enrichment status. The results show that: Most Her2 enriched cases are in the IRS Strong category for Nestin expression.

The chi-square test shows that the correlation between Nestin expression and Her2-neu enriched status is statistically significant (p-value = 0.0022).

Table 22 A: Correlation between Nestin positivity/negativity and Her2 neu enrichment status

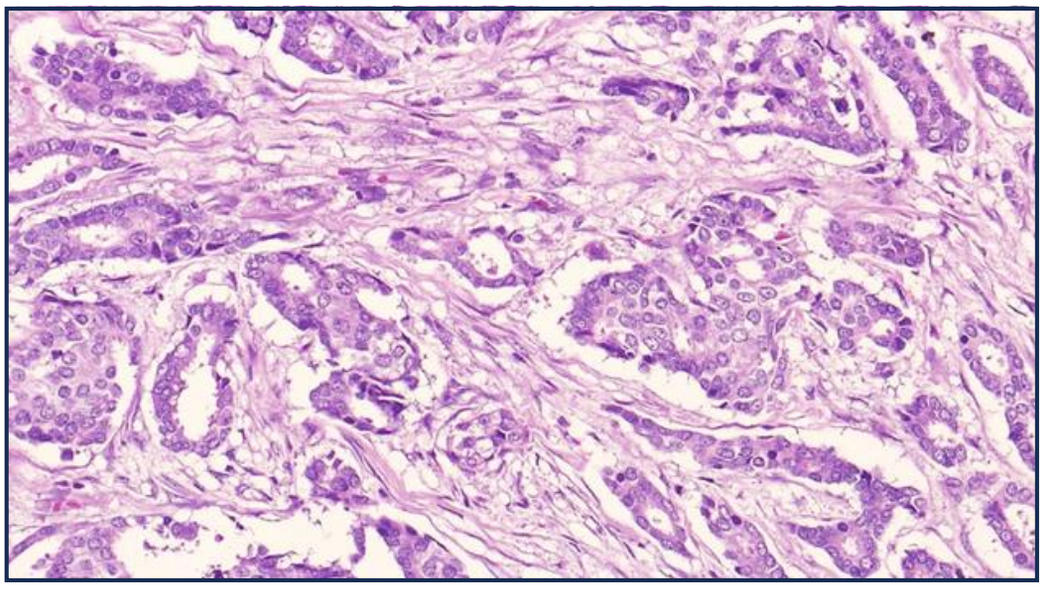
Her2 neu enrichment Status	**Nestin Negative**	**Nestin Positive**	**Total**	**p-value**
Her2 enriched	0	6	6 (40.43%)	0.0022*
Her2 non-enriched	18	29	41 (59.57%)	

Table 22 B: Correlation between Nestin IRS expression and Triple Negative Breast Carcinoma status

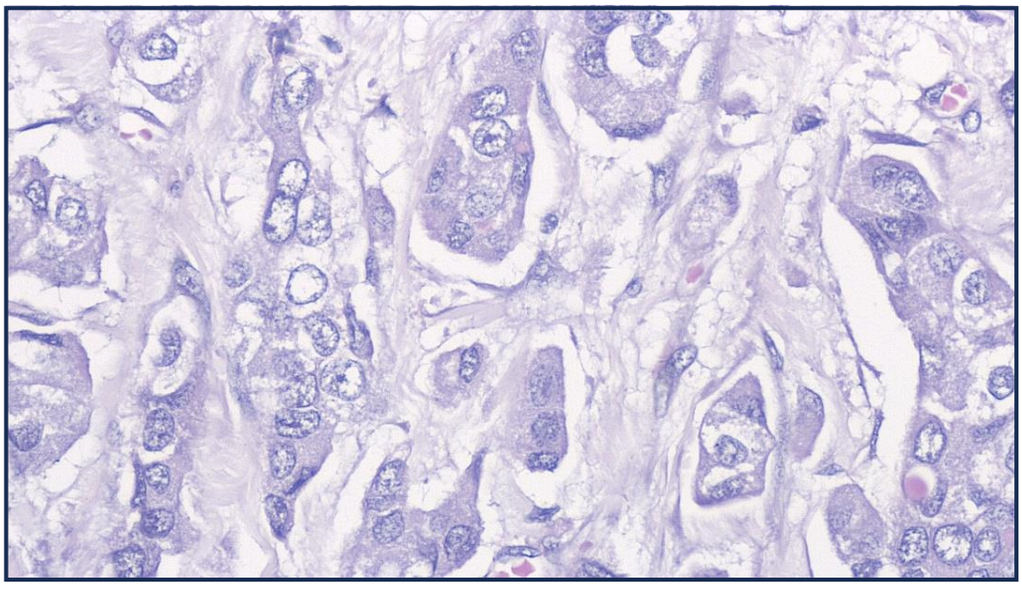
	IRS Negative	IRS Weak	IRS Moderate	IRS Strong	Total	Chi-square	p- value
Her 2 enriched	0	1	2	3	6 (12.77%)	14.26	0.0022*
Her 2 non-enriched	18	15	8	0	41 (87.23%)		
Total	18	16	10	3	47		

(* indicates $p \leq 0.05$ was considered as statistically significant)

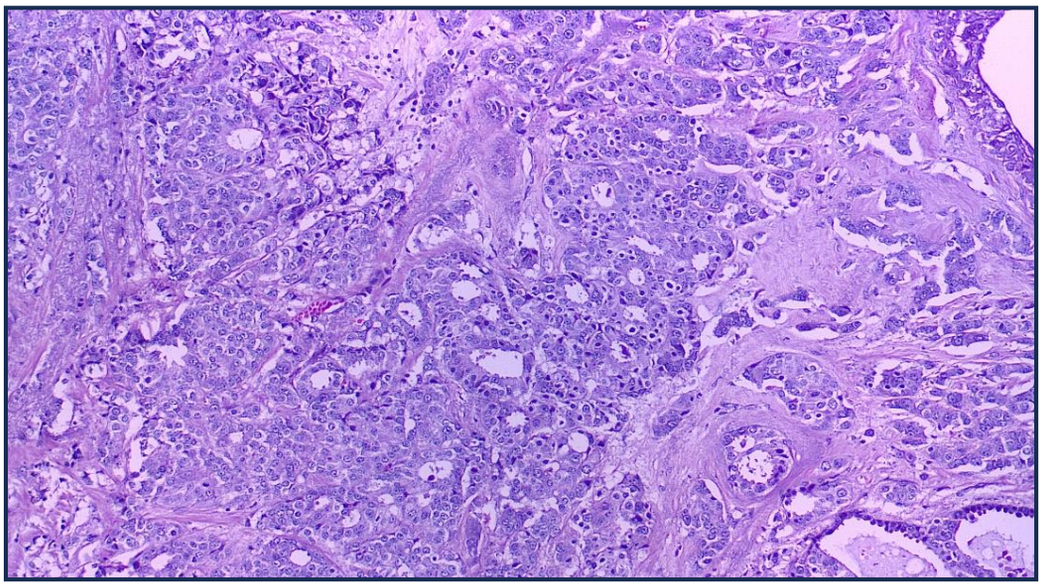
PHOTOMICROGRAPHS



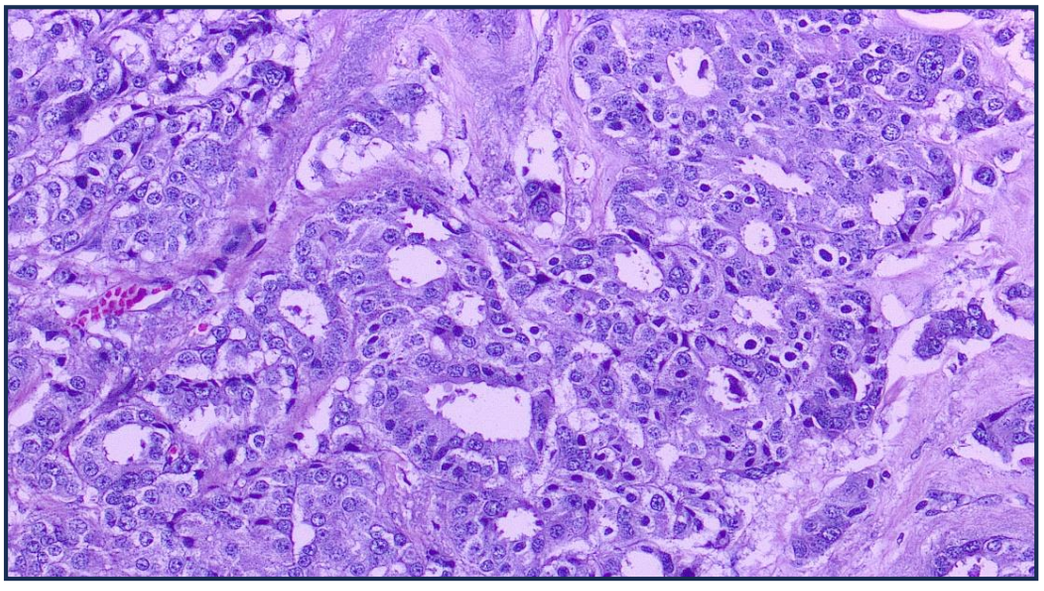
Photomicrograph 2: Grade-I Infiltrating Ductal Carcinoma of breast (H & E, 4x objective)



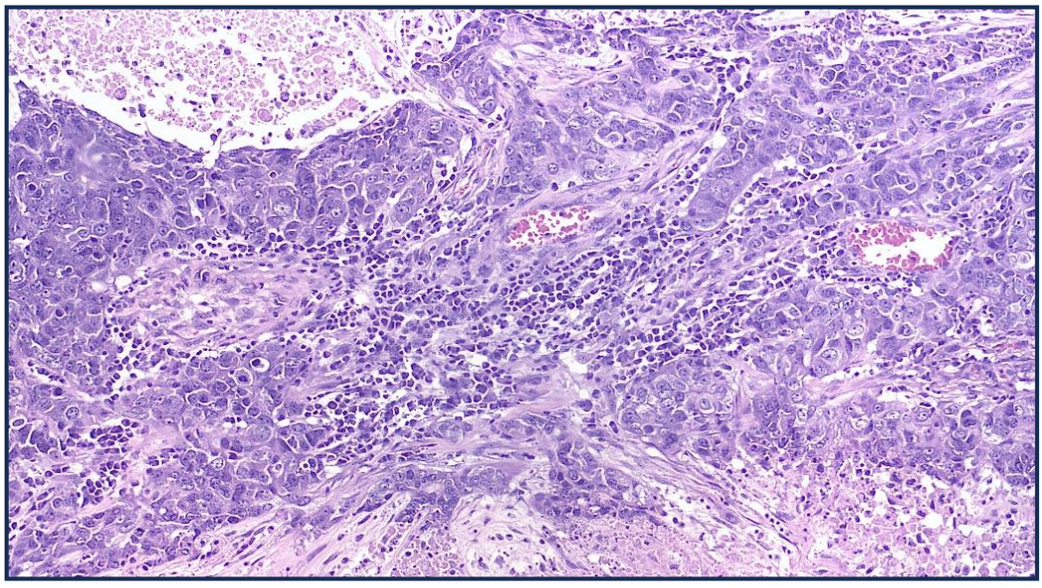
Photomicrograph 3: Grade-I Infiltrating Ductal Carcinoma of breast (H & E, 40x objective)



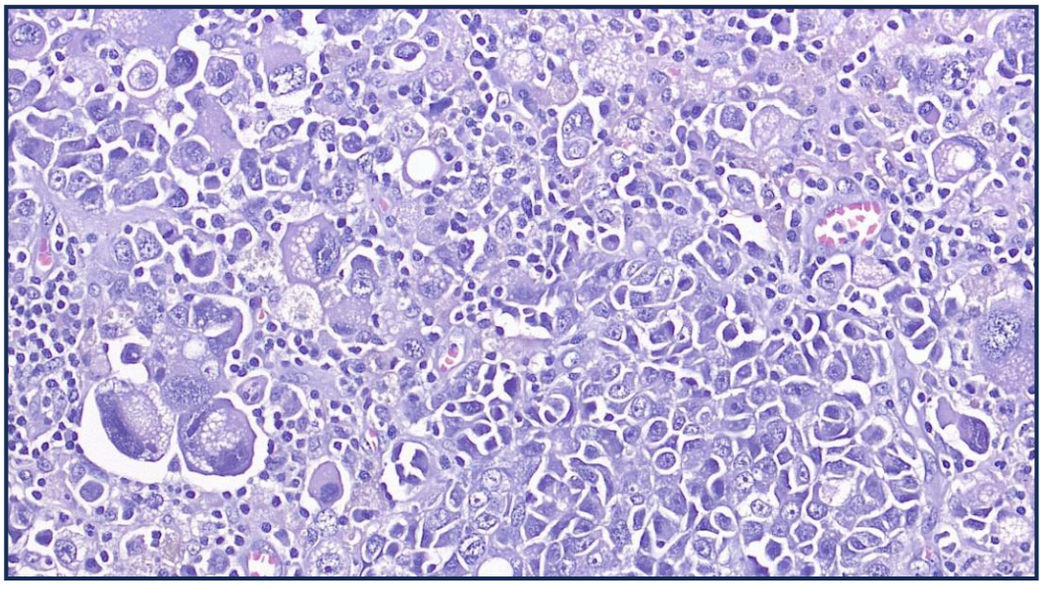
Photomicrograph 4: Grade-II Infiltrating Ductal Carcinoma of breast (H & E, 4x objective)



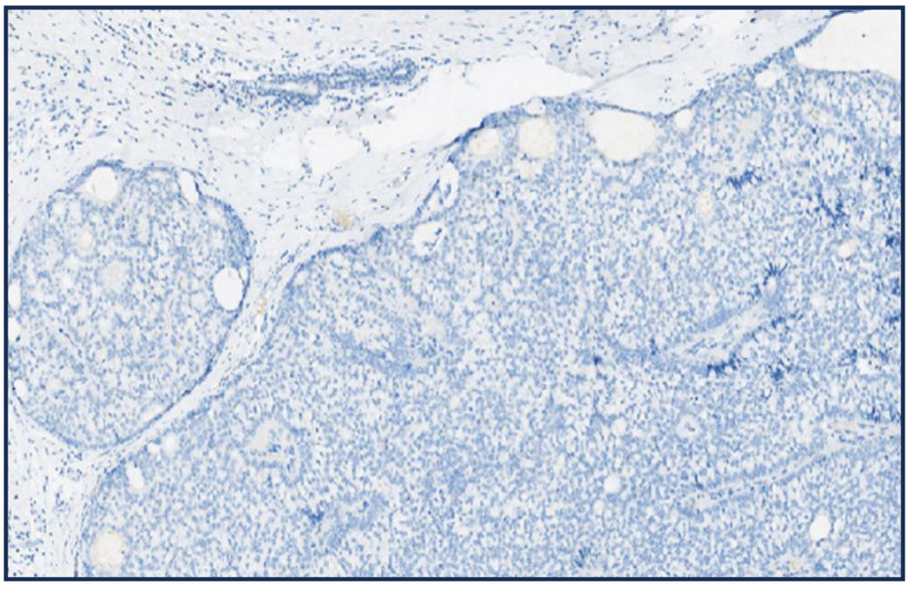
Photomicrograph 5: Grade-II Infiltrating Ductal Carcinoma of breast (H & E, 40x objective)



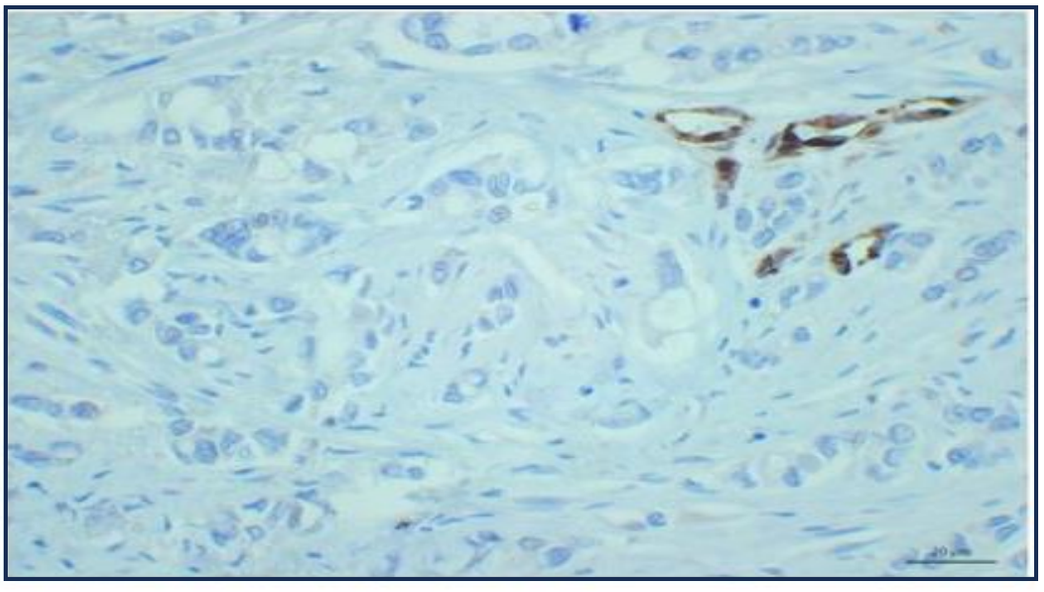
Photomicrograph 6: Grade-III Infiltrating Ductal Carcinoma of breast (H & E, 4x objective)



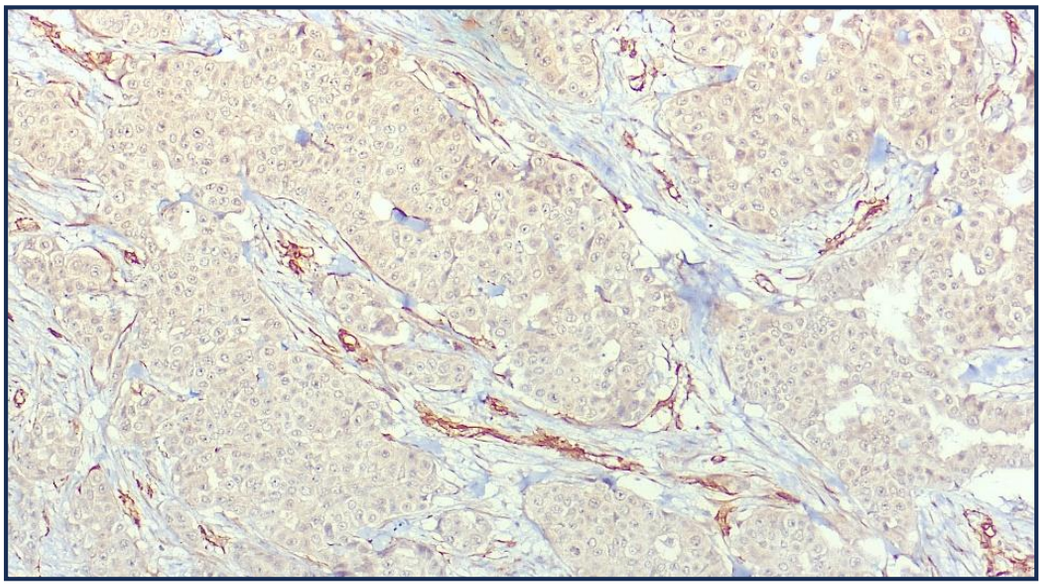
Photomicrograph 7: Grade-III Infiltrating Ductal Carcinoma of breast (H & E, 40x objective)



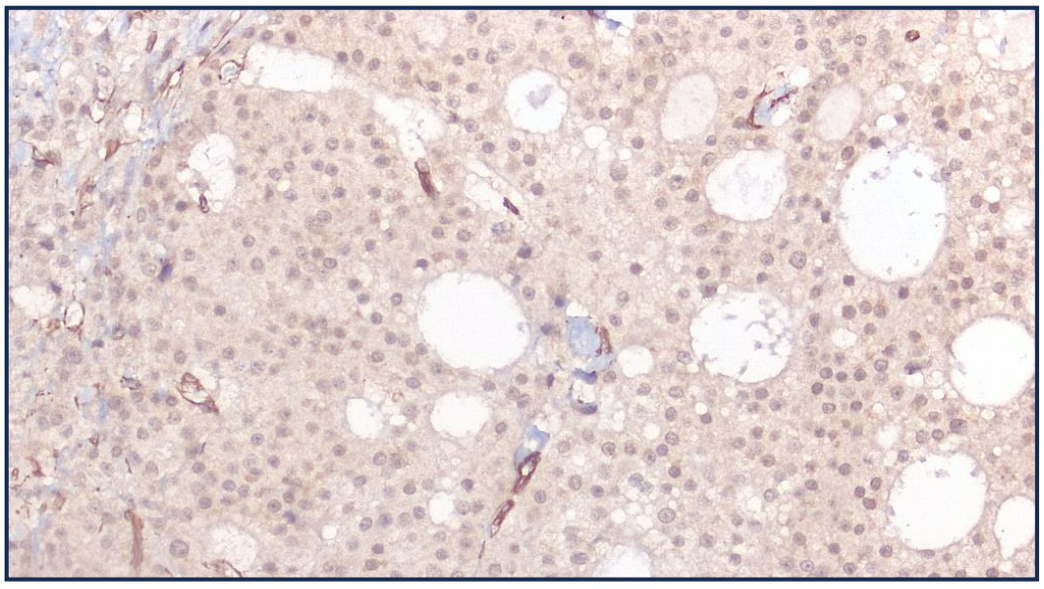
Photomicrograph 8: Grade-I Infiltrating Ductal Carcinoma of breast showing Nestin Negativity. [IRS Score- 0]. (IHC, 4x objective)



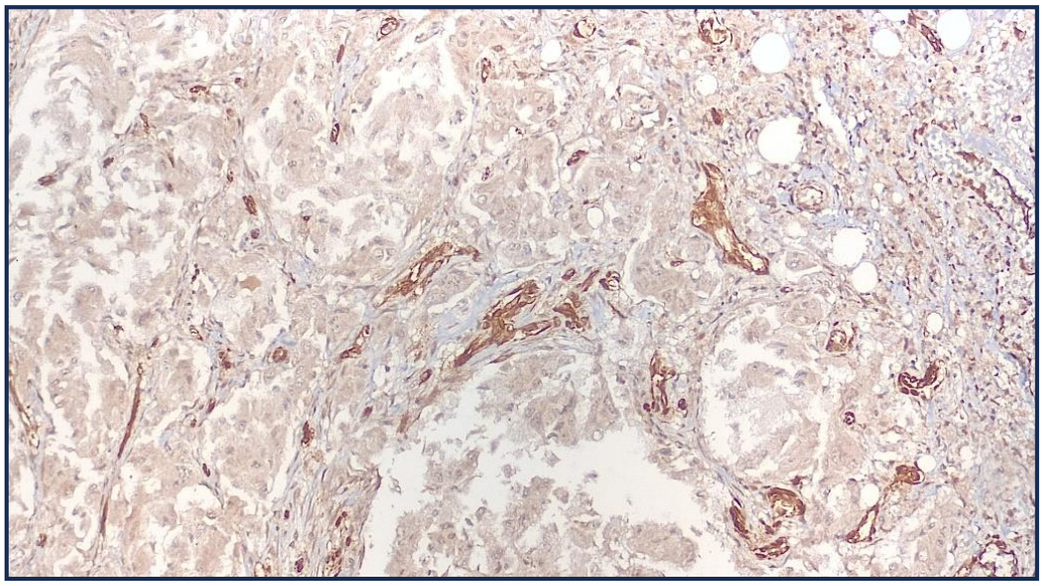
Photomicrograph 9: Grade-I Infiltrating Ductal Carcinoma of breast showing Nestin Negativity. [IRS Score- 0]. (IHC, 40x objective)



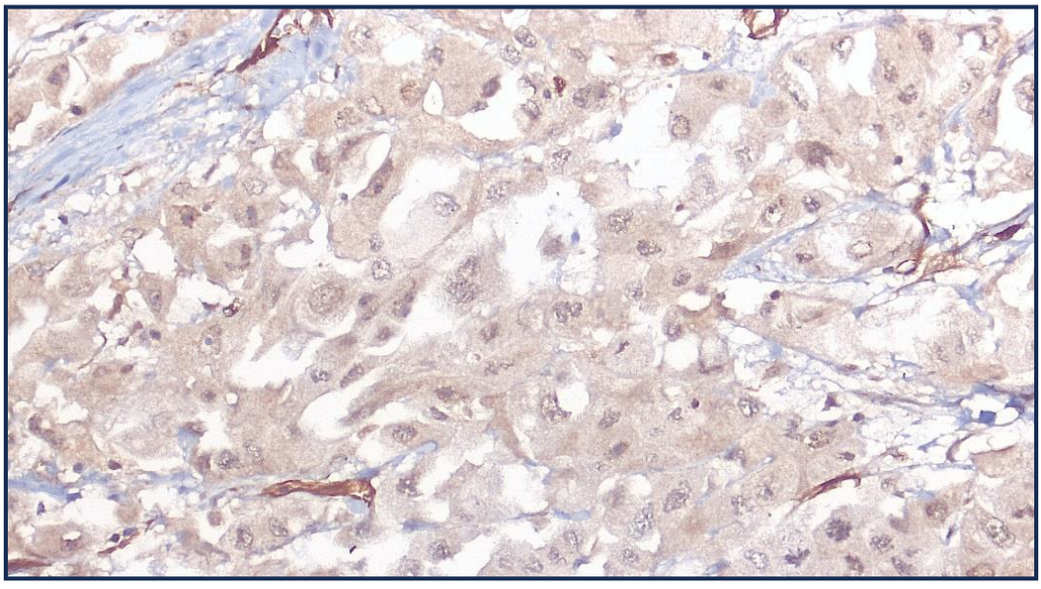
Photomicrograph 10: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Weakly positive [IRS Score- 2]. (IHC, 4x objective)



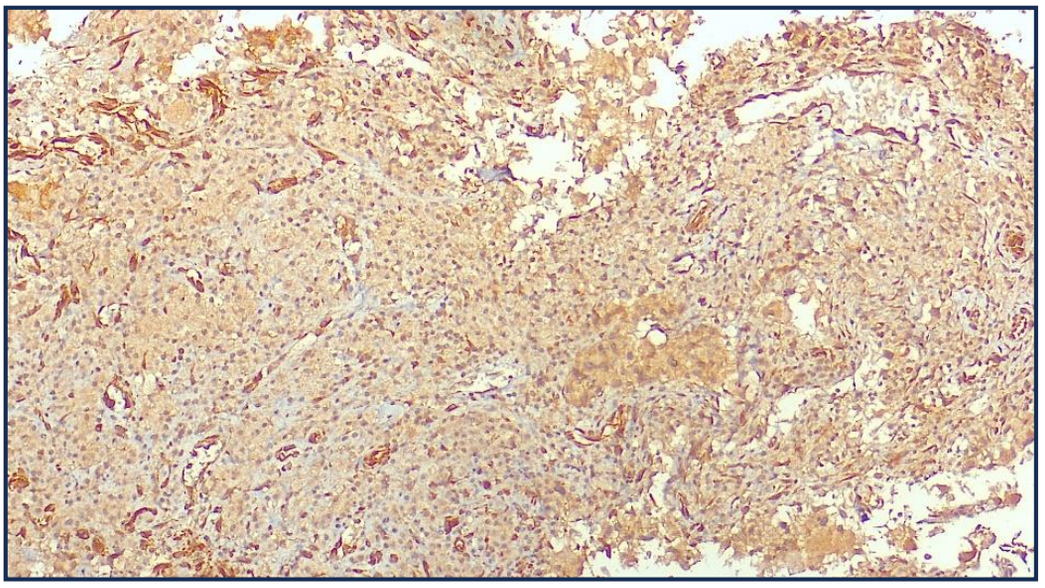
Photomicrograph 11: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Weakly positive. [IRS Score- 2]. (IHC, 40x objective)



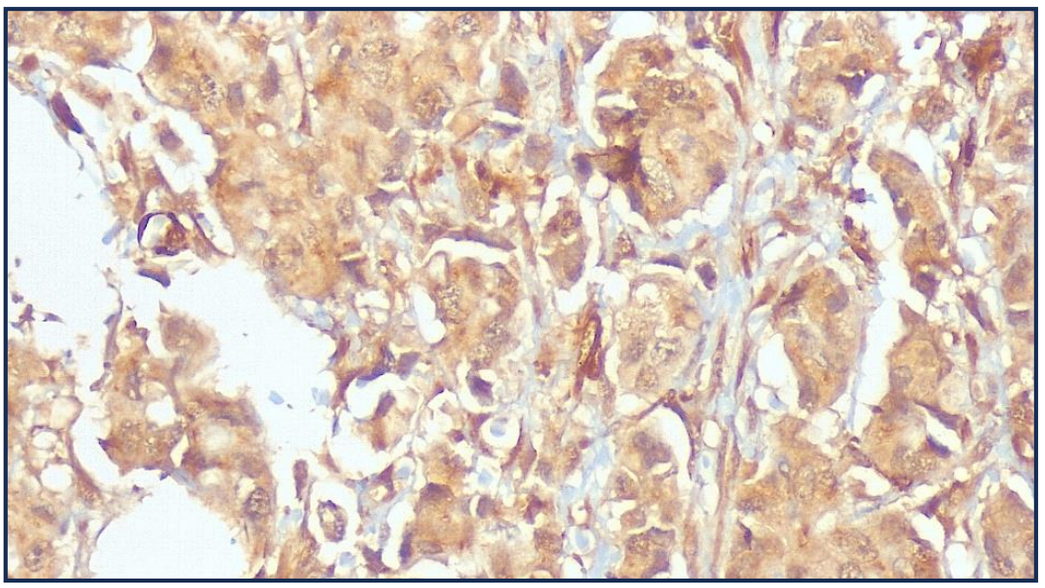
Photomicrograph 12: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Moderately positive. [IRS Score- 4]. (IHC, 4x objective)



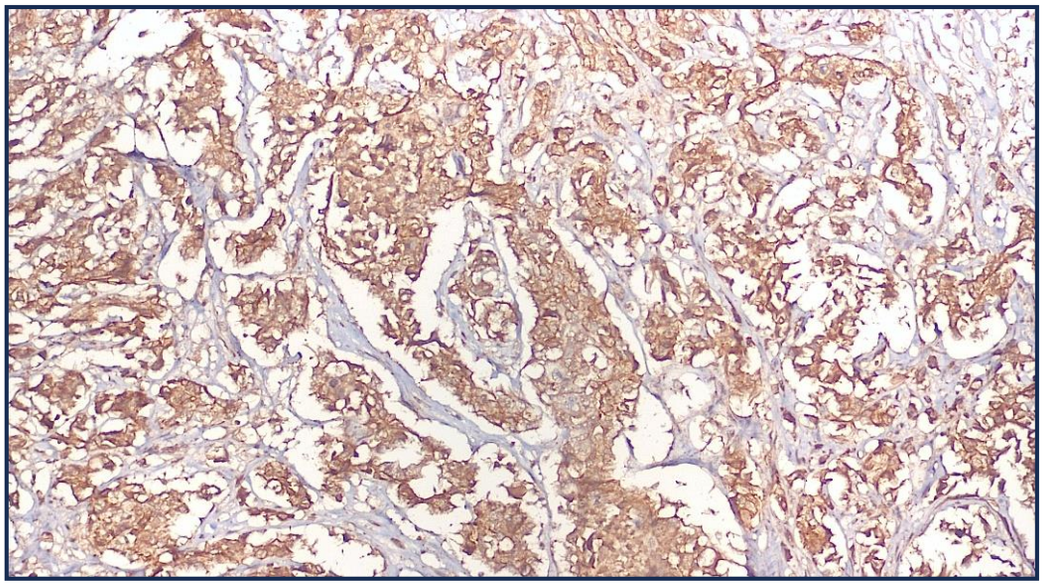
Photomicrograph 13: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Moderately positive. [IRS Score- 4]. (IHC, 40x objective)



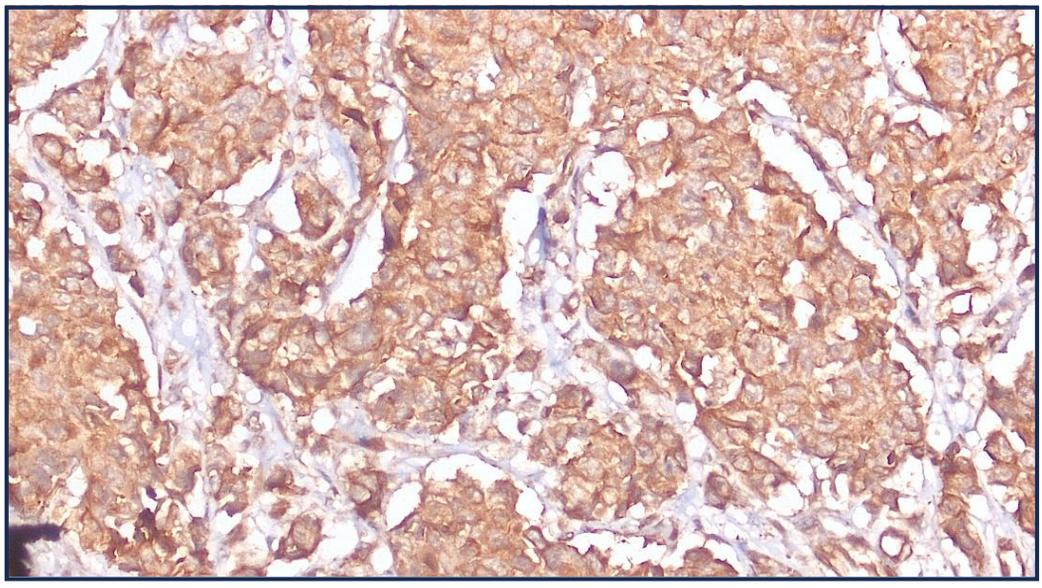
Photomicrograph 14: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Moderately positive. [IRS Score- 6]. (IHC, 4x objective)



Photomicrograph 15: Grade-II Infiltrating Ductal Carcinoma of breast showing Nestin Moderately positive. [IRS Score- 6]. (IHC, 40x objective)



Photomicrograph 16: Grade-III Infiltrating Ductal Carcinoma of breast showing Nestin Strongly positive. [IRS Score- 9]. (IHC, 4x objective)



Photomicrograph 17: Grade-III Infiltrating Ductal Carcinoma of breast showing Nestin Strongly positive. [IRS Score- 9]. (IHC, 40x objective)

DISCUSSION

The aim of this study is to comprehensively evaluate nestin expression in breast cancer and to determine its correlation with histopathological grading and other clinicopathological parameters. By employing a hospital-based cross-sectional design, the study seeks to explore whether nestin - a marker associated with stem cell characteristics and tumor aggressiveness - can serve as a reliable prognostic indicator in breast cancer. The research investigates various dimensions including patient demographics, tumor size, histological grade, receptor status, and invasive features such as lymphovascular and perineural invasion. In doing so, it aims to integrate traditional histopathological evaluation with modern molecular profiling to provide a more nuanced understanding of tumor biology. By correlating nestin expression with key pathological features, the study may help differentiate aggressive tumors from those with a more indolent course, thereby guiding clinicians in selecting appropriate therapeutic interventions. It emphasizes the need to move beyond conventional histological grading alone, suggesting that the incorporation of molecular markers such as nestin may lead to more accurate predictions of patient outcomes and improved clinical management strategies.

Age Distribution

Our study demonstrates a mean patient age of approximately 46.5 years, with the highest incidence in the 41–50 age group (40.40%), followed by the 51–60 age group (19.20%). This age profile is indicative of the well-documented trend that breast cancer predominantly affects middle-aged women. The preponderance of cases in the 41–50 age bracket may reflect a complex interplay between hormonal changes, lifestyle factors, and genetic predispositions that become more prominent as women approach menopause. For instance, Kolia et al. (2024) [75] observed that the majority of breast cancer patients in their cohort were within a similar age range, emphasizing the role of hormonal fluctuations and the transition to menopause in the pathogenesis of the disease. In addition, previous meta-analyses have indicated that breast cancer in younger to middle-aged populations often exhibits distinct molecular profiles, which may have implications for the aggressiveness of the tumor and the expression of biomarkers such as nestin.[77] The age distribution observed in our study aligns with these findings and suggests that factors such as the cumulative exposure to estrogen and the resulting cellular proliferation could be pivotal in tumor development. Overall, the age pattern in our cohort not only provides essential demographic context but also serves as a foundation for understanding the biological behaviour of tumors in different age groups. [75,77]

Laterality

Our analysis reveals that 59.62% of tumors are located in the left breast, while 40.38% are in the right. This left-sided predominance, though modest, is consistent with observations from several epidemiological studies that suggest a slight asymmetry in breast cancer occurrence. The underlying causes of this asymmetry remain a topic of ongoing investigation; some hypotheses propose that anatomical differences, variations in breast tissue density, or even differences in exposure to endogenous hormones may contribute to the higher incidence on the left side. Although specific molecular studies directly linking laterality to nestin expression are sparse, the trend observed in our study is in harmony with previous reports that have noted a similar left-sided predominance in breast cancer cases.[74,76] This asymmetry may also be reflective of inherent developmental differences between the left and right breasts, where subtle variations in the number of terminal duct lobular units could predispose one side to a higher risk of malignant transformation. In addition, environmental and lifestyle factors might interact with these anatomical predispositions, thereby accentuating the observed differences in laterality. While the exact mechanisms underlying left-sided predominance remain unclear, our findings support the notion that breast cancer exhibits subtle asymmetries that warrant further investigation, particularly in relation to biomarker expression and tumor biology. [74,76]

Tumor Quadrant Distribution

The distribution of tumors by breast quadrant in our study reveals that 50% of lesions are localized to the upper outer quadrant (UOQ), followed by 19.20% in the central quadrant, 15.40% in the lateral outer quadrant (LOQ), 3.80% in the lateral inferior quadrant (LIQ), and 5.80% each in the upper inner quadrant (UIQ) and in multicentric locations. The predominance of tumors in the UOQ is a well-established finding in breast cancer literature, often attributed to the higher volume of glandular tissue in this region. The UOQ contains more terminal duct lobular units, which may increase its susceptibility to malignant transformation. This observation aligns with previous studies that have documented a similar quadrant distribution, reinforcing the concept that anatomical and physiological factors play a significant role in tumor localization. [77,78] Moreover, the higher incidence of UOQ tumors may have diagnostic and therapeutic implications; for instance, tumors in this quadrant are often more readily detected on screening mammography due to their location and tissue density. The relatively lower incidence of tumors in the LIQ and UIQ could be explained by the comparatively reduced glandular tissue in these areas, which is consistent with the findings of earlier investigations.

The consistency of these findings with prior research further validates the observed patterns in tumor distribution. [77,78]

Menopausal Status Distribution

The analysis of menopausal status reveals that 61.54% of the study population is postmenopausal, whereas 38.46% is premenopausal. This distribution is clinically significant because menopausal status can impact tumor biology, hormone receptor expression, and treatment response. In our cohort, the predominance of postmenopausal patients may influence the molecular profile of tumors, as suggested by Kolia et al. (2024), [75] who reported a higher proportion of ER-positive tumors in postmenopausal women. Although Kolia et al. (2024) [75] did not observe a significant correlation between serum nestin levels and receptor status, tissue-based assessments like ours can reveal subtle differences in biomarker expression influenced by hormonal milieu. Additionally, Nowak et al. (2017) [74] demonstrated that nestin expression in breast tumor cells is linked with aggressive features, particularly in high-grade cases, which are often observed in older, postmenopausal patients. These findings support the hypothesis that hormonal changes following menopause may create a microenvironment that favours nestin upregulation and contributes to tumor aggressiveness. Overall, the postmenopausal predominance in our study underscores the need to consider hormonal status when evaluating nestin as a prognostic biomarker, especially since its expression appears to correlate with more aggressive tumor behavior. [74,75]

Perineural Invasion (PNI) Status Distribution

The evaluation of perineural invasion (PNI) indicates that 77% of cases exhibit PNI, while 23% do not. PNI is recognized as a marker of aggressive tumor behavior, suggesting a higher propensity for local spread along nerve fibres. In our study, the high incidence of PNI supports the overall aggressive profile of the cohort. Although our chi-square analysis did not reveal a statistically significant correlation between nestin expression and PNI status ($p = 0.2448$), the high frequency of PNI remains notable. Krüger et al. (2013) [76] have previously established a strong link between nestin expression and aggressive, basal-like tumors, which frequently demonstrate neural invasion. Similarly, Shaban and El-Goday (2016) [78] reported that high nestin levels are prominent in triple-negative breast cancers, a subgroup known for multiple invasive features including PNI. These earlier observations imply that while our data do not show a direct statistical association, the high rate of PNI may be indirectly related to increased nestin expression. The trend in our findings—where tumors with PNI tend to have higher immunoreactivity scores—suggests that nestin might contribute to invasive mechanisms that

facilitate neural spread. Overall, although our current sample did not yield a statistically significant result, the high prevalence of PNI is consistent with the aggressive phenotype associated with elevated nestin expression as described in previous studies. [76,78]

Lymphovascular Invasion (LVI) Status Distribution

Our findings show that 86.50% of cases are positive for lymphovascular invasion (LVI) compared to 13.50% without LVI, indicating a high propensity for vascular dissemination—a well-established marker of aggressive tumor behavior. This high incidence aligns with the aggressive profile observed in our cohort. Nowak et al. (2017) [74] highlighted that nestin is expressed in both tumor cells and newly forming vessels, with significant correlations to markers such as CD31 and CD34 ($p = 0.002$ and $p = 0.015$, respectively), implying that nestin-positive micro vessels might facilitate vascular invasion. Furthermore, Tampaki et al. (2017) [81] found that nestin expression was linked with increased vascular invasion and higher recurrence rates. Although our direct analysis of nestin versus LVI did not achieve statistical significance ($p = 0.935$), the overall high rate of LVI remains critical in understanding tumor aggressiveness. It is possible that sample size limitations may obscure a more subtle correlation between nestin expression and LVI. Nevertheless, the biological role of nestin in promoting angiogenesis may contribute to the formation of vascular channels that allow tumor cells to disseminate. These mechanisms are supported by literature emphasizing nestin as a marker for poor prognosis and aggressive vascular proliferation. [74,81]

Expression of Nestin Distribution

The expression pattern of nestin in our study shows heterogeneity, with 11.54% of cases negative, 32.69% weak, 44.23% moderate, and 11.54% strong. This diverse distribution underscores the variable role of nestin in breast cancer biology. The predominance of moderate expression in nearly half of the cases suggests that nestin is expressed at clinically relevant levels in a substantial proportion of tumors, potentially reflecting a mixture of cells with varying degrees of invasive characteristics. Nowak et al. (2017) [74] observed that nestin positivity was strongly associated with high-grade tumors, particularly in triple-negative subtypes, thereby supporting the notion that nestin is a marker of tumor aggressiveness. Similarly, Shaban and El-Goday (2016) [78] demonstrated that triple-negative cancers exhibit significantly higher nestin levels ($p < 0.001$), underscoring its role as an indicator of poor prognosis. Additionally, Zhang et al. (2020) [77] conducted a meta-analysis revealing that high nestin expression correlates with increased Ki-67 proliferation indices and reduced overall

survival. Although a subset of tumors in our study shows negative nestin expression, the overall prevalence of positivity—particularly in moderate and strong categories—supports its involvement in tumor progression. The observed heterogeneity may reflect differences in tumor microenvironments, genetic profiles, or stages of tumor evolution. These findings collectively highlight the potential utility of nestin as a marker for aggressive tumor subsets and as a target for therapeutic intervention. Further research is needed to clarify the molecular mechanisms underlying the differential expression of nestin and to explore its clinical applications in risk stratification and treatment planning. [74,77,78]

Distribution of Intensity Score in Nestin Expressing Cells

The assessment of immunohistochemical intensity scores for nestin reveals that lower-grade tumors generally exhibit lower intensity, while higher-grade tumors tend to show increased staining intensity. In our analysis, well-differentiated tumors predominantly had low to moderate intensity scores, whereas poorly differentiated tumors, particularly those classified as high-grade, showed a tendency toward higher intensity scores. Although the intensity scores alone did not reach statistical significance ($p = 0.277$), the observed trend is consistent with previous findings. Nowak et al. (2017) (74) reported a significant association between high nestin intensity and grade 3 tumors ($p = 0.001$), suggesting that stronger immunostaining is indicative of more aggressive tumor behavior. In a similar vein, Krüger et al. (2013) [76] found that high nestin expression correlates with increased proliferation markers such as Ki-67, reinforcing the idea that intensity may reflect the underlying biology of tumor aggressiveness. Shaban and El-Goday (2016) [78] also noted that triple-negative breast cancers—typically high-grade tumors—exhibited significantly elevated nestin intensity. Although our individual intensity data did not achieve statistical significance, possibly due to sample size limitations or interobserver variability, the trend supports the notion that higher nestin expression is associated with more aggressive tumors. Combining intensity with other parameters, such as percentage of positive cells, may provide a more comprehensive view of nestin's role in tumor progression. Overall, our findings contribute to the growing body of evidence that nestin intensity, as part of a multifaceted scoring system, can serve as an important indicator of tumor differentiation and aggressiveness. [74,76,78]

Distribution of Percentage Score in Nestin Expressing Cells

The percentage score, representing the proportion of tumor cells expressing nestin, provides insight into the overall burden of nestin-positive cells within a tumor. In our study, lower-grade tumors tended to have a smaller percentage of nestin-positive cells, while higher-grade tumors showed a higher proportion of positivity. Specifically, the data indicate that a greater percentage of cells in Grade 2 and Grade 3 tumors express nestin compared to Grade 1 tumors, although the chi-square test did not achieve statistical significance ($p = 0.593$). This trend is in line with findings by Zhang et al. (2020), [77] who observed that higher nestin expression is significantly associated with aggressive tumor characteristics, including increased cellular proliferation and reduced survival. Similarly, Krüger et al. (2013) [76] demonstrated that basal-like and triple-negative tumors, which are typically high-grade, display a higher fraction of nestin-positive cells. The increasing percentage score with higher grade suggests that as tumor differentiation decreases, the proportion of cells exhibiting stem-like features, marked by nestin, increases. Although our study did not find a statistically significant difference when considering the percentage score alone, the observed trend supports the hypothesis that nestin may contribute to tumor aggressiveness. The integration of the percentage score with intensity data provides a more robust parameter, as seen in the overall immunoreactive score (IRS), which does show a significant correlation with tumor grade. These findings highlight the potential of the percentage of nestin-expressing cells as a surrogate marker for tumor aggressiveness and underscore the importance of evaluating both qualitative and quantitative aspects of protein expression. [74,76,77]

Distribution of Immunoreactive Score (IRS) in Nestin Expressing Cells

The combined immunoreactive score (IRS) integrates both intensity and percentage of nestin-positive cells, offering a more comprehensive evaluation of nestin expression. In our study, the IRS clearly stratifies tumors by histopathological grade: lower-grade tumors predominantly exhibit negative or weak IRS, while higher-grade tumors tend to show moderate to strong IRS. The statistically significant association ($p = 0.0025$) between IRS and tumor grade underscores that increased nestin expression correlates with poorer differentiation and more aggressive behavior. Nowak et al. (2017) [74] reported a similar finding where high nestin IRS values were strongly linked to grade 3 tumors, as well as with markers of increased proliferation and worse clinical outcomes. Krüger et al. (2013) [76] also noted that elevated nestin IRS was associated with basal-like phenotypes and higher Ki-67 indices, further supporting its prognostic relevance. Shaban and El-Goday (2016) [78] observed that triple-negative breast cancers, which

often correspond to high-grade tumors, consistently exhibit elevated nestin levels as measured by IRS. The integrated nature of the IRS system makes it a particularly valuable tool, as it captures both the intensity of staining and the extent of positive cells. Our findings suggest that the IRS could be used as an independent prognostic marker, helping to stratify patients according to risk and guide therapeutic decision-making. The significant correlation between IRS and histopathological grade in our study supports the potential clinical utility of nestin in breast cancer, particularly for identifying patients with aggressive tumor phenotypes. [74,76,78]

Expression of Nestin with Menopausal Status

The analysis of nestin expression in relation to menopausal status reveals distinct patterns: premenopausal patients (20 cases) predominantly exhibit negative to moderate immunore activity, while postmenopausal patients (32 cases) tend to display a higher proportion of weak and moderate expression, with a few cases showing strong positivity. The chi-square test indicates a statistically significant difference ($p = 0.0031$), suggesting that menopausal status influences nestin expression. Kolia et al. (2024) [75] reported that although serum nestin levels did not correlate with receptor status, tissue-based measurements might reveal differential expression patterns influenced by the hormonal milieu. In line with this, Nowak et al. (2017) [74] observed that higher nestin expression is often associated with aggressive tumor phenotypes, which are more frequently seen in older, postmenopausal women. The lack of strong nestin expression in premenopausal patients might indicate a relatively less aggressive tumor biology, whereas the increased expression in postmenopausal women could reflect a hormonal environment that favours the activation of stem cell-like pathways. This differential expression pattern supports the hypothesis that nestin could serve as a useful biomarker for risk stratification, particularly in postmenopausal patients who may have a predisposition for more aggressive disease. Overall, these findings highlight the importance of incorporating menopausal status into the evaluation of nestin expression, as it may provide additional prognostic information and guide more personalized treatment strategies in breast cancer management [74, 75].

Expression of Nestin with Lymphovascular Invasion (LVI) Status

The investigation into nestin expression relative to lymphovascular invasion (LVI) shows that tumors with LVI (45 cases) have a varied immunoreactive score (IRS) distribution, with a trend toward higher scores compared to tumors without LVI (7 cases). Although the chi-square

analysis did not reach statistical significance ($p = 0.935$), the overall pattern suggests that higher nestin expression may be more common in tumors exhibiting LVI. Nowak et al. (2017) [74, 79] demonstrated that nestin-positive microvessel density is significantly associated with advanced disease and higher histological grades, indicating that nestin may contribute to an angiogenic microenvironment conducive to vascular invasion. Tampaki et al. (2017) [81] also found that nestin expression is linked with aggressive vascular features and higher recurrence rates. The lack of a statistically significant difference in our study could be due to sample size limitations or interobserver variability. Nevertheless, the trend toward higher IRS scores in LVI-positive tumors is consistent with the notion that nestin may facilitate the formation of vascular channels through which tumor cells can disseminate. While our data do not definitively establish a direct relationship between nestin expression and LVI, they do align with previous findings that emphasize nestin's role in promoting angiogenesis and tumor invasiveness. Further research with larger cohorts is needed to clarify this relationship and to determine whether nestin could serve as a reliable prognostic marker for vascular invasion in breast cancer. [74, 79, 81]

Expression of Nestin with Perineural Invasion (PNI) Status

The assessment of nestin expression with regard to perineural invasion (PNI) indicates that tumors exhibiting PNI (46 cases) have a slightly higher immunoreactive score compared to those without PNI (6 cases), although the difference is not statistically significant ($p = 0.2448$). This suggests that while nestin is involved in aggressive tumor biology, its expression may not be directly correlated with neural invasion. Krüger et al. (2013) [76] have previously demonstrated that nestin is associated with aggressive, basal-like tumors, yet the specific link between nestin and PNI has been less clear. Shaban and El-Goday (2016) [78] similarly noted that although high nestin expression is frequently observed in triple-negative breast cancers—often characterized by various invasive features—a direct association with perineural invasion was not explicitly established. In our study, tumors with PNI tend to show higher IRS scores, suggesting a potential trend that may become statistically significant in larger cohorts. The biological rationale for a relationship between nestin and PNI could be related to nestin’s role in facilitating tumor cell migration and invasion. Despite the non-significant p-value, the trend observed is consistent with the overall literature that positions nestin as a marker of invasive and aggressive tumor behavior. [76, 78]

Expression of Nestin with DCIS component in IDC breast

The correlation between nestin expression and the presence of a ductal carcinoma in situ (DCIS) component in IDC breast shows that tumors with DCIS (24 cases) tend to have higher nestin expression compared to those without DCIS (28 cases). The chi-square analysis reveals a statistically significant difference ($p = 0.0012$), indicating that the presence of DCIS is associated with elevated nestin levels. This finding suggests that even early in the process of tumor invasion, as represented by DCIS, there may be activation of pathways associated with stemness and aggressive behavior. Nowak et al. (2017) [74] observed that nestin expression is strongly correlated with high-grade tumors and aggressive phenotypes, which may also extend to tumors with an in situ component. Zhang et al. (2020) [77] further demonstrated that high nestin expression is linked to poor survival outcomes, implying that tumors with DCIS and elevated nestin may be predisposed to a more aggressive clinical course. Shaban and El-Goday (2016) (78) similarly found that high nestin levels are frequently observed in triple-negative cases, which can sometimes coexist with DCIS. The statistically significant association in our study suggests that nestin could serve as a one of the factors for aggressiveness of DCIS component within IDC breast. [74, 77, 78]

Correlation between Nestin Expression and SBR Grade

Our data indicate a strong correlation between nestin expression and histopathological grade, as determined by the Scarff Bloom Richardson (SBR) system. Lower-grade tumors predominantly exhibit negative or weak nestin expression, whereas higher-grade tumors show increased positivity with more cases demonstrating moderate to strong expression. The statistical analysis reveals a significant association ($p = 0.0042$), confirming that elevated nestin expression is linked to poor differentiation and aggressive tumor behavior. Nowak et al. (2017) [74] similarly found that high nestin expression was strongly correlated with grade 3 tumors and was associated with higher Ki-67 indices and poorer survival outcomes. Krüger et al. (2013) [76] also reported that 78% of high-grade tumors were nestin-positive, suggesting that nestin may be a reliable marker for dedifferentiation and aggressive biology. Shaban and El-Goday (2016) [78] further demonstrated that triple-negative breast cancers, which are typically high-grade, exhibit significantly elevated nestin levels. The progressive increase in nestin positivity from low- to high-grade tumors in our study supports the concept that nestin expression reflects the biological aggressiveness of the tumor. The significant difference in immunoreactive scores across different SBR grades underscores the potential of nestin as a prognostic biomarker. This information could be pivotal for patient stratification, as higher nestin expression may identify

individuals who require more aggressive therapeutic interventions. Future studies should investigate the mechanistic pathways by which nestin contributes to tumor dedifferentiation and explore its potential as a therapeutic target in high-grade breast cancers. [74, 76, 78]

Correlation between Nestin Expression and Tumor Size

Our analysis of the relationship between nestin expression and tumor size categorizes tumors as pT1, pT2, and pT3, with the majority being pT2 (75%). Despite the predominance of intermediate-sized tumors, the distribution of nestin expression does not vary significantly across these categories, as indicated by a chi-square p-value of 0.786. This suggests that nestin expression may be more reflective of intrinsic tumor aggressiveness rather than the physical dimensions of the tumor. In contrast, Zhang et al. (2020) [77] reported that high nestin expression was significantly associated with larger tumor size ($p = 0.002$), highlighting a potential discrepancy that could be due to sample size differences, tumor heterogeneity, or methodological variations. Nowak et al. (2017) [74] have also emphasized that nestin's prognostic impact is more closely related to histological grade and proliferative activity rather than mere tumor size. The lack of a significant correlation in our study suggests that while larger tumors might harbour more aggressive subclones, nestin expression itself does not necessarily increase with tumor size. This observation reinforces the idea that nestin's role in tumor progression is independent of bulk tumor dimensions and is more intimately linked with biological aggressiveness and the tumour's microenvironment. [74, 77]

Correlation between Nestin Expression and Lymph Node Involvement

Our findings demonstrate a significant correlation between nestin expression and lymph node involvement. Patients with lymph node metastasis exhibit a higher immunoreactive score (IRS) compared to those without nodal involvement, with statistical significance achieved ($p = 0.0131$). The trend observed indicates that tumors with advanced nodal status (pN2 and pN3) are more likely to display moderate to strong nestin expression. Nowak et al. (2017) [74] reported that high nestin expression was linked to advanced tumor stage and poorer overall survival, suggesting that nestin may play a role in facilitating metastatic spread. In addition, Krüger et al. (2017) [80] noted that aggressive tumor markers, including nestin, are more frequently expressed in cases with extensive lymphatic involvement, supporting our findings. Although Zhang et al. (2020) [77] found no statistically significant association between nestin expression and lymph node metastasis ($p = 0.08$) in their meta-analysis, the trend in our study suggests that higher nestin levels may be predictive of nodal spread in a subset of patients. The

observed increase in nestin expression with higher nodal involvement may reflect its role in promoting cell motility and invasiveness, contributing to the metastatic process. This significant association reinforces the potential clinical utility of nestin as a prognostic biomarker, particularly in identifying patients at higher risk for nodal metastasis. Future research with larger sample sizes and longitudinal follow-up is warranted to further elucidate the mechanisms by which nestin influences lymphatic spread and to validate its role as a predictor of metastatic potential in breast cancer. [74, 77, 80]

Correlation between Nestin Expression and Estrogen Receptor (ER) Status

The evaluation of nestin expression in relation to estrogen receptor (ER) status reveals significant differences in immunoreactivity. In our study, ER-positive tumors exhibit a distinct IRS distribution compared to ER-negative tumors, with the latter showing a trend toward higher nestin expression. This association is statistically significant ($p = 0.0244$) and indicates that nestin expression is inversely correlated with ER positivity. Zhang et al. (2020) [77] reported a strong inverse relationship between nestin expression and ER positivity ($p < 0.001$), suggesting that tumors lacking ER expression are more likely to exhibit aggressive characteristics and higher levels of nestin. Shaban and El-Goday (2016) [78] further demonstrated that triple-negative breast cancers, which are by definition ER-negative, consistently show elevated nestin levels, reinforcing the connection between ER negativity and an aggressive tumor phenotype. The differential expression pattern observed in our study may reflect underlying biological differences, where ER-negative tumors often adopt a basal-like phenotype characterized by enhanced proliferation and invasiveness. These findings support the potential utility of nestin as a complementary biomarker in the immunohistochemical evaluation of breast cancer, particularly in cases where hormone receptor status suggests a higher risk profile. Incorporating nestin assessment alongside conventional receptor panels may improve prognostic accuracy and assist in the stratification of patients for targeted therapies. Future studies should further investigate the mechanistic links between ER signaling and nestin expression to determine whether modulation of nestin could offer therapeutic benefits for ER-negative breast cancers. [77, 78]

Correlation between Nestin Expression and Progesterone Receptor (PR) Status

The relationship between nestin expression and progesterone receptor (PR) status in our study reveals a statistically significant association ($p = 0.0144$). PR-positive tumors tend to have a different immunoreactive score distribution compared to PR-negative tumors, with the latter

often exhibiting higher nestin expression. This inverse correlation aligns with the findings of Zhang et al. (2020), [77] who observed that high nestin expression is associated with PR negativity ($p < 0.001$), suggesting that the loss of progesterone receptor signalling may contribute to a more aggressive tumor phenotype. Shaban and El-Goday (2016) [78] similarly reported that triple-negative breast cancers, which lack both ER and PR, display significantly higher nestin levels, further supporting the notion that the absence of hormone receptor signalling is linked with enhanced nestin expression. The differential nestin expression between PR-positive and PR-negative tumors implies that PR status may modulate tumor biology, influencing the expression of markers associated with invasiveness. Clinically, this finding is significant because PR status is a key factor in determining therapeutic approaches, and the addition of nestin evaluation could refine prognostic assessments and treatment planning. The significant difference in immunoreactive scores between the two groups suggests that nestin could serve as an additional biomarker for identifying high-risk patients, particularly those with hormone receptor-negative disease. Future research should explore the molecular mechanisms underlying this relationship and assess whether targeting nestin could provide therapeutic benefits for PR-negative breast cancers. [77, 78]

Correlation between Nestin Expression and Her2 neu Receptor Status

The analysis of nestin expression in relation to Her2 neu receptor status in our study indicates that there is no statistically significant association ($p = 0.142$). HER2-positive tumors and HER2-negative tumors exhibit different distributions of nestin immunoreactivity, but these differences do not reach statistical significance. Zhang et al. (2020) [77] noted that nestin expression is predominantly observed in triple-negative and basal-like breast cancers, rather than in HER2-positive cases. Similarly, Shaban and El-Goday (2016) [78] reported that high nestin levels are more characteristic of triple-negative tumors, suggesting that nestin is less relevant in the context of HER2 overexpression. Our data indicate that while some HER2-negative tumors show strong nestin positivity, the overall association between nestin expression and HER2 status is not robust. This may be due to the complex interplay of molecular pathways in HER2-driven tumors, where other factors play a more dominant role in determining tumor behavior. Despite the lack of a statistically significant correlation, the trend observed in our study contributes to the broader understanding of nestin's role in different molecular subtypes of breast cancer. At present, the evidence supports the notion that nestin is more closely associated with triple-negative and basal-like phenotypes rather than with HER2-driven tumors. [77, 78]

Correlation between Nestin Expression and Ki67 Expression Status

The assessment of the relationship between nestin expression and the Ki67 proliferation marker shows that higher nestin expression is significantly correlated with increased Ki67 levels, as evidenced by a statistically significant association ($p = 0.0043$). In our study, tumors with high Ki67 expression exhibit higher immunoreactive scores for nestin compared to those with low or borderline Ki67 levels. Nowak et al. (2017) [74] demonstrated that elevated nestin expression is strongly associated with high Ki67 proliferation indices ($p < 0.001$), linking nestin to rapid tumor cell division and aggressive clinical behavior. Krüger et al. (2013) [76] also reported that aggressive, basal-like tumors with high nestin expression show increased Ki67, reinforcing the relationship between nestin and proliferation. Additionally, Zhang et al. (2020) [77] found that high nestin expression predicts poorer recurrence-free survival, partly due to its association with a high proliferative index. The significant correlation in our study suggests that nestin may contribute to tumor aggressiveness by promoting cell cycle progression. Clinically, assessing both nestin and Ki67 could improve patient stratification and suggests that its evaluation could be integrated into routine pathological assessments to better predict tumor behavior. [74, 76, 77]

Correlation between Nestin Expression and Luminal A/B Status

The comparison of nestin expression between molecular subtypes reveals distinct differences between Luminal A and Luminal B tumors. In our study, Luminal A tumors tend to show lower nestin immunoreactive scores, with most cases exhibiting negative to moderate expression, while Luminal B tumors are more likely to demonstrate higher levels, including strong positivity. This difference is statistically significant ($p = 0.0125$), suggesting that nestin expression may help distinguish between these two subtypes. Shaban and El-Goday (2016) [78] reported that more aggressive subtypes, including Luminal B, exhibit elevated nestin expression, which is consistent with our findings. Moreover, Zhang et al. (2020) [77] found that high nestin expression is associated with more aggressive tumor characteristics, supporting the notion that Luminal B tumors, generally known for higher proliferation and poorer prognosis, have increased nestin levels. The differential expression pattern indicates that nestin could serve as a valuable biomarker for molecular subtyping and may provide additional prognostic information beyond conventional hormone receptor assessment. The significantly higher frequency of strong nestin positivity in Luminal B tumors suggests that these tumors possess enhanced stem cell-like properties, contributing to their aggressive behavior. Clinically, incorporating nestin evaluation in routine diagnostic panels could improve risk stratification and guide therapeutic decisions, particularly in identifying patients within the Luminal B

subgroup who might benefit from more aggressive treatment strategies. Future research should aim to validate these results in larger cohorts and further elucidate the mechanistic pathways linking nestin expression to the distinct biological behavior of Luminal A versus Luminal B tumors. [77, 78]

Correlation between Nestin Expression and TNBC Status

The evaluation of nestin expression in triple-negative breast carcinoma (TNBC) reveals a pronounced association between high nestin levels and TNBC status. In our study, TNBC tumors demonstrate a strong immunoreactive score pattern, with the majority exhibiting strong nestin positivity, whereas non-TNBC tumors display a more moderate expression profile. The statistical analysis confirms a significant association ($p = 0.0020$), underscoring that TNBC, known for its aggressive clinical behavior, is characterized by elevated nestin expression. Shaban and El-Goday (2016) [78] reported that TNBC cases consistently exhibit higher nestin levels compared to other subtypes ($p < 0.001$), a finding that is corroborated by Krüger et al. (2013) [76], who associated nestin expression with basal-like features. This robust association supports the hypothesis that nestin is a marker of aggressive tumor biology and may contribute to the stem cell-like properties observed in TNBC. The high frequency of strong immunoreactive scores in TNBC cases suggests that nestin could be used as a prognostic indicator and may help in stratifying patients who are at a higher risk of recurrence and poor outcomes. Given the limited treatment options for TNBC, the identification of nestin as a potential therapeutic target is of particular interest. Future investigations with larger patient cohorts are warranted to further validate nestin's role in TNBC and assess its potential as a predictive marker for therapeutic response. [76, 78]

Correlation between Nestin Expression and Her2 neu Enrichment Status

The investigation into the association between nestin expression and Her2 neu enrichment reveals a statistically significant difference ($p = 0.0022$). Our data indicate that a subset of Her2-enriched tumors exhibit elevated nestin expression, with a higher proportion of cases demonstrating moderate to strong immunoreactivity, compared to Her2 non-enriched tumors where strong expression is rare. This finding suggests that, while nestin is predominantly associated with triple-negative and basal-like phenotypes, it may also play a role in the aggressive behavior of certain Her2-positive tumors. Nowak et al. (2017) [74, 79] documented that nestin-positive microvessel density is linked to aggressive tumor features, implying that nestin may contribute to angiogenesis and tumor progression in various molecular contexts.

Furthermore, Krüger et al. (2017) [80] reported that aggressive tumor markers, including nestin, are frequently observed in tumors with enhanced growth factor signalling, a hallmark of Her2 neu enrichment. The significant association in our study indicates that nestin could serve as an additional biomarker for identifying high-risk patients within the Her2-positive subgroup. The observation that Her2-enriched tumors with elevated nestin expression may have a more aggressive clinical course supports the potential utility of integrating nestin evaluation into routine molecular profiling. This could eventually lead to more tailored therapeutic strategies that target both Her2 and nestin-associated pathways. Future research should focus on elucidating the molecular interplay between Her2 signalling and nestin expression and determining whether nestin can serve as a predictive marker for response to combined targeted therapies in Her2-positive breast cancer. [74,80]

LIMITATIONS

While this study offers valuable insights, it is important to acknowledge several limitations that may affect the interpretation of the findings.

- **Interobserver variability in grading Nestin** may still influence the results.
- **Cross-sectional design** limits the ability to infer causal relationships between nestin expression and clinical outcomes.
- **Relatively smaller sample size**, may not be sufficiently powered to detect subtle differences in certain subgroups, particularly when stratifying by molecular subtypes or rare clinicopathological features.

Addressing these limitations will enhance the clinical relevance of the findings and contribute to the development of more effective management strategies for breast cancer patients.

FUTURE ASPECTS

Future research should aim to address these limitations by conducting large-scale, multicentre, prospective studies with standardized methodologies. Integrating advanced molecular techniques, such as genomic and proteomic profiling, may also uncover additional pathways associated with Nestin expression and its impact on tumour behaviour.

It is recommended to incorporate a more comprehensive assessment of the tumour microenvironment, including the evaluation of angiogenesis and immune cell infiltration, to better understand the complex interactions that drive breast cancer progression.

SUMMARY

In the present study, 52 histopathologically diagnosed cases of Infiltrating Ductal breast Carcinoma were studied from time period January 2023 to December 2024 at Department of Pathology, JNMC, Belagavi. All the cases were MRM specimens received in histopathology laboratory at KLE's Dr Prabhakar Kore hospital and medical research Centre, Belagavi.

Paraffin embedded blocks of all 52 cases were subjected to immunohistochemical staining for Nestin and its result was correlated with clinicopathological parameters.

The peak incidence was between 41-50 years with most of the cases presenting over Left Upper outer quadrant with 75% of cases with T2 type of tumor according to TNM classification. Most of the cases were G2 (moderately differentiated) according to Scarff Bloom Richardson grading system.

88% or 46 out of 52 cases of IDC breast carcinoma in this study have shown positivity for Nestin immunostaining. However, cases varied in intensity of staining and percentage positivity of tumor cells. A majority of the cases, 44.23% or 23 out of 52 cases showed moderate immunoreactivity of Nestin expression.There were positive significant associations found between nestin expression and various factors such as menopausal status, DCIS status , lymph node involvement, estrogen receptor, progesterone receptor, Ki67 proliferation index, molecular subtypes including Luminal A/B , triple-negative breast cancer, and HER2 neu enrichment. There was no significant correlation found between IHC score and age, IHC score and LVI/PNI status, and percentage or intensity of positive cells. No correlation was found between IHC score and individual clinicopathological variables when evaluated separately.

The findings of the study suggest that Nestin can be used as a biomarker for assessing tumor progression and prognosis.

CONCLUSION

This study has provided an in-depth analysis of the expression of Nestin in breast cancer and its correlation with various clinicopathological parameters, offering critical insights into its potential as a prognostic biomarker and therapeutic target. The findings reveal that the patient cohort, with a mean age of 46.5 years and a predominance of cases in the 41–50 years range, reflects a typical middle-aged population affected by breast cancer. The distribution of tumors favoured the left breast and primarily the upper outer quadrant, which aligns with the anatomical prevalence of glandular tissue in these areas. The menopausal status of the patients, with 61.54% being postmenopausal, underscores the potential influence of hormonal changes on tumor biology and receptor expression profiles.

Histopathologically, the study noted that 30.80% of tumors were classified as Grade 1, 50% as Grade 2, and 19.20% as Grade 3, indicating a spectrum of differentiation, with the majority being moderately differentiated. Tumor size analysis revealed that most tumors were T2 (75%), suggesting an intermediate stage at presentation. The lymph node involvement data showed that nearly half of the patients were node-negative, while the remaining cases demonstrated varying degrees of nodal spread, a factor that is critical in determining prognosis. In addition, the high prevalence of perineural invasion (77%) and lymphovascular invasion (86.50%) emphasizes the aggressive nature of the tumors in this cohort.

A central focus of the study was the expression of Nestin, which was found to be heterogeneously distributed among the tumors: 11.54% negative, 32.69% weak, 44.23% moderate, and 11.54% strong. The detailed immunohistochemical scoring, which incorporated intensity, percentage, and overall immunoreactivity score (IRS), revealed that although the intensity and percentage scores alone did not show significant differences across tumor grades, the composite IRS demonstrated a strong and significant correlation with higher histopathological grades ($p = 0.0025$). This suggests that Nestin expression, when measured comprehensively, is closely linked with tumor aggressiveness. The study further demonstrated significant correlations between high Nestin expression and several clinical parameters, including menopausal status, the presence of a DCIS component, increased lymph node involvement, and adverse molecular characteristics such as ER negativity, PR negativity, high Ki67 proliferation indices, and more aggressive subtypes like triple-negative and HER2-enriched tumors.

These findings have several important implications. First, the clear association between elevated Nestin expression and markers of aggressive disease suggests that Nestin could be a valuable biomarker for prognostic stratification. Patients with high Nestin expression might be identified as having a higher risk of poor clinical outcomes, thus potentially benefiting from more aggressive or targeted treatment strategies. Moreover, the differential expression of Nestin across various molecular subtypes indicates its potential utility in refining the categorization of breast cancer beyond conventional markers, thereby contributing to the evolving field of personalized medicine.

In conclusion, this study not only reinforces the potential of Nestin as a critical biomarker for aggressive breast cancer but also paves the way for its incorporation into future diagnostic and treatment protocols, ultimately contributing to more personalized and effective management strategies in breast oncology.

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ANNEXURE I - INFORMED CONSENT FORM

"EXPRESSION OF NESTIN IN BREAST CANCER AND CORRELATION WITH HISTOPATHOLOGICAL GRADING: HOSPITAL-BASED CROSS-SECTIONAL STUDY "

Principal investigator: Reg no. BN0122006

Guide: Dr.

Objective: The purpose of this study is to evaluate the expression of nestin in breast carcinomas and correlate with histopathological grading and assess its role in invasiveness and prognosis of breast carcinoma.

Introduction: Breast carcinoma is the most common and deadly malignancy of women globally and the majority of deaths are caused by invasion and metastasis. This study may help to evaluate the potential role of nestin in tumor aggressiveness, hence leading to development of potential predictive and prognostic biomarkers as well as novel treatment interventions.

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Withdrawal from participation in the study: Participation in this study is 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: This study may give opportunities for novel targeted therapies.

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 from identifying 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.

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: 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 waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "**EXPRESSION OF NESTIN IN BREAST CARCINOMA AND CORRELATION WITH HISTOPATHOLOGICAL GRADING: 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:

Signature of the investigator:

ANNEXURE II- PROFORMA

NAME:

AGE:

BRIEF CLINICAL HISTORY:

SURGICAL PROCEDURE DONE:

DATE OF COLLECTION:

PAST HISTORY:

MENOPAUSAL STATUS:

H/O MEDICAL ILLNESS:

EXAMINATION:

• SIDE

• SIZE

• SKIN CHANGES

• AXILLARY LYMPH NODE INVOLVEMENT

• NIPPLE, AREOLA

• OTHERS

➢ GROSS FINDINGS

• Size-

• Quadrant involved-

• Margins-

• Nipple and areola-

• Skin involvement-

➢ HAEMATOXYLIN AND EOSIN FINDINGS:

• Histological type-

• Grade-

• Desmoplastic response-

• In-situ component-

• Lymphovascular involvement status-

• Lymph node metastasis-

• Perineural invasion-

• Any other-

➢ IMMUNOHISTOCHEMISTRY FINDINGS:

• Nestin

• ER

• PR

• HER2

• Ki67

ANNEXURE III - IHC MANUAL STAINING PROTOCOL

Tissue sections of 3 microns is cut with microtome and then placed on a coated slide.

Steps for IHC staining for Nestin is as follows:

1.The slides were baked at 60 degree centigrade for 1 hour prior to start deparaffinisation.

2. Then deparaffinised in fresh xylene for two changes 10minutes each.

3. Rinsed in absolute alcohol for two changes 10minutes each.

4. Then were rinsed in water for-5minutes, followed by distilled water for-1minute.

5. They were subjected to antigen retrieval using pressure cooker method using TRIS EDTA buffer solution.

6. After that they were allowed to cool at room temperature for 15minutes.

7. Rinsed with wash buffer 2times with gap of 30seconds each.

8. Placed in 0.5% Hydrogen peroxide-8 to 10 minutes to inhibit endogenous peroxidase activity.

9. Then washed with wash buffer 3times with gap of 30seconds each.

10. Primary antibody incubated for 45-60 minutes at room temperature in a closed room

11. Then they were washed with wash buffer 3times with gap of 30seconds each

12. HRP polymer was applied and incubated for 25-30 minutes at room temperature in a closed chamber

13. Washed with wash buffer 3times with gap of 30seconds each

14. DAB substrate was applied to sections for 10minutes

15. Washed in water-2minute, followed by wash with distilled water-1minute

16. Counter stained with Haematoxylin-3minutes

17. Washed in running water for 2 minutes.

18. Blotted and cleared in xylene & mounted with DPX.

Antibody in the study: Nestin
Localisation: Cytoplasmic
Clonality: Anti-Nestin Monoclonal Mouse Antibody Clone 10C2
Dilution: Ready to use
Manufacturer: Dako 27

ANNEXURE IV- MASTERCHART

Sr No	Block no	Age	Laterality	Quadrant	Menopausal status	SBR Grade	T1/T2/T3	LVI	PNI	Nestin Expression	ER Expression	PR Expression	Her2 Expression	Ki67 index	Molecular Subtype
1	1009/23	65	R	Centre	POST	G1	T2	YES	YES	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
2	1144/23	43	R	UOQ	PRE	G1	T3	YES	YES	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal A
3	1416/23	70	L	UOQ	POST	G2	T1	YES	YES	WEAK	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A
4	2282/23	60	L	Centre	POST	G2	T2	YES	YES	STRONG	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
5	2469/23	56	R	LOQ	POST	G3	T2	YES	NO	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	BORDERLINE	TRIPLE NEGATIVE
6	2269/23	38	R	UOQ	PRE	G2	T2	YES	YES	WEAK	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
7	3278/23	57	L	Multicentric	POST	G2	T3	YES	YES	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	LOW	TRIPLE NEGATIVE
8	2780/24	39	L	UOQ	PRE	G2	T2	NO	NO	MODERATE	POSITIVE	POSITIVE	POSITIVE	HIGH	Luminal A
9	3831/23	57	L	Multicentric	POST	G2	T3	YES	YES	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
10	2361/23	46	L	UOQ	PRE	G1	T2	YES	YES	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A
11	4077/23	45	L	LOQ	POST	G2	T2	NO	NO	WEAK	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A
12	5049/23	50	L	Centre	POST	G3	T2	YES	YES	STRONG	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
13	2409/23	45	L	LOQ	POST	G2	T2	YES	YES	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	LOW	TRIPLE NEGATIVE
14	1552/23	46	L	Centre	POST	G1	T3	YES	YES	MODERATE	POSITIVE	POSITIVE	POSITIVE	BORDERLINE	Luminal A
15	5388/23	78	L	UOQ	POST	G2	T3	YES	YES	MODERATE	POSITIVE	POSITIVE	POSITIVE	BORDERLINE	Luminal A
16	3357/23	67	L	Centre	POST	G1	T2	NO	NO	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
17	2300/23	38	L	Centre	PRE	G3	T2	YES	YES	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
18	5416/23	58	R	UOQ	POST	G2	T2	YES	YES	MODERATE	NEGATIVE	NEGATIVE	POSITIVE	HIGH	Her2 enriched
19	4593/23	54	L	LOQ	POST	G2	T2	YES	YES	MODERATE	NEGATIVE	NEGATIVE	POSITIVE	LOW	Luminal A
20	2269/23	38	R	UOQ	PRE	G2	T2	YES	YES	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
21	5217/23	47	L	UIQ	PRE	G1	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A
22	5243/23	69	R	LOQ	POST	G3	T2	YES	YES	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
23	2568/24	48	L	UOQ	PRE	G1	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	POSITIVE	LOW	Luminal A
24	203/24	60	R	LIQ	POST	G3	T2	YES	YES	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
25	2780/24	39	L	UOQ	PRE	G1	T2	YES	NO	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
26	996/24	77	R	Centre	POST	G1	T3	YES	NO	WEAK	POSITIVE	POSITIVE	POSITIVE	LOW	Her 2 enriched
27	3319/24	65	L	UOQ	POST	G2	T2	NO	NO	MODERATE	NO DATA	NO DATA	NO DATA	NO DATA	NO DATA
28	1891/24	48	L	LOQ	POST	G1	T2	YES	YES	WEAK	POSITIVE	POSITIVE	POSITIVE	HIGH	Her 2 enriched
29	1371/24	46	R	UOQ	POST	G2	T2	YES	YES	WEAK	NO DATA	NO DATA	NO DATA	NO DATA	No data
30	1271/24	45	L	UOQ	POST	G2	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	NEGATIVE	BORDERLINE	Luminal A
31	1176/24	41	R	LIQ	PRE	G2	T3	YES	YES	STRONG	NO DATA	NO DATA	NO DATA	NO DATA	No data
32	390/24	53	L	UOQ	POST	G2	T2	YES	NO	MODERATE	POSITIVE	POSITIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
33	714/24	36	R	UIQ	PRE	G2	2	YES	YES	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
34	1763/24	58	R	LOQ	POST	G1	T2	YES	YES	WEAK	POSITIVE	POSITIVE	NEGATIVE	LOW	TRIPLE NEGATIVE
35	197/24	41	R	Multicentric	PRE	G3	T3	YES	YES	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
36	1121/24	46	R	Centre	PRE	G3	T3	YES	YES	WEAK	NEGATIVE	NEGATIVE	POSITIVE	HIGH	Her 2 enriched
37	586/24	67	L	Centre	POST	G2	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	POSITIVE	HIGH	Luminal A
38	129/24	65	R	UOQ	POST	G2	T2	YES	YES	STRONG	NO DATA	NO DATA	NO DATA	NO DATA	NO DATA
39	894/24	44	L	UIQ	PRE	G1	T2	NO	NO	MODERATE	POSITIVE	POSITIVE	POSITIVE	LOW	Luminal A
40	1760/24	50	L	Centre	POST	G2	T3	NO	NO	WEAK	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
41	3482/24	40	L	UOQ	PRE	G3	T2	YES	NO	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
42	2568/24	48	L	UOQ	PRE	G1	T2	YES	YES	WEAK	NO DATA	NO DATA	NO DATA	NO DATA	NO DATA
43	4755/24	44	R	UOQ	PRE	G2	T2	YES	YES	MODERATE	NEGATIVE	NEGATIVE	NEGATIVE	LOW	TRIPLE NEGATIVE
44	4945/24	60	R	UOQ	POST	G2	T2	YES	YES	WEAK	POSITIVE	POSITIVE	NEGATIVE	HIGH	Luminal B
45	2666/24	48	L	LOQ	POST	G1	T2	YES	YES	MODERATE	POSITIVE	NEGATIVE	NEGATIVE	LOW	TRIPLE NEGATIVE
46	3265/24	90	R	UOQ	POST	G3	T2	YES	YES	STRONG	POSITIVE	POSITIVE	POSITIVE	HIGH	Luminal B
47	3486/24	62	L	UOQ	POST	G2	T3	YES	YES	MODERATE	NEGATIVE	NEGATIVE	POSITIVE	LOW	Her 2 enriched
48	3486/24	62	L	UOQ	POST	G2	T3	YES	YES	WEAK	POSITIVE	NEGATIVE	POSITIVE	LOW	Her 2 enriched
49	3482/24	40	R	UOQ	PRE	G3	T2	YES	YES	STRONG	NEGATIVE	NEGATIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
50	2568/24	50	L	UOQ	PRE	G1	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A
51	2780/24	39	L	UOQ	PRE	G1	T2	NO	NO	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	HIGH	TRIPLE NEGATIVE
52	4870/24	45	R	UOQ	PRE	G2	T2	YES	YES	MODERATE	POSITIVE	POSITIVE	NEGATIVE	LOW	Luminal A