

## Molecular Subtypes of Breast Carcinoma and Their Association with Clinicopathological Features and Survival Outcomes

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### Abstract

Breast carcinoma is a biologically heterogeneous disease characterised by distinct molecular subtypes with variable clinicopathological features and survival outcomes. Molecular classification has improved risk stratification and prognostic assessment. To evaluate the association between molecular subtypes of breast carcinoma and clinicopathological characteristics and to compare overall and relapse-free survival across subtypes using the METABRIC clinical dataset. A retrospective secondary analysis was conducted using the METABRIC dataset comprising 2,509 patients. After excluding cases with missing molecular subtype data, 1,980 patients were analysed. Descriptive statistics, Chi-square tests, ANOVA, and Pearson correlation analysis were performed to assess associations between molecular subtypes and clinical variables. Survival differences were evaluated using mean overall survival (OS) and relapse-free survival (RFS). Luminal A was the most prevalent subtype (35.35%), followed by Luminal B (23.99%). Basal and HER2-enriched subtypes were associated with larger tumour size, higher histologic grade, and increased lymph node involvement. Luminal subtypes demonstrated high ER positivity, whereas HER2-enriched tumours showed increased HER2 expression. Luminal A exhibited the longest mean OS (140.27 months) and RFS (124.89 months). Tumour size and lymph node positivity were negatively correlated with survival duration. Molecular subtypes are significantly associated with distinct clinicopathological features and survival outcomes. Intrinsic subtyping provides valuable prognostic information and supports subtype-based risk stratification in breast carcinoma.

**Keywords:** Breast carcinoma, Molecular subtypes, Clinicopathological features, Overall survival, Relapse-free survival  
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### 1. Introduction

Breast carcinoma is also one of the most widespread malignancies in women throughout the world and is a biologically heterogeneous disease with a wide range of clinical responses and treatment outcomes. Conventionally the taxonomy of breast cancer depended mainly on histopathological characteristics but recently with the development of molecular biology it has been proven that tumours having similar morphology may vary dramatically in genetic signature and prognosis (do Nascimento & Otoni, 2020). Intrinsic molecular subtypes have been developed as a consequence of the acknowledgement of molecular heterogeneity and have greatly enhanced the risk stratification and treatment planning. There are several intrinsic molecular subtypes that are well recognized, including Luminal A, luminal B, HER2-enriched, Basal-like, Claudin-low, and

Normal-like. These categories were first discovered using the gene expression profile, and today they are often estimated with the help of immunohistochemical markers (Kondov et al., 2018). The molecular classification will give information on tumour biology, possibility of metastases, and response to systemic treatments (Fragomeni et al., 2018). Such basal-like forms of breast cancer as luminal ones are commonly typified by ferocious clinical results and lower survival rates in contrast to the luminal types (Milioli et al., 2017). Likewise, HER2-enriched tumours are characterised by different molecular profiles and prognostic meanings (Agostinnetto et al., 2021). The recent studies have focused on the importance of further refinement of molecular subclassification to enhance the accuracy of prognostics. The reclassification plans that involve the use of other biomarkers like the

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expression of p53 has also emphasised the heterogeneity of the existing subtypes (Abubakar et al., 2019). Such developments underscore the complexity of breast carcinoma and the need to have a thorough molecular characterisation.

Molecular subtypes are characterised by a high level of clinicopathological differences. Luminal subtypes are typically linked to hormone receptor positivity and better outcomes whereas Basal-like and some tumours with an enrichment of HER2 have higher histological grades and aggressiveness (Mohammed, 2021). The biological basis of subtype classification is differences in estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, which have a direct impact on treatment choices (Al-Thoubaity, 2020). Molecular subtypes have also been found to be variable in terms of tumour grade, stage, and lymph node involvement. Basal-like and HER2-enriched cancers are associated with higher rates of high-grade tumours, which is indicative of a high level of proliferation and the probability of metastasis (Elidrissi Errahhali et al., 2017). Researchers have shown that molecular subtypes and nodal involvement have high relationships, recurrence potential, and long-term survival rates (Buonomo et al., 2017). Moreover, the clinical and pathological presentation and prognosis of triple-negative and Basal-like tumours vary compared to other subtypes, and in most cases, they have a poor prognosis (Liao et al., 2018). There are emerging signs that also indicate that tumour-infiltrating lymphocytes and other microenvironmental parameters could be predictive and prognostic in certain molecular subtypes (Gao et al., 2020). Also, differences in receptor patterns, such as single hormone receptor-positive tumours, also contribute to the difference in survival (Li et al., 2020). There are also genetic alterations like PTEN loss, which is linked to poor clinicopathological features and poor survival (Li et al., 2017). Taken together, these results seem to point to the clinical significance of the knowledge about subtype-specific patterns.

The data need to be small and poorly annotated (to test the hypothesis of clinopathological features and their connection to molecular subtypes). The METABRIC dataset has a rich amount of molecular subtype classification, clinicopathological variables and long-term follow-up survival data. These data sets enable a strong study of the subtype distribution and survival rates of various groups of patients. The differences in the clinical behaviour of histological subtypes such as invasive ductal and lobular carcinoma were already revealed in previous studies and the necessity of integrated clinicopathological and molecular analyses is underlined (Oesterreich et al., 2022). In the same manner, microinvasive and ductal carcinoma in situ comparisons have demonstrated that pathological features match prognosis (Kim et al., 2018). Although this has been made, additional analysis through massive international cohorts is desirable to confirm and correct subtype-based prognostic testing. The objective of the current research was to determine the relationship between the molecular subtypes of breast cancer and

clinicopathological features and survival outcome with the use of the METABRIC clinical dataset. Through the intrinsic subtype distribution analysis and the tumour grade, stage, lymph node involvement, receptor status, and the survival measures, this study aimed at giving an overall evaluation of the subtype-related clinical patterns and the prognostic significance.

### Objectives of the Study

1. To evaluate the association between molecular breast subtypes carcinoma and clinicopathological characteristics.
2. To compare overall survival and relapse-free survival across different molecular subtypes.

## 2. Materials and Methods

### 2.1 Study Design

This study was conducted in the form of a retrospective observational study with the help of a secondary analysis method. The research was designed on the foundation of previously existing clinical data that was publicly available. No direct patient recruitment and intervention took place. The designed study provided an opportunity to evaluate the relationship between molecular subtypes of breast carcinoma and clinicopathological features and survival. No direct interaction with the patients was made because the dataset was de-identified and available to the public. The framework of retrospective permitted systematic assessment of the data available to determine trends and correlations between molecular classification and clinical variables.

### 2.2 Data Source

The data was taken when it was published on The cBioPortal hosts the Molecular Morphology of Breast Cancer by the International Consortium (METABRIC) dataset platform with the study identifier of brca\_metabric. The data consisted of clinical data of 2509 patients with primary invasive breast carcinoma. The database contained demographic data, tumour features, receptor features, treatment outcomes and survival data. The data was downloaded and filtered using completeness. Clinical variables that were available in the dataset were only utilised. External data of any genomic, transcriptomic, or mutation level have not been included in the current analysis.

### 2.3 Study Population

The target population was the patients with primary invasive breast carcinoma in the METABRIC clinical dataset. Inclusion criteria- were patients who had both molecular subtype based on PAM50 and Claudin-low classification and reported survival data. Patients with unavailable molecular subtype information or without survival data had not been included in comparative studies. Upon the application of these criteria, some of the cases were identified as eligible to undergo statistical analysis. This allowed comparing and analysing the survival of subtypes using patients who

have all the necessary and dependable data on the variables being examined.

### 2.4 Variables Assessed

Analysis extracted variables in the form of clinical and pathological variables in the dataset. The PAM50 plus Claudin-low subtype was part of the molecular variable. The following were classified as clinicopathological variables: age at diagnosis, tumour size in millimetres, tumour stage, tumour grade, positive lymph nodes, histological subtype, and receptor status progesterone receptor (PR), estrogen receptor (ER) and HER2 status. Some of the variables that were used in the treatment included; the type of surgery, chemotherapy, hormone therapy and radiotherapy. The variables that were used as survival variables were overall survival months, overall survival status, relapse-free survival months, relapse-free status and vital status.

### 2.5 Statistical Analysis

The variables of the study were summarised with the help of descriptive statistics. Frequencies and percentages were used to draw the categories of variables. Mean +/- standard deviation was used to express the continuous variables. Associations between molecular subtypes and categorical clinicopathological variables were tested with Chi-square test. In comparing a pair of continuous variables, independent t-tests were used when there were two groups. Pearson correlation analysis was conducted to assess the relationship between continuous variables and Spearman correlation

was applied when the data was not normally distributed. A p-value of less than 0.05 was taken to be statistically significant.

### 2.6 Survival Analysis

The general survival months and the Survival without relapse months were the variables used to assess survival outcomes. The mean survival and median survival periods were estimated in each of the molecular subtypes. The differences in mean survival months between subtypes were evaluated by using the one-way ANOVA. The Chi-square test was used to compare survival status distributions of subtypes. Linear association was also addressed by the correlation analysis between the tumour size and the survival months. Survival analyses were performed without sophisticated modelling and followed the variables and simple statistical techniques arranged in the study protocol.

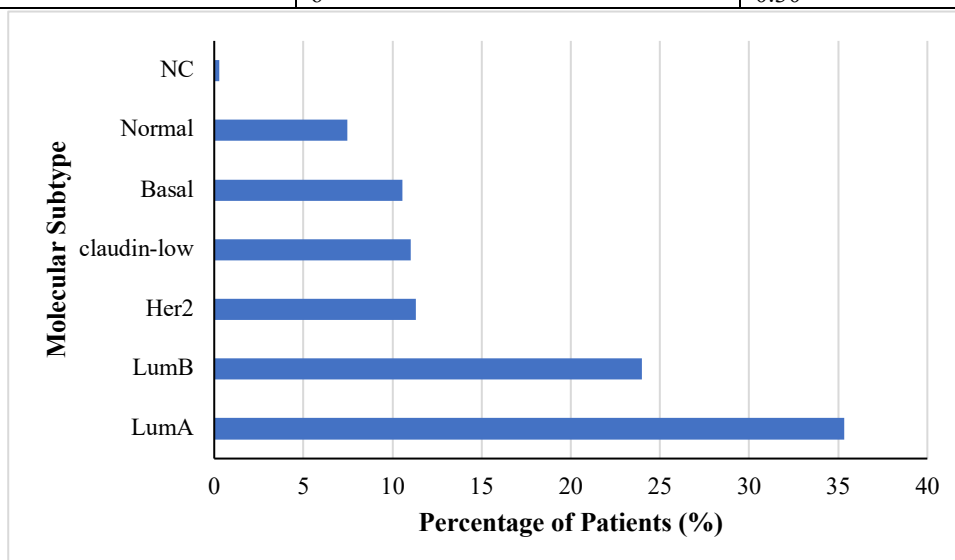
## 3. Results

### 3.1 Distribution of Molecular Subtypes

The dataset of METABRIC included 2,509 patients. The final analysis included 1,980 patients after filtering out those cases that lacked the molecular subtype information. The two most prevalent molecular subtypes were Luminal A (35.35%) and Luminal B (23.99%). HER2-enriched (11.31%), claudin-low (11.01) and Basal (10.56) subtypes were equally represented, whereas the Normal and NC subtypes were underrepresented (Table 1).

**Table 1. Distribution of Molecular Subtypes (n = 1,980)**

Molecular Subtype	Frequency (n)	Percentage (%)
LumA	700	35.35
LumB	475	23.99
Her2	224	11.31
claudin-low	218	11.01
Basal	209	10.56
Normal	148	7.47
NC	6	0.30



**Figure 1. Distribution of Molecular Subtypes in the Study Cohort**

Figure 1 displays the percentage composition of inherent molecular subcategories within the analysed patients (n= 1,980). The highest incidence was Luminal A with Luminal B coming in second place, then HER2-enriched, claudin-low, and Basal subtypes were moderately common whereas the frequency of Normal and NC subtypes was lower.

### 3.2 Association Between Molecular Subtypes and Clinicopathological Characteristics

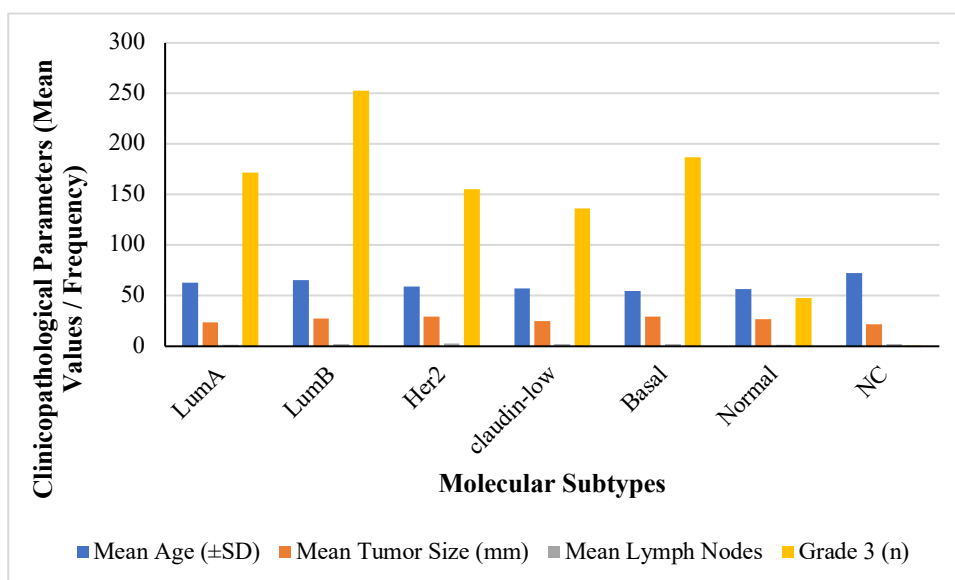
#### 3.2.1 Age, Tumour Size, and Lymph Node Involvement

The mean age at which people had their diagnosis was 60.39 +/- 13.19 years. The Luminal B patients were older

with a mean age of 65.33 +/- 11.42 years and the Basal subtype patients were younger (54.69 +/- 13.92 years). There were different subtypes in tumour size. Both HER2-enriched (29.27 mm) and Basal (29.21 mm) tumours proved to have larger mean tumour sizes than Luminal A tumours (23.95 mm). HER2-enriched tumours (2.84) and Luminal A tumours (1.50) had the highest and the lowest mean number of positive lymph nodes respectively. The most common were high-grade (Grade 3) tumours, as Basal (187 cases) and HER2-enriched (155 cases) subtypes had more aggressive pathology (Table 2).

**Table 2. Clinicopathological Characteristics According to Molecular Subtype**

Subtype	Mean Age (±SD)	Mean Tumour Size (mm)	Mean Lymph Nodes	Grade 3 (n)
LumA	62.86 ± 12.47	23.95	1.50	172
LumB	65.33 ± 11.42	27.55	2.24	253
Her2	58.82 ± 13.18	29.27	2.84	155
claudin-low	57.27 ± 12.26	24.73	2.28	136
Basal	54.69 ± 13.92	29.21	2.19	187
Normal	56.69 ± 12.34	27.13	1.68	48
NC	72.37 ± 5.73	21.83	2.00	1



**Figure 2. Clinicopathological Characteristics Across Molecular Subtypes**

Figure 2 involved a comparison of mean age, mean tumour size, mean lymph node involvement, and prevalence of Grade 3 tumours in molecular subtypes. Basal and HER2-enriched subtypes have greater tumour size and frequencies of Grade 3, and Luminal subtypes have lesser tumour aggressiveness and relatively positive clinicopathological features.

### 3.3 Association Between Molecular Subtypes and Receptor Status

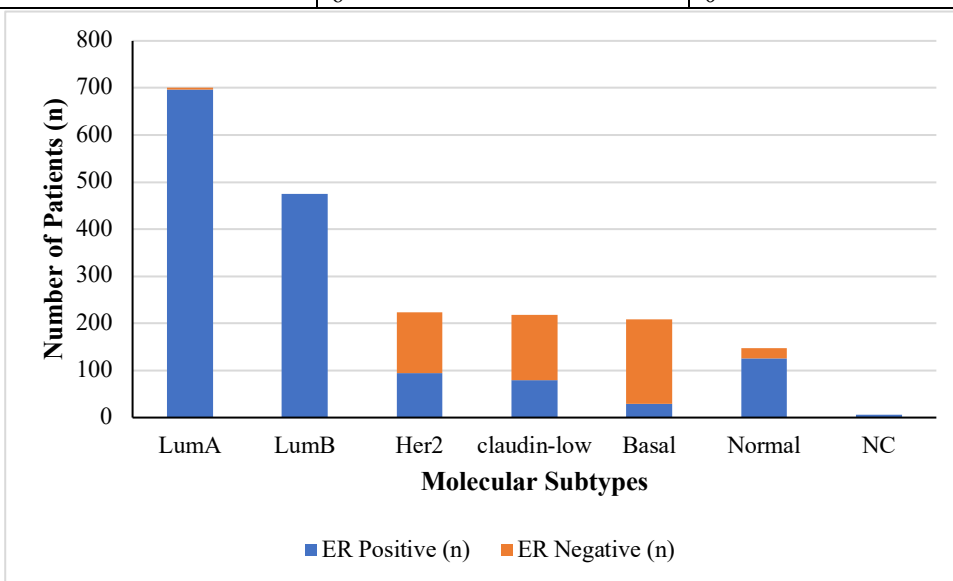
There were significant variations in the pattern of hormone receptor and HER2 expression amongst molecular subtypes. The Luminal A and B tumours were mainly ER-positive with 696 and 475 positive cases being the Luminal A and B tumors respectively. The Basal type, conversely, had a high percentage of ER-negative (180 cases) tumours. The most significant subtype was HER2-enriched (128 positive cases), and Luminal subtypes were mainly HER2-negative (Table 3).

**Table 3. Receptor Status According to Molecular Subtype ER Status**

Subtype	ER Positive (n)	ER Negative (n)
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LumA	696	4
LumB	475	0
Her2	95	129
claudin-low	80	138
Basal	29	180
Normal	125	23
NC	6	0



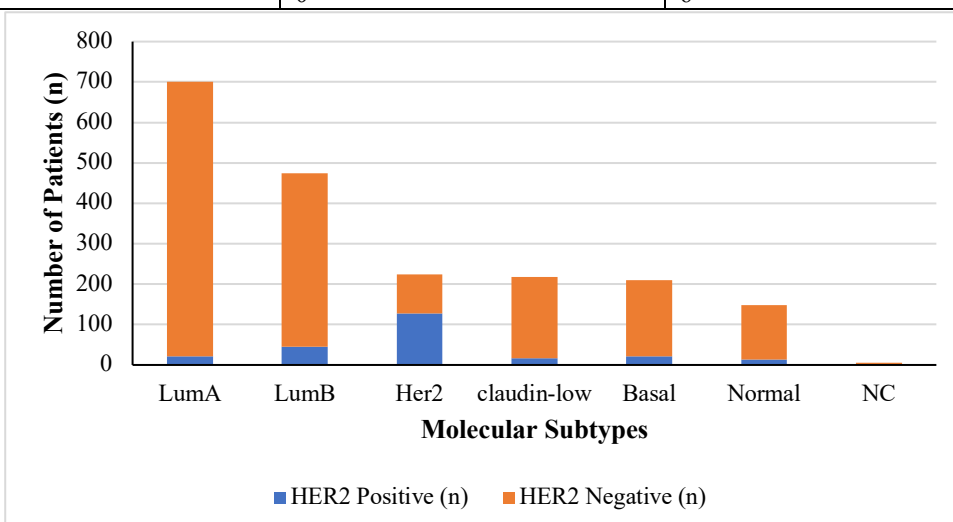
**Figure 3. Distribution of Estrogen Receptor (ER) Status Across Molecular Subtypes**

Figure 3 shows the spread of the cases of ER-positive and ER-negative according to subtypes of molecules. The majority of Luminal A or Luminal B tumors were ER-positive, while the majority of the Basal subtype

tumors were determined to be ER-negative. Claudin-low and her 2 enriched subtypes showed varied ER expression patterns in patients.

### HER2 Status

Subtype	HER2 Positive (n)	HER2 Negative (n)
LumA	21	679
LumB	45	430
Her2	128	96
claudin-low	17	201
Basal	22	187
Normal	14	134
NC	0	6



**Figure 4. Distribution of HER2 Status Across Molecular Subtypes**

Figure 4 presents the pattern of HER2-positive and HER2-negative cases in the molecular subtypes. HER2-enriched tumours showed the greatest percentage of HER2 positivity with Basal, claudin-low, Luminal A, and Luminal B and Normal the largest proportion of HER2-negative showing differences in biological properties across subgroups.

### 3.4 Survival Outcomes Across Molecular Subtypes

#### 3.4.1 Overall Survival

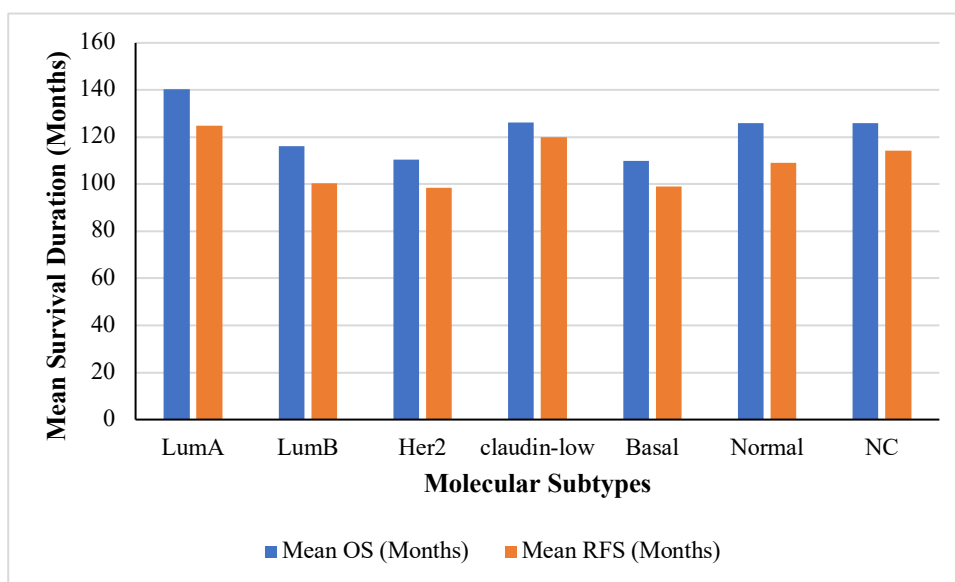
There were differences in the molecular subtypes in the overall survival. Luminal A was the best in overall survival (140.27 months mean). HER2-enriched and Basal subtypes had a relatively short survival (110.48 and 109.76 months, respectively).

#### 3.4.2 Relapse-Free Survival

The same was the case with relapse-free survival. Luminal A had the highest mean period of relapse-free survival (124.89 months), whereas the Basal subtype had lower relapse-free survival (99.03 months) (Table 4).

**Table 4. Survival Outcomes According to Molecular Subtype**

Subtype	Mean OS (Months)	Mean RFS (Months)
LumA	140.27	124.89
LumB	116.18	100.30
Her2	110.48	98.56
claudin-low	126.07	119.94
Basal	109.76	99.03
Normal	125.97	108.95
NC	125.80	114.21



**Figure 5. Comparison of Overall and Relapse-Free Survival Across Molecular Subtypes**

Figure 5 compares mean overall survival (OS) and relapse-free survival (RFS) in terms of molecular subtypes. Luminal A exhibited the greatest survival and Basal and HER2-enriched subtypes exhibited relatively low survival. The general survival was always higher than the relapse-free survival in all subgroups based on the molecular.

### 3.5 Correlation Between Tumour Characteristics and Survival

The Pearson correlation analysis showed that the tumour size and the lymph node involvement showed a positive association ( $r = 0.265$ ). The overall survival showed a slight inverse relationship with tumour size ( $r = -0.189$ ), as the bigger the tumour, the less time the patient survived. The positivity of lymph nodes showed a medium negative association with overall survival ( $r = -0.240$ ). Age or overall survival had a weakly negative correlation ( $r = 0.146$ ) (Table 5).

**Table 5. Pearson Correlation Analysis**

Variables	r value
Tumour Size vs Lymph Nodes	0.265
Tumor Size vs Overall Survival	-0.189
Lymph Nodes vs Overall Survival	-0.240
Age vs Overall Survival	-0.146

#### 4. Discussion

The paper assessed the allocation of the molecular breast subtypes carcinoma as well as their relationship to the clinicopathological features and survival in terms of the METABRIC clinical data. The most common subtype was Luminal A (35.35 per cent) of 1,980 patients analysed, then Luminal B (23.99 per cent), and the HER2-enriched, claudin-low, and Basal subtypes contained similar intermediate frequencies (Table 1). These results show that the subtypes that are predominant in the cohort are the hormone receptor-positive subtypes. The differences in the clinicopathological appearances among molecular subtypes were also apparent (Table 2). Luminal ones were also more diagnosed at a more advanced age and were linked to a smaller size of tumour and a smaller number of positive lymph nodes. Conversely, the Basal and HER2-enriched subtypes exhibited greater mean tumour sizes and a greater lymph node involvement, implying that they revealed aggressive diseases. The fact that Grade 3 tumours are dominating in Basal and HER2-enriched subtypes also confirms the fact that they are associated with increased proliferative and poorly differentiated tumour features.

Status analysis of receptors showed significant patterns of subtypes (Table 3). Both ER-negative and ER-positive Both Luminal A and B tumours were overwhelmingly respectively. The most common was the HER2-enriched subtype, which was HER2-positive. The results of these studies confirm the biological nature of intrinsic subtype classification and confirm the consistency of molecular categorisation between the datasets. The survival analysis indicated that there were significant differences among subtypes (Table 4). Luminal A had the longest mean overall survival and relapse-free survival and Basal and HER2-enriched subtypes had shorter mean survival. Such findings suggest that molecular subtype is a good predictive variable of prognosis. Correlation analysis also revealed that, there were negative associations between tumour size, and lymph node positivity and overall survival (Table 5), and this supports the clinical relevance of tumour burden and node positivity as outcome predictors.

The fact that the Luminal A subtype was predominant in this study is in agreement with previous institutional and population-based studies that indicated that Luminal tumours are the most prevalent molecular type (Pandit et al., 2019). On the same note, cross-population studies have shown similar distributions of subtypes where Luminal A and Luminal B which comprise the largest percentage of breast cancer (Villarreal-Garza et al., 2017). The correlation of Basal and HER2-enriched subtypes with a high tumour grade and tumour size is also consistent with the literature that reported more aggressive clinicopathological characteristics of such groups (Zhao et al., 2020; Xiao et al., 2024). Basal-like tumours have always been associated with an increase in histologic grade and worse differentiation which is a manifestation of the biological aggressiveness. The

observed patterns of receptor distributions in this analysis are related to the known molecular definitions, with Luminal types of subtypes most likely to be ER-positive and HER2-enriched tumours that are characterised by the elevated expression of HER2 (Peiffer et al., 2023). Moreover, higher survival of Luminal A and poorer prognosis in Basal and HER2-enriched types is largely reported in multi-centred studies (Zuo et al., 2017; Wang et al., 2019). Negative correlation between the advanced tumour burden and survival in the present study is also similar to the previous studies which have revealed the prognostic value of nodal involvement and tumour stage (Wang et al., 2018).

The results of this research support the clinical usefulness of molecular subtype classification of breast carcinoma. The unmistakable correlation of subtype and tumour grade, involvement of lymph node, receptor and survival outcome makes it evident that molecular profiling should be part of the routine clinical assessment. The luminal subtypes, especially Luminal A were found to have more favourable prognostic features whereas Basal and the HER2-enriched tumours had employed streakier prognostic behaviours and shorter survival rates. Subtype-specific clinical patterns can be used to understand how various subtypes display distinct patterns of risk and, therefore, to make decisions about therapies. Molecular classification is thus a very important element in informing management plans as well as long-term projections.

There are a number of strengths in this study. To begin with, the sample size is very big which increases the statistical reliability and minimises the error of chance. Second, long-term survival data are available which makes it possible to evaluate prognostic differences among subtypes. Third, the data contains detailed clinicopathological variables, which are essential to combine molecular and clinical variables. Nevertheless, this study has limitations despite its strengths. The data on molecular subtype were lacking in about 21% of the patients and this can result in selection bias. The analysis is retrospective, which restrains the ability to conclude causation. Also, there might have been heterogeneity in the treatment of patients which could have affected the survival rates, and the specificity of therapy-response relationships was not investigated.

But in future research, prospective validation cohorts should be used to validate these results. Subtype-specific prognostic evaluation can be further optimised by the incorporation of other molecular markers and genomic changes. In addition, the study of treatment-response patterns in molecular groups would also offer a deeper understanding of the individualised treatment methods. Multi-omics and longitudinal analysis could enhance the knowledge of tumour biology and enhance the precision medicine in breast carcinoma.

#### 5. Conclusion

This paper has critically analysed the subtypes of molecules distribution between breast cancer and their

correlation Having characteristics of clinicopathology and prognosis through the METABRIC clinical data. One of the most common subtypes of Luminal A was identified in the analysed patients, and Luminal B was the second most common with a middle frequency of HER2-enriched, claudin-low, and Basal subtypes. Categorical variations in tumour characteristics were seen between subtypes. Tumour size, histologic grade and the involvement of more lymph nodes were related to Basal and HER2-enriched tumours, compared to Luminal subtypes which had more favourable clinicopathological outcomes. Patterns of receptor status were highly related to intrinsic subtype classifications, where Luminal tumours were found to be highly estrogen receptor positive and HER2-enriched tumours were found to be highly HER2-enriched. Survival analysis also demonstrated that there were huge disparities between the subtypes Using Luminal A having the best in general and relapse-Survival without restrictions and Basal and HER2-enriched subtypes having relatively worse results. Further, the tumour size and involvement of lymph nodes showed a negative correlation to the duration of survival. All in all, these results support the clinical significance of molecular classification of breast carcinoma. Intrinsic subtyping is a valuable source of prognostic data and is an important instrument of risk stratification and clinical decision-making. Further study, incorporating the molecular and therapeutic response, is likely to be more useful in the future by adding more accuracy to the management plans in breast cancer.

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