

PROGNOSTIC SIGNIFICANCE OF STROMAL CALLA EXPRESSION IN PRIMARY BREAST CARCINOMA IN RELATION TO PATHOLOGICAL RESPONSE AFTER NEOADJUVANT CHEMOTHERAPY

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Abstract

Background: In this study we aimed to assess the IHC expression of stromal CALLA in invasive breast carcinomas as prognostic factor and correlate its relationship with six clinico-pathological parameters by assessing the pathological response to neoadjuvant chemotherapy

Method: Study design is cross-sectional, it conducted on 50 females with breast invasive ductal carcinoma, FFPE tissue blocks collected from the archive of laboratory of Al-Sader medical city - Al Najaf governorate/ Iraq, during the period from September 2023 to September 2024. CALLA immunohistochemistry expression detected by labeled polymers and enhanced polymer system (Dako En-Vision™ Flex) Dako-protocol. it was correlated with age of patients, tumor grade, hormonal expression, her-2/neo expression, Ki-67 expression and molecular profile.

Result: Stromal CALLA immunohistochemistry was expressed in 48%. There is statistically significant positive correlations between stromal expression and response to neoadjuvant chemotherapy ($P = 0.011$), most cases with positive CALLA expression either nonresponding to NAC or achieved partial response while most of CALLA negative cases achieved complete pathological response the same for her-2 positive cases, though statistically non-significant but most CALLA expressed cases were Grade III, her-2 enriched and triple negative molecular profile most of them had partial pathological response (not complete)

Conclusions: Stromal CALLA had been expressed in 48% of invasive ductal carcinoma and there is positive correlation between stromal CALLA expressions and higher tumor grades, her-2 expression and triple negative profile suggesting the effects of stromal CALLA expression on aggressive behavior of breast ductal carcinoma with decreasing a chance of complete pathological response after neoadjuvant chemotherapy

Key words: CALLA immunohistochemistry, CD-10, breast carcinoma, prognostic factor, neoadjuvant chemotherapy, molecular profile.

Introduction:

Breast carcinoma regarded as the 2nd most prevalent cause of cancer mortality in female also most frequent malignancy in women. In 2020, there were over 2.3 million newly reported illnesses and almost 685,000 fatalities, it is regarded as most prevalent malignant tumor in women⁽¹⁾. Thirty percent of females with early-stage BC still develop recurrent disease despite improvements in early identification and thorough therapy. It has a poor prognosis due to metastases and localized recurrences, that are the primary causes of unsuccessful therapy. This issue may be attributed to the biological characteristics and nature of the malignant cells⁽²⁾. It is a diverse illness that has unique inherent subgroups. Utilising the three distinct immunohistochemistry markers estrogenic-receptor (ER), progesterone-receptor (PR), and human-epidermal-growth-factor-receptor 2 (HER-2/Neu), Breast tumors diagnosed clinically are classified into one of four primary subtypes, which are treatable using targeted therapies: luminal A, luminal B, HER2-enriched and triple negative breast cancer (TNBC)⁽³⁾.

The tumor-node-metastasis (TNM) staging system is another extensively utilized classification criterion for identifying clinical aggressiveness of a cancer, predicting prognosis, and directing therapeutic approaches.⁽⁴⁾ Nevertheless, it is noteworthy to consider tumor malignant behaviors are affected not only by cancer cells but also by the tumor microenvironments around them⁽⁵⁾. Tumor micro-environments (TMEs) refer to the reciprocal interactions between stromal cells, tumor cells, and cellular components. Recent studies have demonstrated that TMEs not only influence the growth and spread of tumors, but also affect the biological characteristics of cancer cells⁽⁶⁾. The production of numerous nutrients, growth factors, chemokines, and cytokines by tumor stroma, a significant component of TME, aids in the development of tumors⁽⁷⁾.

Numerous cell types, involving fibroblasts, lymphocytes, epithelial, and mesenchymal stem cells, are present in these microenvironments. In this situation, many biological markers had been identified to divide patients into various subgroups depending on the traits of their tumor stroma. Various subsets of mononuclear-inflammatory-cells and cancer-associated fibroblasts have been distinguished by their production of matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases⁽⁸⁾.

Common acute lymphoblastic leukemia antigen (CALLA) is the name given to CD10, a zinc-dependent metalloprotease with a cell surface molecular mass of 90–110 kDa. The epithelial cells of several organs, like the prostate, colon, liver, and stomach, usually express this enzyme. By reducing the local quantities of peptide substrates accessible for receptor binding, It is well-established that this protease controls the biological functions of numerous peptide substrates. Much research shows that the level of CD10 expression in the stroma is related to how aggressively different epithelial cancers grow⁽⁹⁾.

Numerous studies have revealed a substantial correlation between dysregulation of CALLA expression and tumour growth and aggressiveness in a diverse spectrum of malignancies, including melanoma⁽¹⁰⁾, colorectal cancer⁽¹¹⁾, and nasopharyngeal cancer⁽¹²⁾. although it is still not obvious how CD10 is used for diagnosis or prognosis⁽¹³⁾. In vitro research has identified CD10 as the marker of breast stem-like or bipotent progenitor cells⁽¹⁴⁾.

The aim:

- To correlate CALLA expression with various clinicopathological parameters including age, grade, hormone expression, Her-2/neo, ki-67 and molecular profile.
- To assess the response to neoadjuvant chemotherapy in CALLA positive patient which gives hint about prognostic significance

Material and Method:

The study was design was a prospective cross-sectional study include Iraqi female patients with primary breast invasive ductal carcinoma. All data were kept private. No information was divulged and used for research only. Ethical committee for clinical studies with approval code MEC-198 authorized permission to conduct the study and it followed the tenets of the Helsinki Declaration.

The study was done in the Najaf governorate, department of pathology and Forensic Medicine / Faculty of Medicine - University of kufa

Selection of Cases

After applying inclusion criteria, 50 FFPE tissue blocks and slides were collected from laboratory of Al-Sader medical city - Al Najaf in a convenient way. All slides had been re-examined by two pathologists to confirm the diagnosis on H&E-stained slides, then confirm the ER, PR and HER-2 scoring on the tru-cut biopsy then reviewed the slides of mastectomy or breast conserved surgery after completing neoadjuvant chemotherapy 6-8 cycles

Inclusion criteria

- Core biopsy samples positive for invasive ductal carcinoma NST
- Complete biological profile ER, PR and HER2 with or without Ki67 on the tru-cut biopsy
- Patient scheduled for neoadjuvant chemotherapy.
- Resection after 6-8 cycles neoadjuvant chemotherapy to assess the pathological response

Exclusion criteria

- In adequate material (depleted paraffin blocks)
- with incomplete postoperative information
- Local recurrent tumors.
- Male gender.
- ILC
- Unknown molecular subtype (biological profile).

Slide Preparation:

One slide was prepared for each case on the treatment naive tru-cut biopsy, for staining with primary antibody CALLA immunohistochemistry. (FLEX Monoclonal Mouse Anti-Human CD 10 (Agilent Technologies Singapore International, Denmark))

Procedure of Immunohistochemistry by Envision Antibody Complex

The formalin-fixed and paraffin-embedded blocks were 4 μm thick, cut by a microtome, and mounted on the positively charged slides. Deparaffinization and rehydration, then antigen retrieving using heat induced antigen retrieving, blocking by endogenous enzymes to avoid background staining, primary antibody incubation for 30 minutes, polymer -bound secondary antibody also incubated for 30 minutes which enhance amplification and more chromogenic detection, finally counterstaining and mounting with DPX for visualization under microscope. to evaluate the expression of CALLA in breast carcinoma samples. (< 10% regarded as negative and equal or more than 10% of stromal membranous staining regarded as positive no matter the staining intensity).

Review of Immunohistochemical Scoring Systems

The already ER, PR and Her-2-stained slides were reviewed for scoring; hormone receptor status (ER and PR) is graded according to Allred scoring system while HER2 classification in breast cancer is determined by ASCO / CAP guideline criteria

Follow up data and response to neoadjuvant therapy.

All included patients were scheduled for neoadjuvant therapy. After course completion 6-8 cycles of chemotherapy, patients underwent breast conservative surgery or mastectomy and the pathological response was assessed following the Royal college guidelines {Ellis, 2016 #1507}.

1-Complete Pathological Responses, which could be a) No residual breast carcinoma. or b) No residual invasive malignancy but ductal carcinoma insitu present.

2-Partial Pathological Responses to therapy, which could be a) Minimal residual tumor/near total effects typically (e.g., <10% of tumor remaining in the tumour bed seen as foci of residual fibrosis demarcating the original tumor extents) or b)10%-50% of tumor clusters remaining or c) > 50 % of tumor cells remaining. Comparison with the previous core biopsy sample was helpful.

3-No evidence of response to therapy, most tumor cells still viable

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (IBM SPSS) software-25. data that observed were presented as frequency & percentages. The continuous variables were expressed as mean \pm standard deviations (SD) or range according to data distribution. Statistical comparisons were done using Chi-square tests or Fisher's exact test to assess proportions of nominal/ ordinal variables in different groups. P value of less than 0.05 was regarded as statistically significant

Results:

1- Study group characteristics:

In this study there are 50 cases of female with invasive ductal carcinoma, with a mean age of 49.53 years, standard deviation was 11 years and median was 50.3 year. Age range was between 29 to 71 years, 28 (56%) of cases being 50 years and older. Most of the tumours 37 (74%) were grade II. In almost two third 31 (62 %) of the cases, the estrogen receptors (ER) and progesterone receptors (PR) were positive. While, membranous HER2/neu was positive in 15 cases (30%). A high mitotic index (> 14%) was seen in 5 cases (10 %), low mitotic index (\leq 14%) was seen in 13 cases (26 %) and it was unknown for 32 (64%) of the cases.

Regarding molecular subtype, luminal-A accounted for 5 (10%), Luminal-B accounted for 13 (26%) but there are 15 (30%) of luminal cases were not classified as A or B (no Ki-67). HER2-neu enriched cases accounted 10 (20%) of the cases. While triple negative cases were the least accounted for 7 (14%).

2-Response to neoadjuvant therapy

Complete response was observed in 31 (62%) of the cases. More than a quarter 18 (36%) had partial response and one case showed no response, as further illustrated in Figure 1

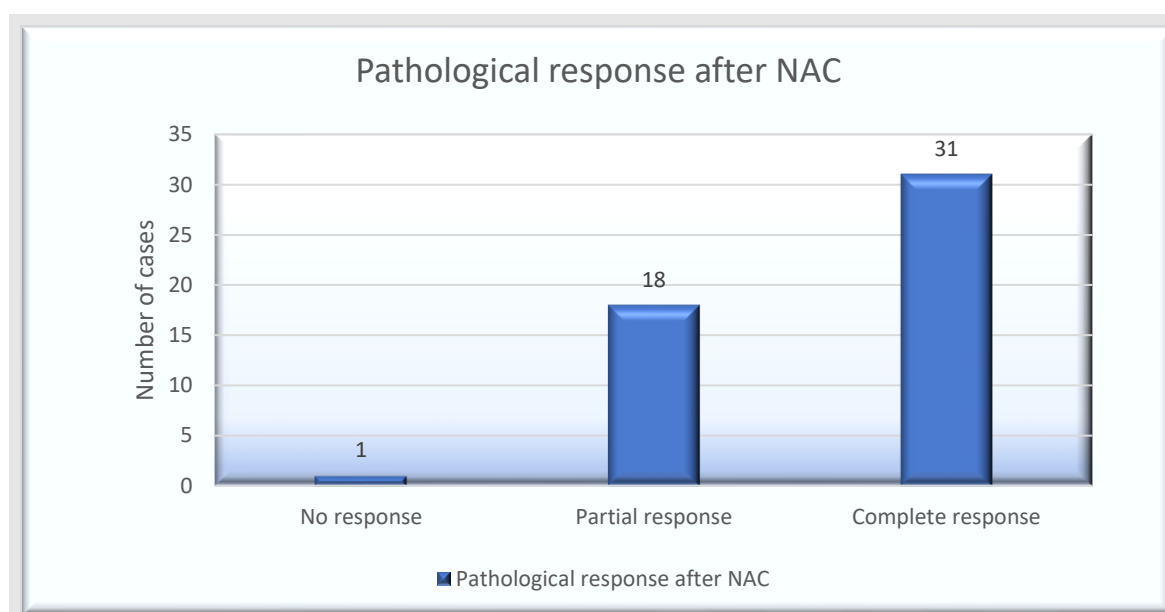


Figure 1: Response to neoadjuvant therapy.

Table 1 shows out of CALLA expressed tumors, 14 (77.8%) had partial pathological response compared to 4 (22.2%) of negative cases. Conversely, the majority of CALLA negative cases achieved complete pathological response 22 (70.97%) as compared to 9 (29.03%) of CALLA positive cases, $P < 0.011$.

Table 1: The association between CALLA expression and response to therapy

Response to NAT	Total	CALLA IHC testing		P value
		CALLA negative No. (%)	CALLA positive No. (%)	
No response	1	0	1 (100)	<0.011
Partial	18	4 (22.2)	14 (77.8)	
Complete	31	22 (70.97)	9 (29.03)	

3-Correlation between CALLA expression, clinicopathological parameters and response to therapy

3-1- Correlation with age:

For young patients (< 50 years old), 13 (59.1%) of them were CALLA negative and 9 (40.9%) were positive CALLA positive with p value 0.003. Malignancy of older female (≥ 50 years old) were more frequently CALLA expressed 15 (53.6%). As in Figure 2, the difference was statistically not significant.

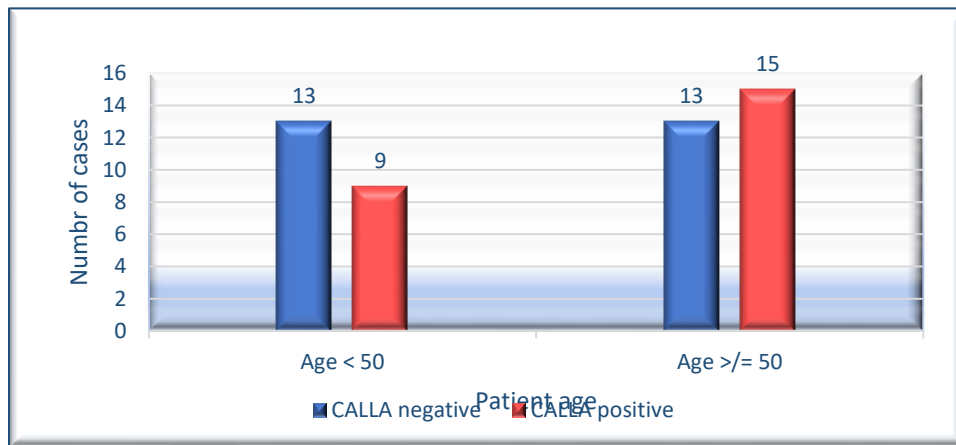


Figure 2: Correlation of patients age with CALLA expression

The correlation between responses to NAC therapy and CALLA expression in both age groups explained in Table 2. Young patients with CALLA positive malignancy revealed a significant correlation with partial response in 6 patients (85.7%) in comparison with those who are negative 12 (85.7%) of them achieved complete response, P=0.003.

Despite statistically not significant (p value 0.102) but older patient had a similar pattern. The majority of them who achieved partial responses 8 (72.7%) had positive CALLA staining while about two-third of those with complete pathological responses 10 cases (58.8%) had negative CALLA staining.

Table 2: The correlation between patient age and response to neoadjuvant chemotherapy with CALLA expression

Age	Response to NAT	Total	CALLA stromal expression		P value
			Not Expressed Number (%)	Expressed Number (%)	
<50	No responses	1	0	1 (100)	0.003
	Partial responses	7	1(14.3)	6(85.7)	
	Complete	14	12 (85.7)	2 (14.3)	
≥50	Partial	11	3 (27.3)	8 (72.7)	0.102
	Complete	17	10 (58.8)	7 (41.2)	

3-2-Correlation with tumor grade:

Most of patients with grade III tumours, were CALLA positive 10 (83.3%), while 2 (16.7%) were CALLA negative. Out of the grade II tumours, 14 (37.8%) were CALLA positive, whereas the remaining 23 (62.2%) were negative. The single (100%) grade I case had negative staining. Grade III tumors had a significantly higher rate of CALLA expression, Figure 3.

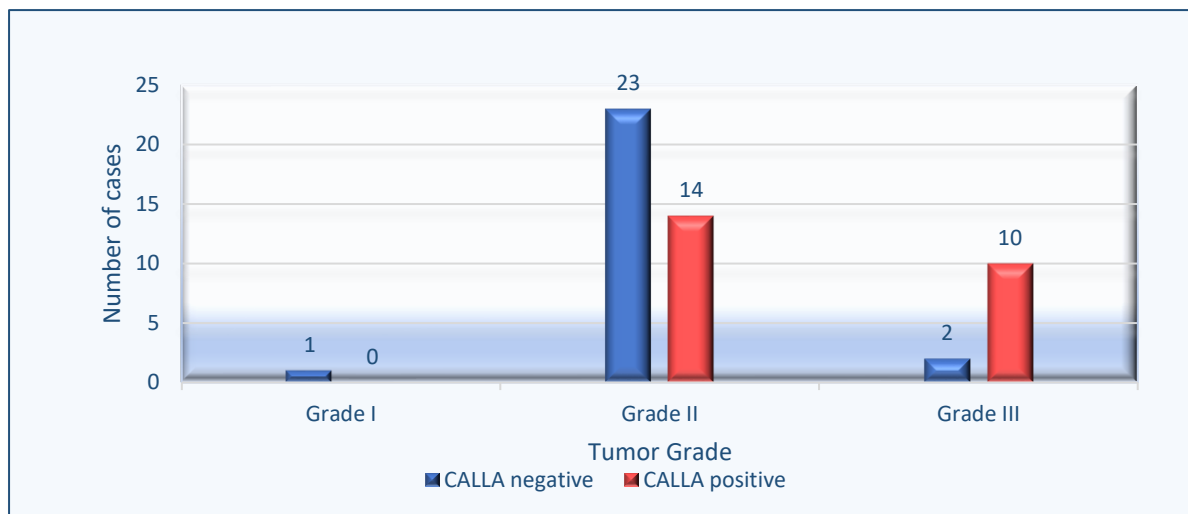


Figure 3: Correlation between tumour grade and CALLA expression

The correlation between response to neoadjuvant therapy and CALLA expression in each tumour grade was illustrated in Table 3. The single grade I patient who had a complete pathological response was CALLA negative. In GII patients, tumours with expressed CALLA had a lower rate of complete pathological response in 8 (29.6%) in comparison to those with negative staining 19 (70.4%). In contrast, CALLA positive achieved partial response 6 (60%) in comparison to CALLA negative tumors 4 (40), P value= 0.190. Regarding Grade III although all cases with CALLA expression achieved only partial responses 8 (100 %) and only 2 cases (66.7) of CALLA negative reached to complete pathological response, the difference did not reach statistical significance (may be due to small sample size).

Table 3: The correlation between tumour grade and pathological response to neo-adjuvant therapy with CALLA expression

Tumour grade	Response to Neoadjuvant therapy	Total	CALLA expression		P value
			CALLA negative No (%)	CALLA positive No (%)	
I	Partial	0	0	0	-
	Complete	1	1 (100)	0	
II	Partial	10	4 (40)	6 (60)	0.190
	Complete	27	19 (70.4)	8 (29.6)	
III	No response	1	0	1 (100)	0.147
	Partial	8	0	8 (100)	
	Complete	3	2 (66.7)	1 (33.3)	

3-3- Correlation with Hormonal receptors, Her-2/neo and Ki-67:

The status was significantly different. Approximately two-thirds of CALLA expressed tumors 14 (82.4%) (77.8%) were hormonal negative (ER and PR negative). In contrast, most of CALLA negative patients 23 (69.7%) were Estrogen Receptor (ER) positive and 22 (68.8%) were Progesterone Receptor (PR) positive, P-value < 0.001. as in Figure 4 and Table 4.

Table 4: The correlation between ER, PR, Her-2/neo and Ki-67 with CALLA expression.

Hormone status		Total	CALLA expression		P value
			CALLA negative No (%)	CALLA positive No (%)	
ER status	Negative	17	3 (17.6)	14 (82.4)	<0.001
	Positive	33	23 (69.7)	10 (30.3)	
PR status	Negative	18	4 (22.2)	14 (77.8)	<0.001
	Positive	32	22 (68.8)	10 (31.2)	
Her2/neo	Negative	35	29 (82.8)	6 (17.2)	0.042
	Overexpressed	15	2 (13.3)	13 (86.7)	
Ki 67	≤14%	13	6 (46.2)	7 (53.8)	0.492
	>14%	5	4 (80.0)	1 (20.0)	
	Unknown	32	13 (40.6)	19 (59.4)	

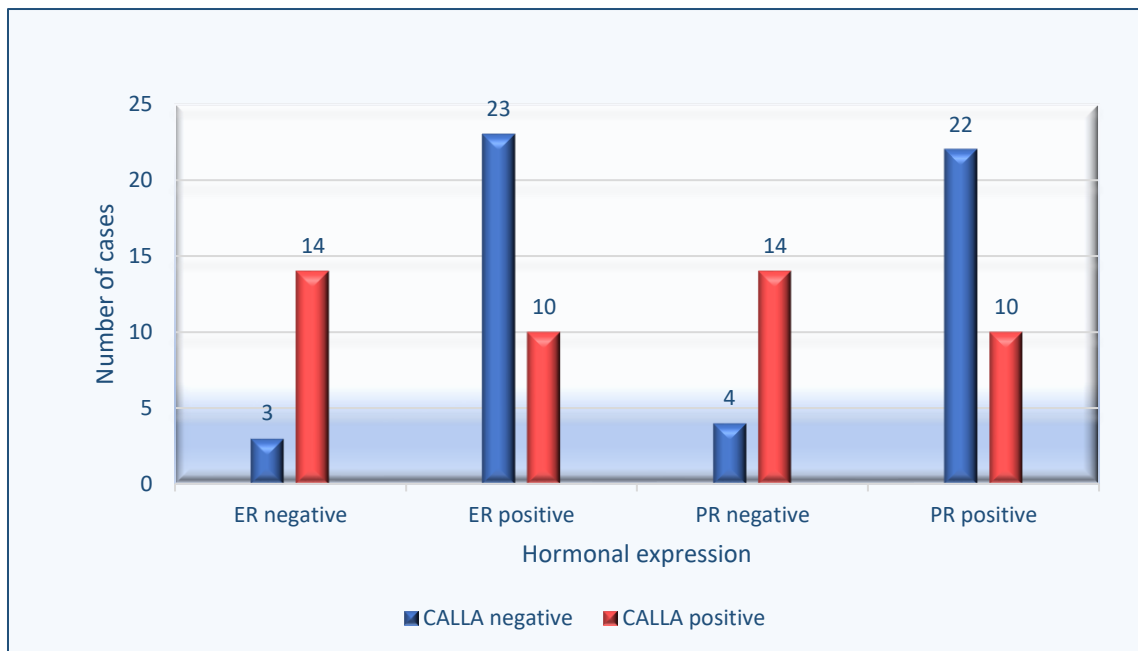


Figure 4: The correlation between hormone receptors ER and PR with CALLA expression, P-value < 0.001.

Regarding Correlation with Her2/neo; A statistically significant correlation was observed between HER2/neo expression and CALLA expression, as in Table 4 and Figure 5. Most patients with overexpressed Her-2/neo were CALLA positive 13 (86.7%); While patients with negative

CALLA expression were significantly associated with HER2 negative expression 29 (82.8%), P=value = 0.042.

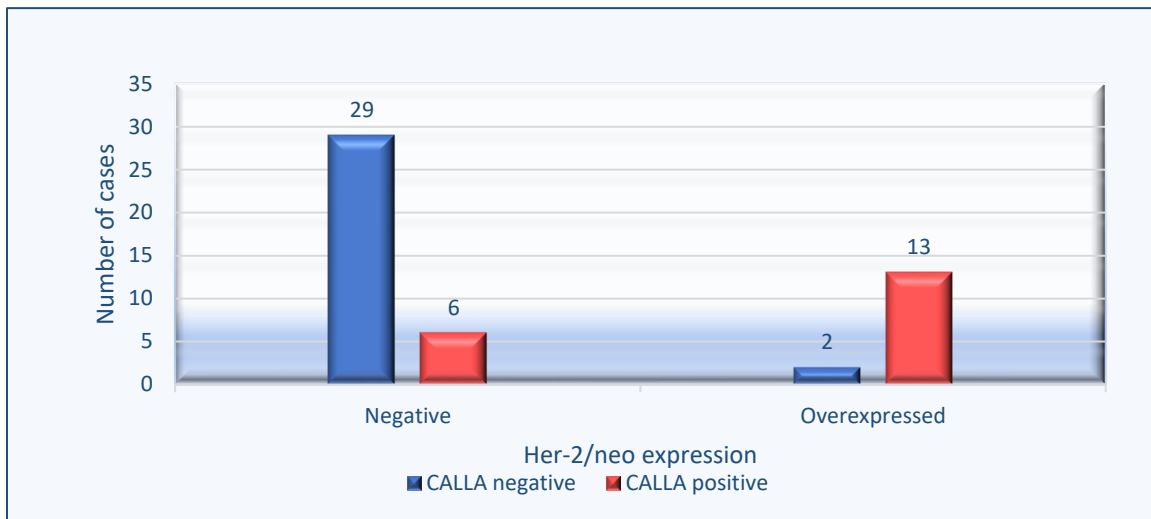


Figure 5: The association between HER2 neu expression and CALLA expression.

While with low Ki-67 index there were no significant differences in the mitotic index between CALLA positive and CALLA negative patients, however there were 32 cases (64%) tumours with unknown Ki-67 expression, 19 (59.4%) of them were CALLA positive. Almost the same proportions of CALLA positive cases 7 (53.8%) and negative cases 6 (46.2%) revealed low mitotic index < 14%.

Despite CALLA negative cases showed a higher mitotic index 4 (80.0%) versus one case (20.0%) the difference was statistically not significant even after exclusion of tumors with unknown Ki-67 expression. The details are shown in Table-4 and Figure-6.

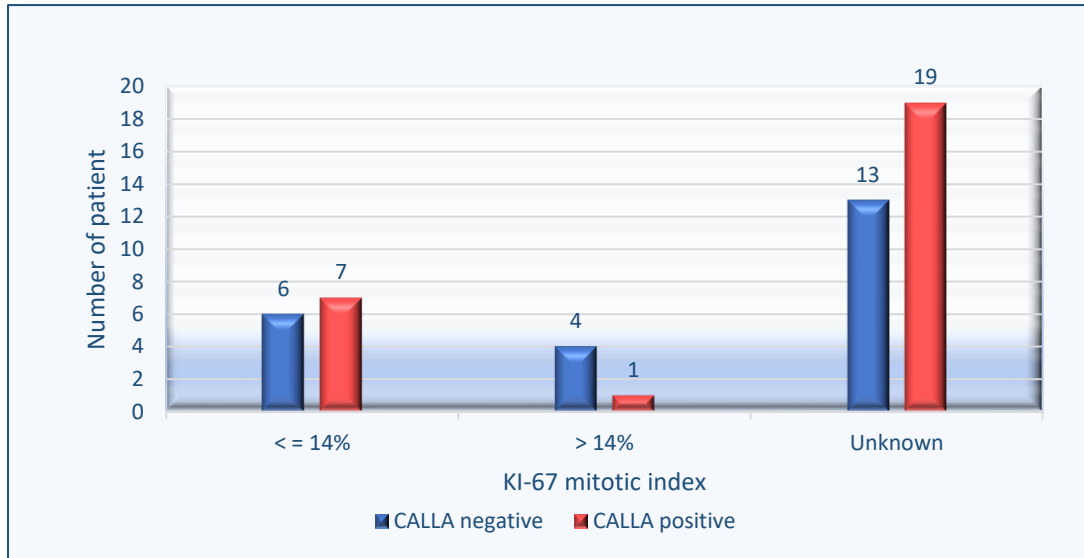


Figure 6: The correlation between Ki67 proliferation index and CALLA expression

3-4 Correlation with Molecular profiles:

A strong association was noted between both HER2/neu-enriched carcinomas and triple negative carcinomas with CALLA expression (P-value =0.016). Table 5 reveals 8 out of 10 HER2/neu enriched carcinomas (83.3 %) and 6 (85.7) out of 7 triple positive carcinomas were CALLA positive, whereas only one (20%) out of 5 of luminal A and 2 cases (13.3%) out of 15 luminal unclassified carcinomas (16.7 %) were CALLA positive, As in Figure 7.

Table 5: The correlation between molecular subtypes of breast carcinoma with CALLA expression

Molecular subtypes	Total	CALLA expression		P value
		CALLA negative No (%)	CALLA positive No (%)	
Luminal-A	5	4 (80)	1 (20)	0.016
Luminal-B	13	6 (46.2)	7 (53.8)	
Luminal unclassified (unknown A or B)	15	13 (86.7)	2 (13.3)	
HER2/neu enriched	10	2 (16.7)	8 (83.3)	
Triple negative	7	1 (14.2)	6 (85.7)	

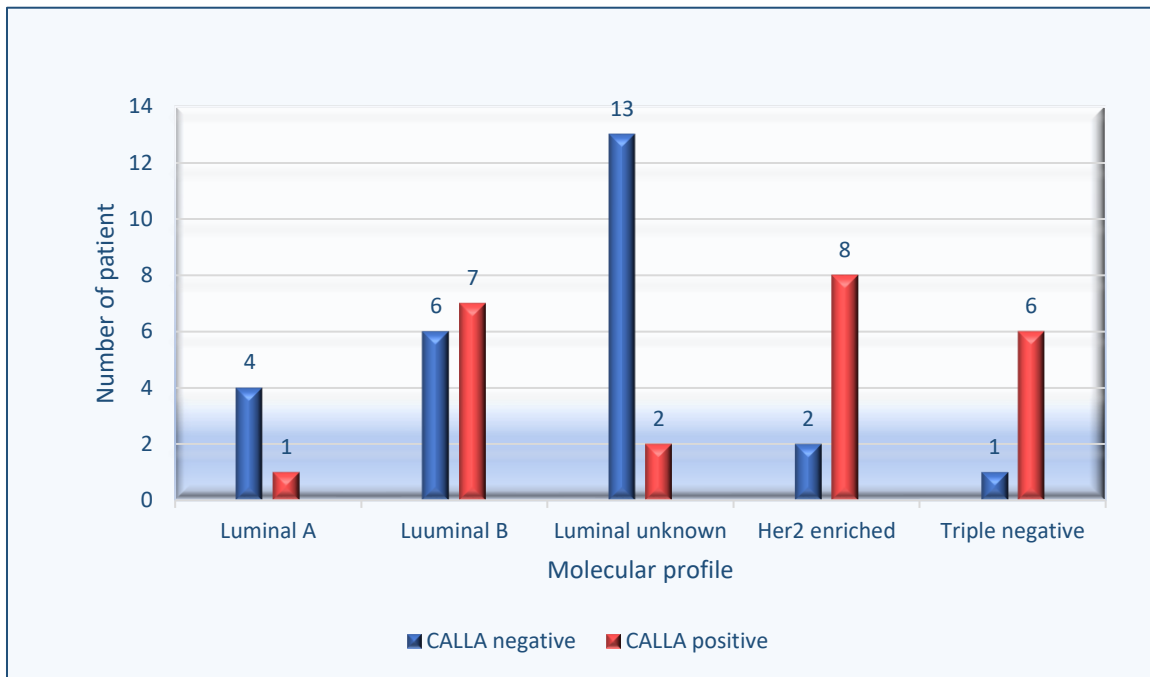


Figure 7: The correlation between molecular subtypes and CALLA expression, *P-value=0.016

3-5- The correlation response to neoadjuvant therapy and CALLA expression in each molecular subtype:

There is complete pathological responses in the patients with CALLA negative tumors in all luminal-A tumors 3 (100%), most Luminal-B cases 5 (55.6%) and luminal unclassified 11 (91.7%) with nonsignificant P value. In her-2/neo all cases 2 (40%) of negative CALLA expression showed complete pathological response while most of positive cases 3 (60%) achieved partial response, in cases with triple negative subtypes, the single case who didn't achieve any response to neoadjuvant therapy was CALLA positive, while all cases with positive CALLA expression showed jut partial pathological rponse 4 (100 %) in compassion to 1(50%) achieved complete response. As showed in Figure 8, and Table 6.

Table 6: The correlation between molecular subtypes and responses to neo-adjuvant therapy with CALLA expression

Molecular subtypes	Response to NAT	Total	CALLA expression		P value
			CALLA negative No. (%)	CALLA positive No. (%)	
Luminal A	Partial	2	1 (50)	1 (50)	0.819
	Complete	3	3 (100)	0 (0)	
Luminal B	Partial	4	1 (25)	3 (75)	0.676
	Complete	9	5 (55.6)	4 (44.4)	
Luminal (unknown A or B)	Partial	3	2(66.7)	1 (33.3)	0.849
	Complete	12	11 (91.7)	1(8.3)	
HER2/neu rich	Partial	5	0	5 (100)	0.429
	Complete	5	2 (40)	3 (60)	
Triple-negative	No response	1	0	1 (100)	0.537
	Partial	4	0	4 (100)	
	Complete	2	1 (50)	1 (50)	

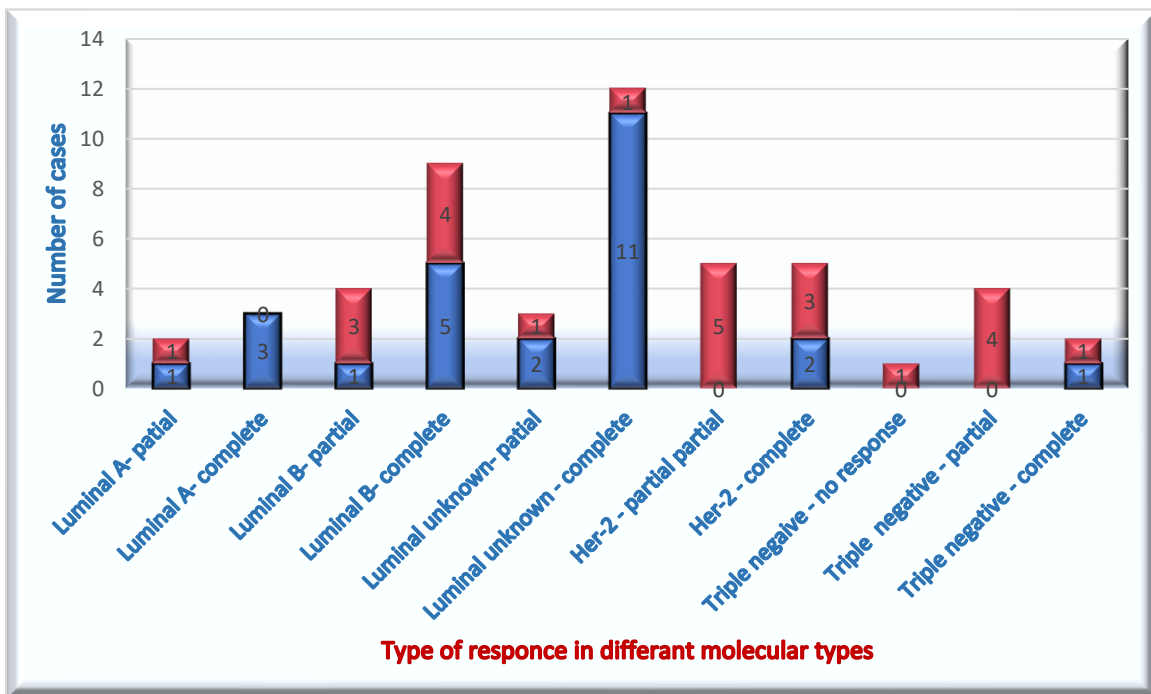
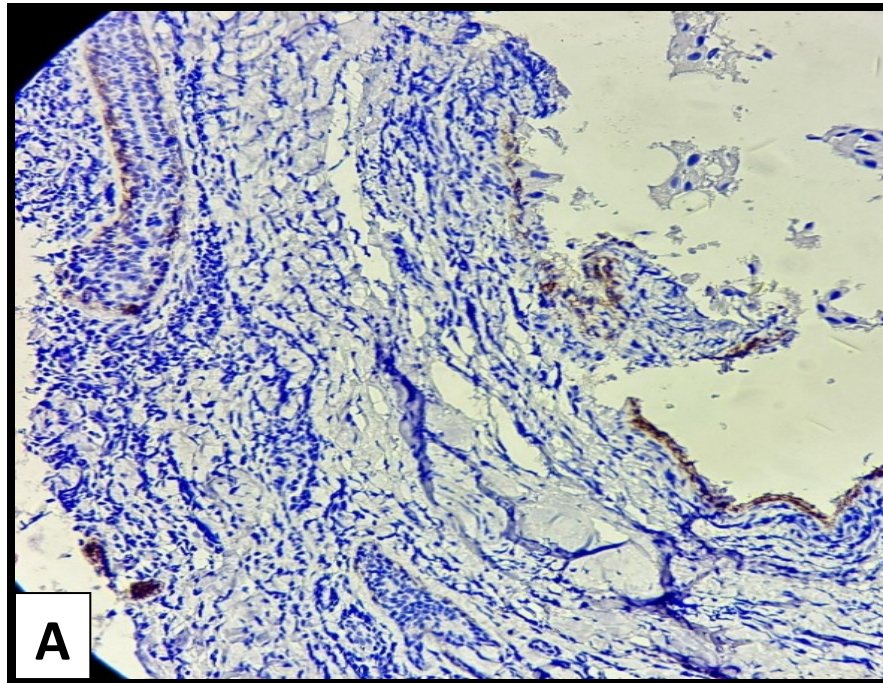
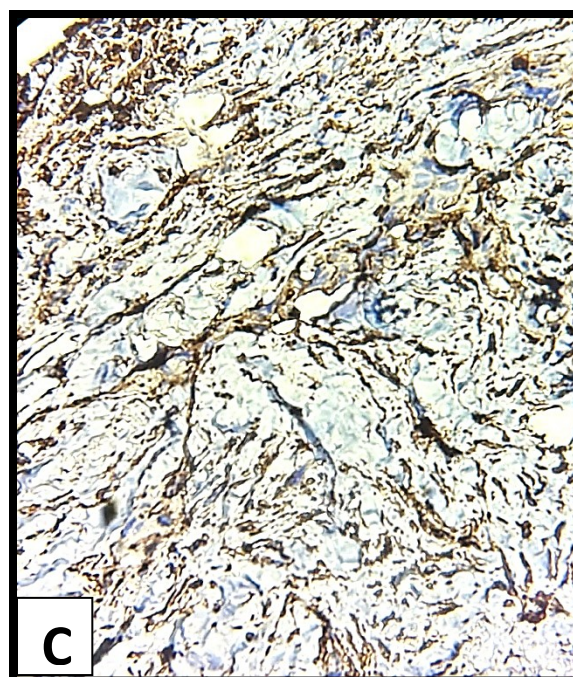
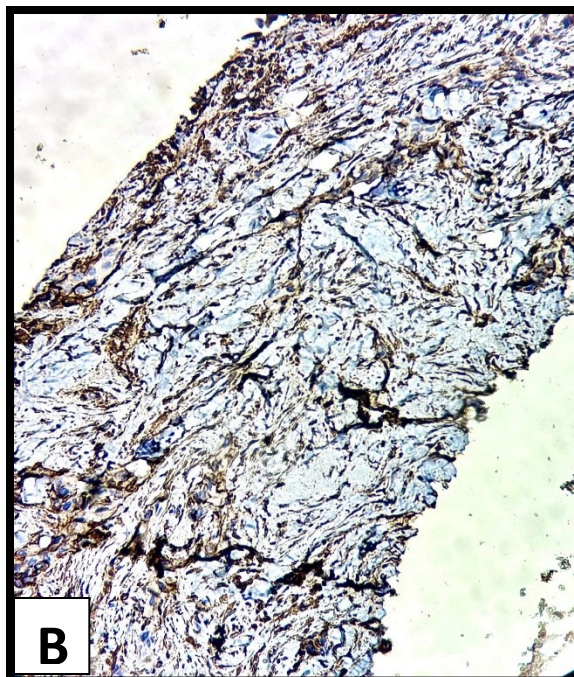


Figure 8: The association between molecular groups and response to neoadjuvant therapy with CALLA expression.



Figures 9: (A- magnification power is 10x) Histological section of CALLA negative immunohistochemistry in the struma (less than 10% of stroma is positive)



Figures 9: (B-magnification power is 20x C-magnification power is 40x) Histological sections of CALLA positive immunohistochemistry in the struma (More than 10% of stroma is positive)

Discussion

Despite the fact that breast carcinoma is epithelial in origin still the stroma plays a role in carcinogenesis and prognosis, many articles revealed that stromal CALLA expression is associated with more aggressiveness and poor survival in patient with breast carcinomas. But the correlation between CALLA expression and response to neoadjuvant chemotherapy is not well established. So, the optimal integration of many important prognostic factors remains a researcher's field, our data included six parameters (age, grade, hormone receptor status, epidermal growth factor receptors (Her-2/neo), mitotic rate and molecular profiles, which have been accepted worldwide as prognostic parameters⁽¹⁵⁾. In the current study, we did immunohistochemistry of CALLA marker on 50 FFPE (Formalin Fixed Paraffin Embedded) tissue blocks and detect the staining microscopically for 50 patients with invasive ductal breast carcinomas. The cut-off score in previous studies for CALLA (CD10) was 10%, 20% or 30 % of the stroma with or without assessment of intensity^(10, 11, 16, 17, 18). these cut off points were used to categorized the tumors into CALLA (CD10 negative CD10 positive); or categorized the CALLA-positive cases into weak & strong positive subgroup. In this study we depend on the same basis with the first cut off point which is regarded as negative (CALLA < 10%), and $\geq 10\%$ regarded as positive regardless the intensity of staining. Despite the CALLA expression had been suggested as important prognostic marker, its expression was very variable in previous studies, it was ranged from 18% (20 /110)⁽¹⁷⁾ to 49.50% (50 /101)⁽¹¹⁾ to 79.50% (205 /258)⁽¹⁰⁾, while in our study the stromal CALLA expression was 48 % among females with IDCs (24/ 50 patient).

Patient and tumour characteristics

In our study the average age of the patient was 49.52 ± 11 years 44% of them were < 50 years old. Which agree with the previous Iraqi studies of epidemiology. In a large cross sectional study included 1093 patient from all governorates of Iraq the median age was 46.40 ± 9.5 ⁽¹⁹⁾. Alwaan et al in (2010), reported that out of 721 patients with breast carcinoma, 580 (80.40%) of them were younger than 60. (54.1%) of them were in the pre-menopausal age group and (22.20 %) were younger than 40 years old⁽²⁰⁾. A recent Kurdistanian study for 429 patients with breast carcinoma revealed that the average age was 49.6 ± 10 years and (53.80) of females were of middle age or younger⁽²¹⁾. According to WHO estimation, about 50% of malignancies in middle east area

occur below the age of 55. As the exposure to the risk factors increase, the age- incidence rates of all malignancy in this area are projected to double ⁽²²⁾. This is differ the reports obtained from developed countries and western nations, which estimated that the peak incidences rates tend to occur decades later ⁽²³⁾.

In the current study the majority of patients 37 (74%) had Grade-II breast carcinoma and about quarter of patients had higher grade disease. Similarity was illustrated by Abdulkareem et al in Iraqi local study ⁽²¹⁾, Nevertheless, American cancer statistics reported a higher prevalence of Grade-I breast carcinoma (21%) and Grade-III (29%) ⁽²⁴⁾. Enviromental and racial factors were established to affect tumour development and behaviour ⁽²⁵⁾.

In this study the examined ER, PR, Her-2/neu and Ki-67 categorized the malignancies as, Luminal-A (10%), Luminal-B (26%), Her-2/neu enriched (20%), and lastly triple negative breast carcinoma (14%). However, there is (30%) of luminal groups categorized as luminal unclassified (Luminal A or B) because absent information about Ki-67. Studies that addressed the molecular classification of breast cancer in Iraqi patients are inconsistent, with variable variation in the Luminal-A and Luminal-B destribution ^(26,27,28).The range of Luminal-A group was between 29 % ⁽²⁸⁾, 45 % ⁽²⁷⁾, and 56 % ⁽²⁶⁾ While the range of Luminal-B group was between 14 % ⁽²⁷⁾, 17 % ⁽²⁶⁾, and 35 % ⁽²⁸⁾. All the Luminal cases in our study were 30 case (60 %) which is within the same range of previous publications. But the Her-2/neu rich cases relatively had higher rate when compered with other local and interaactional studies in which the range was 3 % to 10.30 % ^(24, 26-28).

Relation with CALLA expression

In our study, <half of the cases (48%) were CALLA positive, a zinc-dependent metalloproteinase that is frequently detected on mature neutrophils, pro-B lymphoblasts, bone marrow lymphoid stem cells, renal cell carcinoma, endometrial stromal sarcoma, and pro-B lymphoblasts ⁽¹⁷⁾. it has a role as a regulator of healthy mammary progenitor cells as well as in the degradation and remodeling of extracellular matrix components ^(29,30). It has been established that this molecule is involved in the processes of cell adhesion, migration. It has been shown that tumors with CAFs expressing CALLA (CD10) were more aggressive with rapid progression and inferior prognosis ⁽²⁹⁾. In breast cancer, high CALLA expression has been associated with hormone receptor

negativity and HER-2/neu overexpression. Furthermore, it has been observed that neoadjuvant anthracycline-based chemotherapy induces alterations in the dynamics of stromal CD10 expression (31).

There is discrepancy in CALLA expression which may be partially due to variation in the characteristics of each study group specially the compelling evidence that CALLA expression has a relation to age; the significant association with age may be unnoted in the current study due to smaller sample size⁽³²⁾.

CALLA positive cases were significantly correlated with higher tumor grade (grade-III), negative hormonal expression (ER and PR) and Her-2/neu overexpression. Which agree with other study that correlate with tumor grade such as Boler AK et. al. ⁽³²⁾. Regarding tumor phenotype, Puri V et. al (2011) and Diem Thi-Ngoc Vom, et al indicated, in agreement with our findings, a significant association of CALLA expression with low estrogen receptor and progesterone receptor expression, HER2 immunohistochemistry 3+ and also high Ki-67 ^(33, 34)

In comparison to CALLA negative, the positive cases were significantly less in Luminal-group including luminal unknown (unknown A or B) 23 case vs 10 (46% vs 20%) and more in Her2/neu enriched group (16.70 vs 83.30%). Additionally, 85.7% of triple-negative tumors were CALLA positive. A good deal of studies failed to demonstrate a statistical association between CALLA expression and molecular subtype. But there is scattered studies found a significant correlation between CALLA expression and molecular classification ⁽³⁵⁾. Tahani Louhichi et-al. (2018) demonstrated that CD10 expression usually associated with her-2 enriched and triple negative breast cancer ⁽³⁵⁾

CALLA expression and responses to neoadjuvant chemotherapy

The current study reveals a significant correlation between CALLA expressed carcinoma and limited (partial) responses to NAC (neoadjuvant chemotherapy) in contrast to complete pathological response (P=0.011) in CALLA negative cases. The association between positive cases and partial pathological response was significant in young patients < 50 years old (P value was 0.003), Her-2 expressed carcinomas (86.7%) (P value 0.042), and molecular subgroup (P = 0.016). this was agreed with A.K. Boler, et al (2021) who observed CALLA positivity was significantly

associated with high-tumor grade (P value < 001) and odd ratio 26.00⁽³²⁾, Sayantan H. Jana et-al found a significant correlation between negative expression of CALLA prior to NAC and a higher responses in 375 Her2/neu negative cases. This significant relationship persist in Estrogen receptor negative subgroup⁽³⁶⁾

CD10 expression in the stroma was found to be significantly associated with higher grade of tumors (P value= 0.04), increased mitotic activity (P value- 0.33), worsened prognosis (P value= 0.01), Estrogen Receptors negativity (P value= 0.0001), Her-2/neu positivity (P value =0.19) and finally with molecular classification (CD10 expressed in HER2/neu enriched subtype, and CD10 was negative in Luminal subtype)⁽³⁷⁾

Limitations of the Study:

Small sample size was an issue in our study. Only representative blocks with known molecular profile were subjected to IHC; as a result, there may be some variation in CD10 expression because of the heterogeneity of the tumor. Lack of follow-up at current time which may be done later on to connect with progression-free survival and overall survival

Conclusions:

1. CALLA expression can be regarded as adverse prognostic marker expressed in higher tumors grade, tumors with Her2/neu overexpression and tumors that don't express Hormones receptors (ER and PR negative breast carcinomas)
2. CALLA positive cases had been associated with partial response to neoadjuvant chemotherapy, while tumors that had negative CALLA expression achieved pathological complete response to NAC
3. Statistically significant association between CALLA expression and partial pathological response in patients who were younger than 50 years old
4. Consequently, we discovered that the molecular subtype is closely related with CALLA expression and concluded that CALLA expression was more frequent in Her2neu positive and triple negative cases compared to luminal A and luminal B subtype.

Recommendations:

Study the association between CALLA expression and progression-free survival and overall survival.

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