The prognostic value of triple negative in stage II/III breast cancer

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Abstract

Introduction. Breast cancer is no longer seen as a single disease but rather a multifaceted disease composed of distinct biological subtypes with diverse natural history, clinical, pathological, and molecular features. Recent attention has been directed at the molecular classification of breast cancer.

Objective. To evaluate the prognostic value of triple-negative subtype in stage II/III breast cancer and to define the role of clinical stage in prognosis of breast cancer.

Methods. We used the immunohistochemical technique to divide 255 cases of breast cancer, stages II and III, into four subtypes according to estrogen receptor/progesterone receptor and Her-2 expression.

Results. Triple-negative subtype comprised 76.5% of the cases with 12.3% recurrence rate. Luminal A subtype also carried a poor outcome with 16.7% recurrence rate.

Conclusion. Triple-negative subtype has the worst overall and disease-free survival in stage II/III breast cancer. Clinical stage is still an independent prognostic factor in the breast cancers of all types.

Introduction

Breast cancer is the most common cancer in women in developed western countries[1] and is becoming even more significant in many developing countries.[2] In Egypt, breast cancer is the most common cancer among women, representing 18.9% of the total cancer cases among the Egyptian National Cancer Institute series of 10,556 patients during the year 2001,[3] with an age-adjusted rate 49.6/100,000 population; between 57% and 62% of all breast cancers were diagnosed before the age of 55 years.[4]

Breast cancer is a heterogeneous disease, encompassing a number of distinct biological entities that are associated with specific morphological and immunohistochemical features and clinical behavior.[5–7] Triple-negative breast cancers account for 10–17% of breast carcinomas. The main characteristics of triple-negative cancers include the fact that they more frequently affect younger patients <50 years[8,9] and are significantly more aggressive than tumors pertaining to other molecular subgroups.[10–12] This aggressiveness is best illustrated by the fact that the peak risk of recurrence is between the first and the second years and the majority of deaths occur in the first 5 years following therapy.[9]

From the pathologist's point of view, the differences between triple-negative and non-triple negative breast cancers are not surprising, given that the

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majority of triple-negative cancers are of histological grade III.[13] The majority of triple-negative cancers are high-grade invasive ductal carcinoma of no special type, metaplastic and medullary carcinomas.[14,15]

Her-2 is encoded by the Her2 gene which is located on chromosome 17. Because of its function as an activator of signaling pathway, Her-2 plays a central role in a number of cellular processes including proliferation, motility, and resistance to apoptosis. Her-2 has no known legend and can heterodimerize with other Her-proteins, thus allowing Her-2 to participate in a number of signal transduction pathways in the absence of a specific legend.[16] This effect may be enhanced by the over-expression of Her-2 in cancer cells, leading to increased cell proliferation and decreased cell death as well as changes in cell motility. It appears likely that over-expression of Her-2 protein is linked with Her2 gene amplification; Her-2 status provides both prognostic and predictive information in patients with breast cancer.[17] Over-expression of Her-2 receptor is associated with poor prognosis in patients with breast cancer as well as with aggressive tumor growth and metastasis. Her-2 positivity has also been associated with tumor grade, positive lymph node metastasis at presentation, and high mitotic count.[18] Her-2 status also correlates with relative response to various agents; Her-2 positivity may result in increased resistance to endocrine therapy and a decreased benefit from non-anthracycline–non-Taxane-containing chemotherapy, conversely, Her-2 positive patients may exhibit improved response to anthracycline therapy as well as paclilaxel.[19]

Recent attention has been directed at molecular classification of breast cancer. The immunohistochemistry (IHC) classification provides both therapeutic and prognostic information.

In this study, breast cancer cases were classified into four groups based on the IHC expression of estrogen receptor (ER), progesterone receptor (PR), and Her-2. The groups were: [ER/PR+ve, Her-2+ve, luminal B], [ER/PR+ve, Her-2−ve, luminal A], [ER/PR−ve, Her-2+ve] and [ER/PR–ve, Her-2−ve], or triple-negative group. These groups were evaluated for prognostic factors, recurrence, and metastasis in follow-up period of 2 years.

Patients and methods

From April 2008 to June 2010, 255 female patients with primary breast cancer were enrolled in this study from Sohag Oncology Institute. Eligibility criteria included: (1) pathologically confirmed breast cancer by core needle biopsy; (2) clinically stage IIB (T2 tumor larger than 2 cm but not larger than 5 cm, N1 metastasis to epsilateral axillary lymph nodes) and stage III (T3 tumor larger than 5 cm, N1 metastasis to epsilateral lymph nodes). Initial nodal state was evaluated by physical examination; (3) The pathological tumor staging was assessed according to the criteria established by the 6th edition of AJCC Cancer Staging Manual[20] and the grading of the tumor according to Elston and Ellis classification.[21] All patients were previously untreated.

(4) Adequate initial evaluation includes clinical examination, computed tomography, bone scan, obtaining adequate information regarding bone marrow, complete blood picture, and also cardiac, hepatic, and renal functions. After three to four cycles of chemotherapy, the patients were re-evaluated for their response to chemotherapy. Menopausal state was determined, based on clinical presentations and patient reports.

We examined the biological factors ER/PR and Her-2/neu expression by IHC, and evaluated their association with the clinical outcome within 2 years of follow-up.

The study protocol was reviewed and approved by the Institutional Review Ethical Board at Sohag University.

IHC technique and pathological examination

ER, PR, and Her-2/neu expression were evaluated by the avidin–biotin complex technique: tissue sections from the tumor and axillary lymph nodes were fixed in formalin and paraffin-embedded; and tissue blocks were cut at 4 μm, de-paraffinized in xylene, rehydrated with graded ethanol, and immersed in citrate phosphate-buffered saline (PBS). After an antigen-retrieval process in microwave with PBS at pH 6 for 3×10 min, primary antibodies were used as follows: ER (Dako Corporation, Carpinteria, CA, USA) at 1 : 50; PR (Dako Corporation) at 1 : 50. Because the Her-2 protein is expressed in normal breast epithelial cells, the Her-2 IHC assay is a quantitative rather than a qualitative test. For IHC, a positive Her-2 test is defined as (3+) cell surface protein expression (uniform intense staining of >30% of invasive tumor cells), and an equivocal test as (2+) cell surface protein expression was considered negative.[19] Her-2/neu (Nova Castra Lab) was used in the concentration of 1 : 200. All primary antibodies were mouse monoclonal antibodies. Biotinylated anti-mouse antibody was used as secondary antibody and streptavidin peroxidase methods were used. The cut-off value of 10% or more

and positively stained nuclei in 10 high power fields were used to define ER and PR positivity.[22] Her-2 expression and ER and PR hormonal profiles were done in two separate labs; equivocal Her-2 (2+) confirmed results only were included in the study.

Treatment options for women with axillary node positive breast cancer. Pre-menopausal women with ER/PR positive take chemotherapy + ovarian ablation/GnRH analog + Tamoxifen for 5 years.

Post-menopausal women with ER/PR positive take aromatase inhibitor [AI] during the course of adjuvant chemotherapy to lower the recurrence rate either as a primary therapy or after 2–3 years of Tamoxifen. Duration of [AI] should not exceed 5 years, and the patients took it were selected carefully for cardiac side effects of the drug.

Pre-menopausal and post-menopausal women with ER/PR negative take chemotherapy.[23]

In our Cancer Institute, the following chemotherapy protocol was applied in treating breast cancer stages II and III.

Protocols used as adjuvant chemotherapy in breast cancer (stage II) are given as follows:

1. FAC (repeated every 21 days for six cycles)
   - 5-Fluorouracil (FU): 500 mg/m^2 IV D1
   - Adriablastina (doxorubicin): 50 mg/m^2 IV D1
   - Cyclophosphamide 500 mg/m^2 IV D1
2. AC (repeated every 21 days for six cycles)
   - Adriablastina: 60 mg/m^2 IV D1
   - Cyclophasphamide: 600 mg/m^2 IV D1
3. CMF (repeated every 21 days for six cycles)
   - Cyclophasphamide: 600 mg/m^2 IV D1
   - Methotrexate: 40 mg/m^2 IV D1
   - 5-Fluorouracil: 600 mg/m^2 IV D1
4. EC (repeated every 21 days for six cycles)
   - Epirubicin: 75–100 mg/m^2 IV D1
   - Cyclophosphamide: 600 mg/m^2 IV D1

The patients (60 cases) underwent simple single mastectomy/lumpectomy before starting the course of post-operative adjuvant chemotherapy (mastectomy in post-menopausal and lumpectomy in pre-menopausal patients).

Protocols usually used in locally advanced disease (stage III) as neoadjuvant (pre-operative) chemotherapy are given as follows:

1. FEC (three to four cycles pre-operative)
   - 5-Fluorouracil: 600 mg/m^2 IV, D1
   - Epirubicin: 75–100 mg/m^2 IV, D1
   - Cyclophosphamide 500 mg/m^2 IV, D1
2. TAC (repeated every 21 days for four cycles followed by surgery)

- Docetaxel (taxotere): 100 mg/m^2 D1
- Adriamycin (doxorubicin): 60 mg/m^2 D1
- Cyclophosphamide: 600 mg/m^2 D1

The choice of the regimen combination and the substitution of specific one or more drug with another was determined by a decision of Cancer Institution Medical and Economic Committee and the supervising oncotherapy specialists.

After completion of the neoadjuvant chemotherapy, the patients (195 cases) underwent modified radical mastectomy and received post-operative adjuvant chemotherapy followed by radiation and hormonal therapy if indicated.

Statistical analysis

Results were statistically analyzed using the SPSS version 11, Chi-square test was used to assess the statistical significance with *p*-value <0.05 considered to be significant.

Results

In our study, we evaluated 255 primary breast cancers for the hormonal receptors (ER/PR) and Her-2 expression by immunohistochemical technique. The age of the studied patients was between 20 and 70 years with median age at 41 years (Figure 1).

The breast cancer cases were classified into four groups according to ER/PR and Her-2 receptor expression by immunohistochemical technique: [ER/PR+ve, Her-2+ve (32 cases), luminal B)], [ER/PR+ve, Her-2−ve (16 cases), luminal A], [ER/PR−ve, Her-2+ve (11 cases)], and a [triple-negative group (196 cases)].

Different histopathological types of mammary carcinomas were represented in the study. Infiltrating duct carcinoma (IDC; 182 cases) constituted about 71.3% of the studied cases and it is the most common tumor type, with 132 (72%) diagnosed at stage III and 50 cases at stage II. Lobular carcinoma constituted about 17% (44 cases) and medullary carcinoma about 7.8% (20 cases). The other histopathological types were represented in the study but in smaller numbers (Table 1).

The majority of the cases (207/255 (81.2%)) were hormonal receptors negative (ER/PR−ve) with 147 cases at stage III and 60 cases at stage II, with statistically highly significant correlation between hormonal receptor negativity and advanced clinical stage at diagnosis (*p* = 0.0001).

In this study, the number of triple-negative cases was 196 (76.7%), divided into 136 cases (69%) at
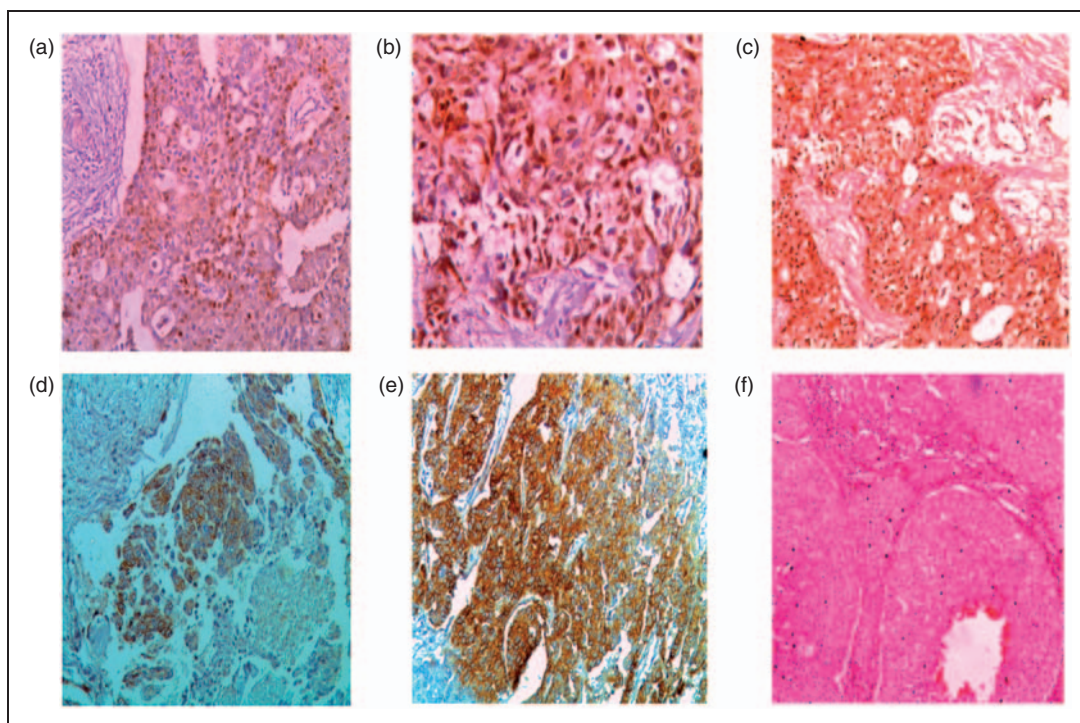
Figure 1. IHC expression of ER: (a) 10× and (b) 40×; (c) IHC staining of PR positive expression section (10×); IHC staining of Her-2 positive expression (3+): (d) 4× and (e) 10×; (f) H&E staining (4×) showed breast cancer cases with nuclear grade II, central necrosis, and moderate nuclear pleomorphism.
ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; H&E: Hematoxylin and eosin stain.

Table 1. Clinicopathological features of the studied patient

Diagnosis	No.		
Pre-menopausal ♀	93 (36.5%)		
Post-menopausal ♀	162 (63.5%)		
IDC	182 (71.3%)		
Lobula	44 (17.2%)		
Medullary	20 (7.8%)		
Mucoid	5 (1.9%)		
Papillary	4 (1.7%)		
Clinical stage		Recurrence	Metastasis
Stage III	195 (76.4%)	24 (12.3%)	2
Stage II	60 (23.6%)	2 (3.3%)	1
Patient groups			
Triple(−)	196 (76.8%)	20	3
ER/PR(+)	32 (12.5%)		
Her-2(+)			
ER/PR(+)	16 (6%)		
Her-2(−)		6	
ER/PR(−)	11 (4.7%)		
Her-2(+)			

stage III and 60 cases at stage II (31%) (Table 1), with statistically significant relation between the triple-negativity and the advanced clinical stage of the disease at diagnosis ($p = 0.001$).

Her-2 positive expression was detected in 27 cases (10.6%) and all were at stage III, while Her-2(−ve) constituted about 89.4% of the cases. There is statistically significant relation between Her-2 positive expression and advanced clinical stage ($p = 0.002$).

Recurrence was detected in 26 cases within the follow-up period and constituted 10% of the studied cases. Of them, 24 cases (92.3%) were at stage III and only 2 cases at stage II, with statistically highly significant correlation between recurrence rate and clinical stage ($p = 0.002$).

There were three cases of tumor metastasis that constituted about 0.02% of the studied cases, and they were in the triple-negative group, with significant statistical correlation between metastasis and triple negativity ($p = 0.001$).

Discussion

An estimated one million cases of breast cancer are diagnosed annually worldwide; of these, more than 170,000 are described as triple-negative and have significant clinical implications.[24]

The clinical course of breast cancer patients treated with neoadjuvant chemotherapy remains difficult to predict, because histologically homogenous breast cancers may vary in response to therapy and have divergent outcomes. As a result, many researchers have tried to identify prognostic factors to give these patients the optimal treatment options and prolonged survival. Triple-negative breast cancer accounts for approximately 15% of breast cancers.[25]

Two-hundred and fifty-five breast cancer cases were classified into four groups according to ER/PR and Her-2 receptor expression by immunohistochemical technique: {ER/PR(+ve) Her-2(+ve)}, {ER/PR(+ve) Her-2(−ve)}, {ER/PR(−ve) Her-2(+ve)} and {ER/PR(−ve) Her-2(−ve)}, or triple-negative group.

This classification was correlated with intrinsic gene expression microarray categorization which also classified breast cancer into the four main groups luminal A, luminal B, Her-2 +, and triple-negative groups.[26]

Perou et al.[27] classified breast cancer into luminal class that are hormonal receptor positive and clustered hormonal receptor negative tumors into three groups; Her-2 positive, basal-like, and hormonal receptors, Her-2, basal-like negative groups. Another more recent study[28] had classified breast cancer into the same four groups as in this study, based on IHC profile: ER/PR(+), Her-2(+), ER/PR(+), Her-2(−), ER/PR(−), Her-2(+), ER/PR(−), and Her-2(−). This study had shown both the triple-negative and ER/PR(−), Her-2(+) subtype to have poorer clinical, pathologic, and molecular prognosis.

In our study, Her-2/neu-positive cases constituted 10.6% of the studied cases. This finding is in agreement with Ross et al.,[29] who reported that approximately 10–34% of breast cancers over-expressed the Her-2/neu receptor and is referred to as Her-2 + tumors. However, in this study, Her-2/neu positivity increased according to the stage of the disease. Her-2/neu-positive cases were all at the advanced stage III with statistically significant relation between Her-2 expression and advanced clinical stage ($p = 0.002$). This finding suggested that as the disease stage of the breast cancer is advanced, Her-2/neu positivity increases and Her-2 positive expression is closely associated with higher stage of breast cancer that is corresponding to poor prognosis. This finding is in agreement with Kim et al.,[30] who examined Her-2 positivity in relation to the disease stage. They found that the positive rate became higher as the disease stage progressed ($p = 0.0009$) and the significance of Her-2/neu positive as a prognostic factor could be confirmed only in stage III breast cancer.

A study compared the tumors that expressed Her-2/neu and had positive lymph nodes and extra-capsular extension with those tumors which were Her-2/neu negative and lymph node positive with extra-capsular extension. It was found that Her-2/neu over-expression is associated with a more aggressive subtype of cancer.[31]

Triple-negative breast cancer is an important area of research for both researchers and clinicians because triple-negative breast cancer is a poor prognostic factor for disease-free and overall survival. No effective specific targeted therapy is readily available for them, and there is a clustering of triple-negative cases in the pre-menopausal women and the overlap of BRCA-1-associated breast cancers with triple-negative phenotype is significant.[32]

Rhee et al.[33] reported that in node negative breast cancers, triple-negative cases have a higher relapse rate and more aggressive clinico-pathologic characteristics than non-triple-negative cases. In a study on 345 breast cancers, triple-negative cancer has a high histological grade, more metastasis, more local recurrence and contra-lateral breast cancers, and worse overall survival.[34]

In our study, ER was negative in 207 cases (81.2%), with (57.6%) of them at clinical stage III,

and 23.5% at stage II, with statistically highly significant relationship between the ER expression and the clinical stage at diagnosis ($p = 0.0001$). It seemed that ER expression alone cannot predict outcome and this finding in our study was in agreement with a recent study that divided ER positive expression tumors into two subtypes. They concluded that two major groups within the ER+ cancer can be recognized at the molecular level, one that corresponds to high grade, highly proliferative tumors (luminal B). This group is less sensitive to endocrine therapy and more sensitive to cytotoxic drugs with poor prognosis and adjuvant chemotherapy may improve the outcome. The other group is low-grade cancers, with low proliferation rate, and excellent prognosis with endocrine therapy alone; it does not appear to benefit from adjuvant chemotherapy (luminal A). The histological grade, Her-2 status, and Ki67 may be used to estimate prognosis and chemotherapy sensitivity in ER+ cancers.[35]

In a population-based cohort study, Grann et al.[36] found that ER/PR expression is an independent prognostic factor in breast cancer. They concluded that patients with ER/PR positive tumors have a better survival than hormone receptor negative tumors with a 5-year overall survival at all stages of 83% in the ER/PR positive group *versus* 69% in the double negatives.[36]

It has been observed that approximately 80% of BRCA-1-associated breast cancers are negative for ER/PR and Her-2/neu (triple negative), and cluster with basal-like breast cancers by DNA microarray while 80% of BRCA-2-associated breast cancers are ER/PR+ but Her-2/neu negative and luminal.[37]

In this study, six cases of tumor recurrence were detected in the {ER/PR(+ve), Her-2(−ve)} group, with a poor clinical out come than the {ER/PR(+ve), Her-2(+ve)} subtype. This could be explained by the fact found in a retrospective study using 58 Her-2 amplified tumors, unsupervised gene expression analysis. It reported that Her-2 is not a single protein but, three separate subtypes independent of stage, histological grade, and ER status. Importantly, one of these subtypes (cluster 2) had a significantly worse clinical outcome, with overall survival 12% in the poor-prognosis group compared with 50–55% in the good prognosis groups over a 10-year follow-up period.[38]

Triple-negative breast cancers was associated with increased risk for visceral metastasis ($p = 0.0005$) and shorter post-recurrence survival ($p < 0.0001$) in another study.[39] In this study, three cases of metastasis had been detected at the follow-up period; all were in

the triple-negative subtype. The lung, bone, and ovaries were the site of metastasis with two cases diagnosed at stage III. There was no statistical relation between hormonal receptor negativity and the site of metastasis. This could be due to the small number of the studied cases. In a recent study, the triple-negative subtype was significantly associated with breast tumors with bone and brain metastasis when compared with breast tumors without metastasis. There was a significant association with tumor size >2 cm (T2) in breast tumors with bone and visceral metastasis compared with breast tumors without metastatic disease. When compared with breast tumors without metastasis, bone metastasis was significantly associated with ER and E-cadherin positive breast tumors and brain metastasis were significantly associated with ER and PR positive breast tumors. They concluded that using IHC, a standard panel of molecular markers of breast carcinomas can be of significant value in predicting sites of metastasis.[40]

In this study, the tumor relapse was detected within 2 years after completing the course of therapy with good initial response to chemotherapy. This finding was in agreement with that of Carey et al.,[41] who reported that the clinical response to anthracycline-based chemotherapy, doxorubicin-cyclophosphamide, was higher in the ER/PR(−ve), Her-2(+ve) (70%), and triple-negative (85%) subtypes than in the luminal ER/PR(+) (47%; $p < 0.0001$). Despite displaying initial chemosensitivity, patients with triple-negative and ER/PR(−ve), Her-2(+ve) subtypes had worse disease-free survival ($p = 0.04$) and overall survival ($p = 0.02$) than those with the luminal subtype. In contrast, the recurrence risk for non-triple-negative group remained constant over time.[8] The majority of triple-negative breast cancers are characterized by an aggressive clinical history, shorter survival, and a relatively high mortality rate.[9,42]

In our study, a statistically significant difference was detected in clinical features and the outcome between the subtypes of breast cancer using the most common subtype (ER/PR(+) Her-2(−) luminal A), as a reference. The triple-negative subtype had a worst overall survival (tumor recurrence and metastasis). This classification should be complemented with many other tumor traditional prognostic variables as age, tumor sizes, stage, and lymph node status and adjuvant chemotherapy.

This study was a cohort study, conducted in a single institution (Sohag Cancer Institute) which is a referral center, with limited economic resources. Most of the patients were poor, diagnosed at other medical centers. This leads to delayed diagnosis, and starting therapy

with the majority of cases at an advanced clinical stage (71% of cases at stage III). All the studied patients were diagnosed before therapy by Tru-cut needle biopsy, and were treated with adjuvant/neoadjuvant chemotherapy, then re-evaluated clinically, before and after surgery. The effect of chemotherapy on the tumor cells was not predictive and the histopathological features, nuclear grading and tumor type, showed various changes, with the precise grading of the tumor could not be reached in all cases. Excessive necrosis, hyalinosis, dense fibrosis, and scattered bizarre neoplastic cells were observed in most cases after neoadjuvant therapy. Considering immunohistochemical hormonal profiling, the intensity of the stain was not homogenous in whole sections, and the results of IHC usually give lower frequencies.

Conclusions

It has been concluded from this study that the triple-negative subtype has the worst overall and disease-free survival in stage II/III breast cancer compared to other subtypes. Despite short duration of follow-up, the results showed statistical superiority of initial clinical stage in predicting survival than the hormonal expression. IHC classification of breast cancer seemed to be valuable as a clinical tool, because ER/PR and Her-2 testing are widely available, at reasonable cost. This classification is clinically useful, therapeutically informative, and based on immunophenotype, and it is prognostic as well as predictive.

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