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Invasive breast carcinoma: Correlation with the molecular subtypes and pathological response to neo-adjuvant chemotherapy

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Abstract

Introduction: Breast cancer is the most commonly occurring cancer in females and leading cause of cancer related deaths worldwide. The analysis of gene expression data have suggested that breast carcinoma can be divided into molecular subtypes which have distinct clinical features, different prognosis and clinical outcome. Claudins are members of a large family of tight junction proteins that regulate cell adhesion. Currently there are 40 claudins that are variably expressed in several cancers. Only few studies have examined their expression in breast carcinomas. Recently subtype of claudin with low expression has been described that has a prognostic and predictive indication in relation to response following neoadjuvant chemotherapy.

Aim: The aim of this study was to classify breast tumors into the molecular patterns based on the expression of estrogen receptor, progesterone receptor and her-2 neu and to evaluate the expression of claudin, ki-67 and p-53 in invasive breast carcinoma. Pathological response following neoadjuvant chemotherapy was assessed in different molecular patterns of invasive breast carcinomas correlating with expression of claudins.

Materials and Method: A retrospective analysis of 100 breast carcinomas immunostained with ER, PR and her-2-neu were performed and tumors were subtyped into molecular patterns. Immunostaining with ki-67 and p-53 was done in 43 cases to assess the pathological response to neoadjuvant chemotherapy. Claudin 1, 3, 4, and 7 was tested in 43 cases by RT-PCR method.

Results: Of 100 cases of invasive breast carcinomas diagnosed and immunostained during the 5-year period from 2012-2016, basal-cell type accounts to 20 cases, followed by her-2 enriched type (17 cases), luminal -A (47 cases) and luminal-B (16 cases). Claudin was expressed in luminal A (44.2%), luminal B (26%), basal (16.3%) and her-2 enriched (14%) subtype. Incidence of claudin low was seen in 14% of T2-4 lesions, 7% nodal metastasis and 9.3% of high grade tumors. Pathological response was better for basal-like type when compared to other subtypes.Ki-67 expression was high among triple negative tumors when compared to non-triple negative tumors. Pathological response was good in basal-like subtype when p-53 was expressed.

Conclusion: The histopathological examination of the tumor helps in assessing the response following neoadjuvant chemotherapy and can be correlated with expression of claudin, ki-67 and p-53.Claudin-low subtype can be seen in triple negative, luminal A& B and her-2 enriched type. More number of cases need to be studied in future to assess the response rate in claudin –low tumors as this has direct impact on current therapeutic strategies and follow-up is mandatory to look for recurrences.

Key words: Breast Carcinoma, Molecular Patterns, Claudin, ki-67, Pathological Response.

Introduction

Breast carcinoma represents 15% of cancer related deaths in women second only to lung carcinoma as a cause of cancer death.[(1)] It is a heterogeneous group of tumors with a variety of morphologic features.[(2,3)] This clonal heterogeneity confers resistance to chemotherapy and radiotherapy. In 2009, Parker and colleagues[(5)] developed an efficient 50-gene classifier, called Prediction Analysis of Microarray (PAM50).[(4,5,6)] Global gene expression profiling studies have produced a new molecular classification of breast carcinomas with four distinct subtypes luminal A& B, her-2 enriched, basal like and normal breast like.[(7-10)] Each of these subtypes has unique biologic and prognostic features. As microarray gene expression analysis is not routinely available, breast carcinomas can be classified based on immunohistochemistry. Triple negative breast carcinoma represents 10-20% of breast carcinoma and are associated with young age at diagnosis, more advanced disease, stage, higher grade, increased mitotic rate and BRCA1 mutation and poor prognosis.[(6,11,12,14)] Recent gene expression studies have identified a novel claudin low subtype of breast carcinoma that has adverse prognostic factor with high risk of recurrence especially with triple negative breast carcinoma.[(15,16,17)] Triple negative tumors have significant biological heterogeneity and hence need exists to develop targeted and less toxic therapies for these subtypes.[(11,15,18,19)] The pathological complete response(PCR) after neoadjuvant chemotherapy is a surrogate marker for a favourable prognosis in breast carcinoma patients.[(10,20,21)] Ki-67 has predictive and prognostic value in patients receiving neoadjuvant chemotherapy.[(22,23)] The distribution of TP-53 mutation is linked to the molecular subtypes and correlated with the pathological response to chemotherapy.[(35)]

Materials and Method

100 cases of breast carcinoma immunostained with ER, PR and her-2 neu were classified based on their molecular patterns and subtyped into 4 molecular patterns as follows:[(10)]

Luminal A & B: ER positive, PR positive, her-2 neu negative

her-2 positive: ER negative, PR negative, her-2 neu positive

Basal-like: ER, PR and her-2 neu negative
Normal breast like

Immunohistochemical staining was performed using the Thermoscientific/lab vision monoclonal mouse antibody for estrogen receptor (clone:ER-SP1), progesterone receptor (clone:PR-SP2) and her-2 neu (clone:C-erb2-SP3) diluted with phosphate buffered saline(PBS). The latter 43 cases were selected and tested for claudin 1,3,4,7 by RT-PCR method .ki-67 and p-53 was performed in all the 43 cases to assess the response to therapy. Immunohistochemical staining for Ki-67 was performed using Dako mouse anti Ki-67 monoclonal antibody; clone (MIB-1:M7240) pre-diluted in phosphate buffered saline (1:25 dilution) on both control and test sections according to manufacturer's instructions. Staining for p-53 was performed using biogenex monoclonal p-53 mouse antibody; clone (DO7) diluted in PBS. ER and PR stain were considered positive if staining was seen in more than 1% of tumor nuclei. For her-2 neu status tumors were considered positive if scored as 3+ according to the guidelines of the American Society of Clinical oncology/College of American pathologists.[2,7,14,24)]

Tumors with low expression of all claudins were defined as low claudin. Pathological complete response to neoadjuvant chemotherapy was studied using histopathological parameters in 13 cases with claudin expression.

Results

Four hundred and thirteen cases of breast lesions were diagnosed during the 5-year period from 2012-2016. Of these benign fibroadenoma accounts to 190 cases, followed by malignant breast lesions (136 cases), fibrocystic changes (43cases), gynecomastia (28 cases), abscess (7 cases), phyllodes tumor (6 cases), tuberculosis (2 cases) and one case of duct papilloma. Out of 136 malignant cases, infiltrating ductal carcinoma (IDC) accounts to 132 cases followed by one case each of invasive lobular carcinoma (ILC), mixed carcinoma with ductal and lobular features, medullary and metaplastic carcinoma. Tumors were graded and scored according to the modified Bloom Richardson grading system into grade1-3. Tumors treated with neoadjuvant chemotherapy were followed by mastectomy and pathological staging (ypTNM) was done based on the tumor size, nodal involvement and clinical metastasis. Table1 depicts the distribution of cases pertaining to clinicopathological parameters. Immunohistochemical staining for ER, PR and her-2-neu was performed on 100 cases and subtyped into basal like (20cases); luminal A (47cases); 16 cases of luminal B and 17 cases of her-2 enriched subtype. (Fig. 1)Neoadjuvant chemotherapy was given to 29 patients. Testing with claudin 1,3,4,7 was done by RT-PCR method for 43 patients and was correlated with molecular patterns and clinicopathological parameters (Table 2, Fig. 2). Out of 43 cases 19 cases (44.2%) were luminal A, 11(26%)were luminal B, followed by 7 cases (16.3%)of basal like and6 cases(14%) of her-2 neu pattern(p value<0.002). Low proliferative index was seen in 14 cases of luminal A and high proliferative index was seen in 6 cases of basal-like subtypes. (Fig. 3) In our study we examined the expression of claudin 1.3, 4 and 7 in 43 cases in order to characterize the claudin low group. Tumors were categorized into low normal and high for each type of claudin. Chi-square analysis revealed that the luminal A contain significantly more tumors that were claudin positive (19 cases; p<0.002) and were statistically significant. Low claudin was seen in 4 cases of basal like subtype, 1 case each in her-2 enriched and luminal B subtypes and none in luminal A type. Out of 7 triple negative cases claudin expression was low in only 4 cases. High claudin was observed in two cases of luminal subtype. Out of 43 cases, claudin expression seen in 97.7% of tumors were categorized into T2-T4, 44.2% were positive in lymph nodes and 11.6% metastasized. (Fig. 4). Incidence of claudin low in T2-4 is 14% and 7% with nodal deposit. Among these groups, neoadjuvant chemotherapy was given to 13 cases and subsequent pathological clinical response was assessed. (Fig. 5) ki-67 is an independent prognostic and predictive parameter. Cut-off was calculated as 15% in our study and correlated with pathological clinical respose. The histopathological evidence of chemotherapeutic respose was graded from H&E sections on the basis of parameters used by Chevallier[1,25)] According to chevalier system it is divided as follows:

PCR (pathological complete response): Defined as disappearance of all the tumor or DCIS in breast with no invasive carcinoma and negative lymph nodes.

PPR (pathological partial response): Defined as presence of invasive carcinoma with stromal alterations.

PNR (pathological no response): Defined as little modification in original tumor appearance

Only invasive carcinoma and lymph nodes were graded based on this criteria. Lymphovascular emboli and in-situ were noted separately. Pathological complete response was studied in 13 cases using histological parameters by assessing the cellularity and scored as low, intermediate and high; sclerosis, necrosis, lymphocytic infiltrate and hemosiderin laden macrophages (Fig. 6, Fig. 7).[1,19,20)] The mean age of the study population was 47 years with a range between 33 and 58 years. In our study out of 13 cases none of them had pathological complete response. All the cases were invasive ductal carcinoma. Pathological partial response was assessed in 9 cases (<50yrs) and 4 cases (>50yrs). T2 lesions <5cm were 5 in number and 8 cases were T3 lesions >5cm in size. In our study claudin expression was normal in metaplastic carcinoma. Ki-67 was performed in all 43 cases to assess the response to therapy. Tumors with high proliferating index and p-53 expression are associated with better pathological response in basal-like subtypes. (Fig. 8, Fig. 9, Fig. 10, Fig. 11)

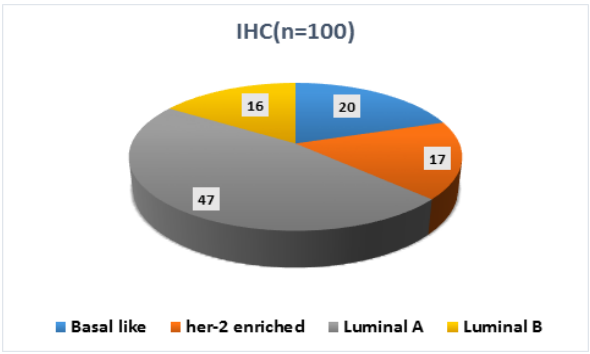

Fig. 1: Distribution of molecular patterns based on immunohistochemical markers

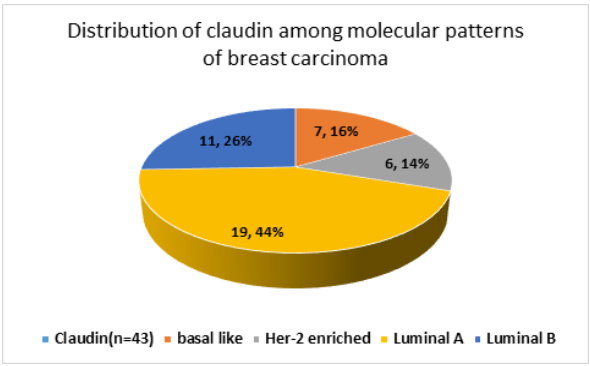

Fig. 2: Distribution of claudin among molecular patterns of breast carcinoma

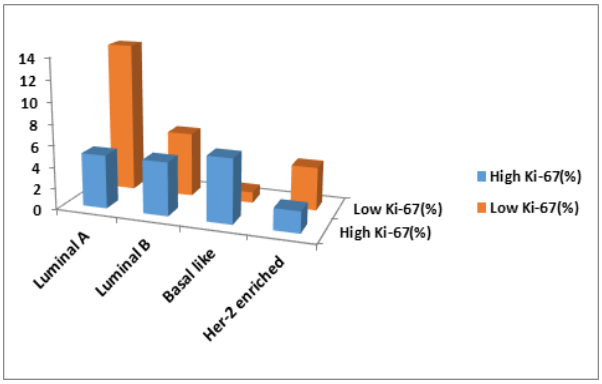

Fig. 3: Distribution of ki-67 proliferative index among molecular patterns of breast carcinoma

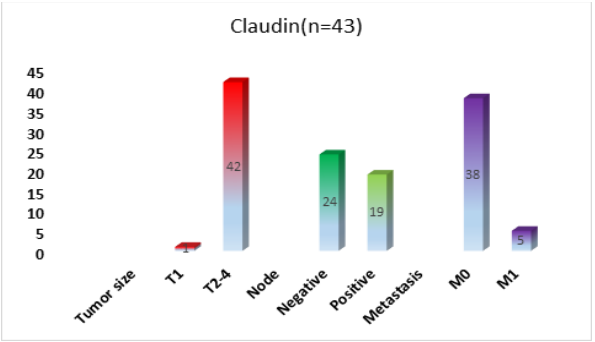

Fig. 4: Expression of claudin based on TNM staging

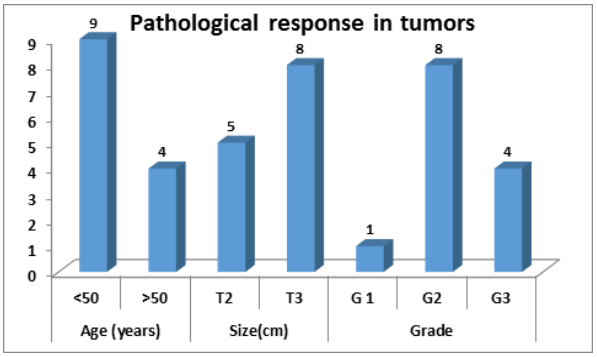

Fig. 5: Pathological response among tumors based on clinical parameters

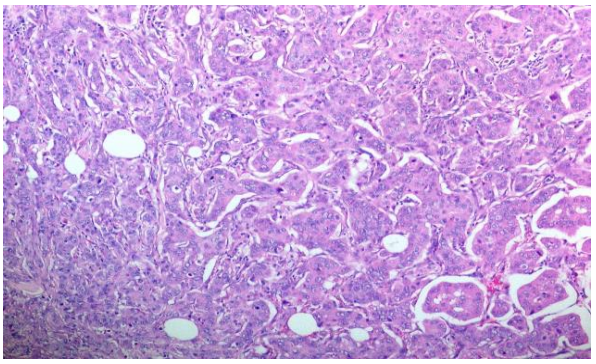

Fig 6: Microphotograph of invasive breast carcinoma (H&E, X200)

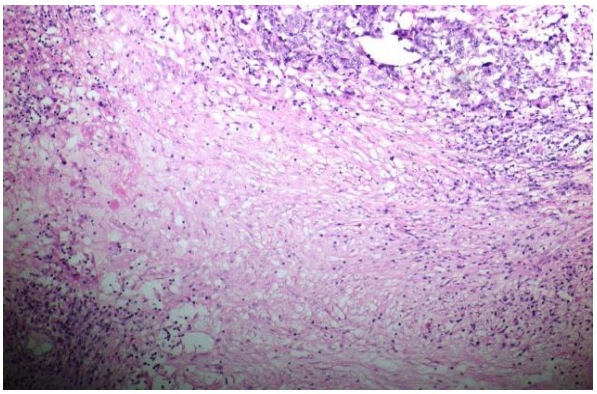

Fig. 7: Microphotograph of tumor with fibrosis and inflammatory response following NAT

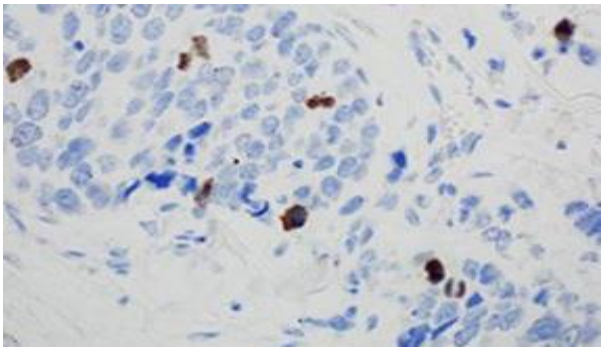

Fig. 8: Microphotograph of invasive breast carcinoma (ki-67 x200)

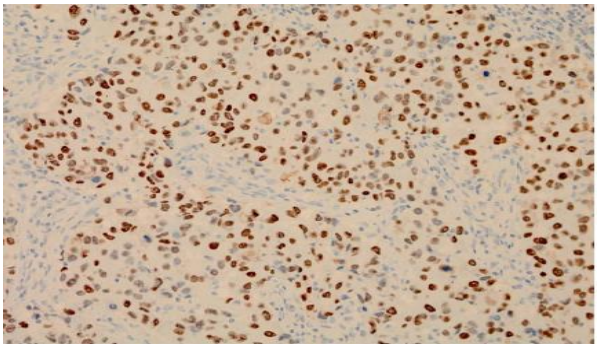

Fig. 9: Microphotograph of invasive breast carcinoma (p-53 x200)

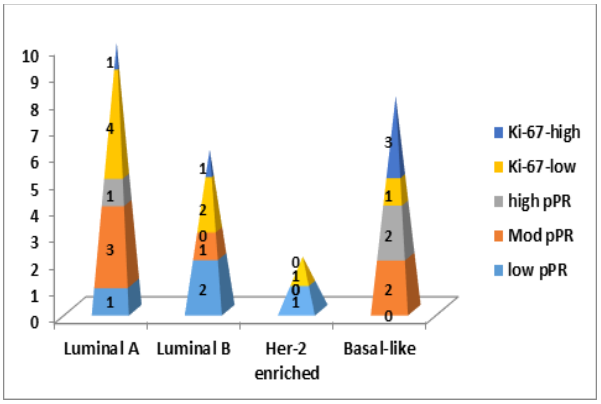

Fig. 10: Distribution of pathological response of tumors based on ki-67 proloiferative index

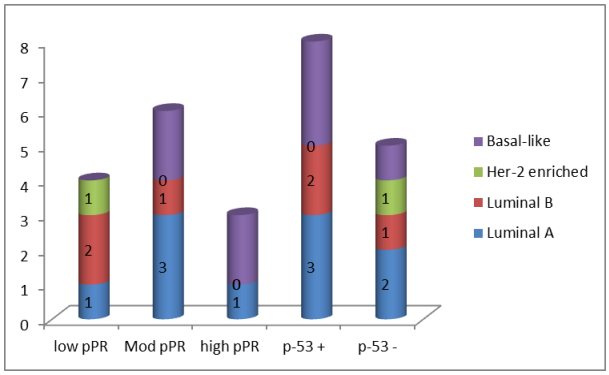

Fig. 11: Distribution of pathological response of tumors based on p-53 expression

Discussion

Molecular subtypes of breast carcinoma has significant differences in incidence, survival and response to therapy.[2,4] In 2000, Perou et al have produced a new molecular classification of breast carcinoma with four distinct subtypes by global gene expression profiling studies: luminal A & B, her-2 enriched, basal like and normal breast like.[12,26] Claudin –low subtype was also identified by Herschkowitz et al in both mouse and human breast tumors subsequently.[8,27,28] Each of these subtypes has unique biologic and prognostic features. Selection of claudin was based on the molecular evidence describing low gene expression of claudin 3, 4, 7 in claudin low subtype. Claudin 1 was selected based on our previous observation of decreased expression of these tight junction proteins in colonic, gastric and renal carcinomas[16,17,19,29]

Triple negative breast carcinoma is a subgroup lacking ER and PR expression and Her-2 amplification. It has histologic subtypes that range from salivary gland type tumors with low grade histologic features and low grade behaviour to medullary carcinoma with high grade histologic features and aggressive clinical behaviour.[11] Basal like subtype of triple negative is defined via gene expression microarray analysis and hence used only for research. Claudin- low breast carcinoma is one of the subtype of triple negative tumors characterized by low expression of genes involved in tight junctions and cell-cell adhesion including CLD 3,4, and 7, occludin, E. cadherin showing high expression of epithelial to mesenchymal transitional genes and stem cell feature[8,13,14,19]

Vasudevan et al studied 48 cases and PCR was seen in 27.1% (13 cases) and PPR in 70.9% (35 cases).[1] In lymph node PPR was seen in 93% cases PCR in 7% cases. 27% of cases belonged to age group less than 59 yrs and only 2 cases (18.2%) were above 60 yrs. Mean clinical size being 3.75cm. 26(70.3%) cases were5 cm and 10 cases (90.9%) were above 5cm.In our study PPR was seen in all the cases with mean age being 47yrs. and all the cases belonged to age group less than 59yrs and were below 5cm. Younger patient responded with PCR and this correlates with our study also[1] Our study also correlates with the study conducted by Saxena et al where the mean age in Indian population was 48yrs.[9] Chin et al showed PCR in 10% cases and tumors presented in T2 stage[30] but the mean age was 52 yrs. Smith et al and Baer et al showed PCR in 19-31% cases.[31,32] Study done by Kulka et al showed PCR in 14.1% (13 cases).[24] In study done by Vasudevan et al one case of grade 3 in age group 40.Rest were low grade in elderly (60-80 yrs.). Vasudevan et al studied that PCR was more for TN tumors than non-TN and this correlated with our study. Thus PCR appropriate marker for basal like and claudin- low subtype (CL) that represent 80% of all TN types. Vinnicombe et al studied that in clinically proven complete resolution mammography showed residual tumor in 5 cases and hence concluded that histopathological examination is the gold standard to note the response following neoadjuvant therapy (NAT).[33] Perou et al studied 470 breast carcinomas. PCR was 7% in luminal type, basal type (43%) and her-2 neu (38%).[34] In another study done by Sabatier et al PCR was studied in 1294 cases out of 5447 cases. Of 228 cases PCR after NAT was close to 32%, basal (33% and her2 (37%), luminal A 7% and luminal B(18%) this is similar to Prat and collegues in series of 133 cases where out of 18 CL PCR was 39% in CL, 79% in basal and 39% in her 2 type.[15,19] Among 228 CL PCR was 32%. In luminal (7%) luminal B (18%), basal (33%) and her-2

(37%). PCR was high in high grade tumors. Incidence of CL tumor was 12.4% similar to 7-14% reported by Prat and collegues.[19] In our study PPR was seen in all 6 claudin- low cases. Claudin low was expressed in 4 cases of basal like, one case each in her-2 enriched type and Luminal B type. Sabatier et al studied that CL was seen in 62% of T2T3, 54% node and 56% of grade 3 tumors and was close to that of Prat (65%, 47% and 62%). In our study claudin low is seen in 14% of T2-4 and7% of positive nodes Sabatier et al analysed triple negative in 67% of cases, Prat is 52%. Triple negative cases were 20% in our study. Claudin-low is heterogeneous than basal and luminalA. Ki-67 influences molecular subtype. Ki-67 was divided into 3 groups A to C for correlating PCR.[22] Ki-67 was seen in 60.4% of TN, 22% of luminal and 25.4% of her-2. Out of 363 cases ki-67 was correlated with clinicopathological characters. Ki-67 >40% correlated with worse prognosis in TNBC irrespective of tumor size and lymph node status. Cheang et al told ki-67 was 14% in luminal B had poor survival than luminal A that had ki-67 <14 %.[19,22,23] In our study ki-67 <15% was seen in 14% of luminal A and 6% of luminal B. Ki-67>15% was seen equally among luminal A &B, 6% of basal like pattern and 2% of her2-enriched pattern.p-53 was expressed in all the molecular subtypes except Her-2 enriched which was correlated with low pathological response. In a study done by Montagna et al PCR was 27.1% and PPR was 70.9% following NAT and is the best predictor of overall survival.[20] Minckwitz et al concluded that tumor stage after NAT was significantly associated with prognosis. Patients with ypN2a (tumor deposit involving 4-6 axillary nodes) and ypN3a (tumor deposit involving more than 10 axillary nodes) had a median overall survival (OS) or disease free survival (DFS) of 70 and 30 months respectively.[10] Claudin- low has poor outcome compared to Luminal A. Poor prognostic subtypes are LUMB, her2 and basal when compared to Luminal A.

Conclusion

The histopathological examination of the tumor is the gold standard for assessing chemotherapeutic tumor response; PCR is an indicator of overall survival rate by several studies. Younger patients, low grade, tumor size <5cm and high proliferating tumors (ki-67>15%) benefit better with neoadjuvant chemotherapy. Claudin low subtype is heterogeneous and can be seen in triple negative, luminal A&B and her-2 enriched type. Claudin low tumors are aggressive tumors and warrant further study to better understand this mysterious subtype with more cases in future. Therefore intense search for markers that may be crucial in the course of disease; especially those with prognostic and therapeutic purposes will be needed to develop targeted & personalized treatment.

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