INTRODUCTION

Thyroid nodules of various biological natures occur in approximately 10% of the population worldwide, predominately adult females [1].

Clinically detected thyroid nodules include non-neoplastic lesions such as hyperplastic nodules (HN) and neoplastic lesions such as follicular adenoma (FA), follicular carcinoma (FC) and follicular variant of papillary thyroid carcinoma (FVPC). These lesions histologically share a follicular-patterned morphology and are traditionally grouped under the umbrella term “follicular thyroid lesions” [1-3].

The “gold standard” in the diagnosis of thyroid nodules remains to be the routine histopathological examination of hematoxylin and eosin stained sections. Nevertheless, the histopathological interpretation of thyroid lesions with a follicular growth pattern poses a challenge to pathologists; mainly the distinction between HN and FA, FA and minimally invasive FC as well as FA/FC and the FVPC [1,3,4].

Previous studies have variably employed several ancillary techniques as immunohistochemistry, morphometric studies and molecular methods in order to achieve a definitive diagnosis of different types of follicular thyroid lesions [2-7].

Researchers have investigated the expression of galectin-3, cytokeratin 19, p53, bcl-2, HBME-1, and Ki-67. In addition, certain mutations have been observed such as PET mutations and mutations in the first exon of K-ras in PTC and mutations in codon 61 of H-ras and N-ras in FC [8-12].

Although some of these techniques are useful, they should be used cautiously as they are neither consistent nor reliable [8-12]. This justifies the continuous pursuit of new
markers that would ensure an accurate diagnosis of follicular thyroid lesions.

CD56, a neural cell adhesion molecule, also known as Anti-Leu-19, was originally identified as a glycoprotein on natural killer cells [13].

CD56 is ubiquitously expressed in the central nervous system (CNS), peripheral nerves and skeletal muscles, most types of neuroendocrine cells, and various epithelia (e.g. enterocytes and the newly formed bile ductular cells). It is also expressed in ovarian stromal cells, uterine smooth muscle cells and osteoblasts [14].

CD56 has been used as a diagnostic marker in certain tumors such as acute and chronic myeloid leukemia, ovarian sex cord-stromal tumors, Wilms' tumor, neuroblastoma and ganglioneuroblastoma, phaeochromocytoma/paraganglioma and certain CNS tumors [13,14].

Furthermore, CD56 was proved to have a prognostic impact in certain malignancies. Loss of CD56 correlates with poor prognosis in colonic and pancreatic carcinomas [15,16].

The aim of the present study was to investigate the diagnostic utility of CD56 protein expression in different follicular thyroid lesions using immunohistochemistry.

MATERIALS AND METHODS

Patients and Tissue Samples

The present study comprised 68 retrospective cases of follicular thyroid lesions retrieved from the archives of the Pathology Department, Faculty of Medicine, University of Alexandria from the year 2005 to the year 2012. Complete clinical and operative data were available for all patients. Histopathological diagnosis was done according to the WHO criteria [17].

Immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked by hydrogen peroxide. Antigen retrieval was done by immersion of tissue sections in a 10 mM citrate buffer (pH 6.0).

Immunohistochemical staining was performed using an avidin-biotinylated immunoperoxidase methodology. The primary mouse monoclonal antibody (CD56, Clone 123C3, Dako, Denmark) was applied in a 1:100 dilution. The bound antibody was detected by the Ultra-Vision Detection System anti-polyvalent, HRP/DAB (ready-to-use) (Thermo Scientific™, USA). Negative and positive controls (neuroblastoma) were included in all runs.

Evaluation of Immunohistochemical Staining

Cases were considered CD56 positive if they showed cytoplasmic ± membranous staining in ≥10% of the cells [18].

The immunostained slides were evaluated for the extent (percentage) of positive cells (0: <10%, 1+: 10-25%, 2+: >25-50%, and 3+: >50%) and intensity of staining reaction (1 = weak, 2 = moderate, 3 = strong). A total score was calculated by summation of the aforementioned extent and intensity scores [18].

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS® Statistics 20, International Business Machines Corporation, New York, USA). Quantitative data were described using median, minimum and maximum, as well as mean and standard deviation (SD). Qualitative data were described using number and percentage. Various statistical tests (Chi-square test [$\chi^2$], independent t-test [t], Mann–Whitney [U], Kruskal–Wallis test [H], Pair-wise follow-up analysis, and Spearman’s correlation coefficient [ρ]) were used to evaluate and correlate the results. Significance test results were judged at 5% level.

A logistic regression model was built to predict malignancy among thyroid lesions using the patient’s age and the total CD56 staining score. The diagnostic performances of patient’s age, total CD56 staining score and the developed model were evaluated using receiver operating characteristic (ROC) curve analysis. Youden Index was used to estimate the cut-off point at which the highest diagnostic accuracy was reached.

RESULTS

Clinicopathological Findings

The age of the patients ranged from 20 to 70 years ($M = 45$, $SD = 10.8$). 64 patients (94.1%) were females, and four patients (5.9%) were males with a M: F ratio of 1:16.

All the studied cases had normal serum levels of T3, T4 and TSH. Ultrasound examination was done for all patients, and pre-operative fine-needle aspiration cytology was performed in 63 patients (92.7%). 52 patients (76.5%) underwent total thyroidectomy, 11 patients (16.2%) underwent hemithyroidectomy, 3 patients (4.4%) underwent subtotal thyroidectomy and 2 patients (2.9%) underwent total thyroidectomy with radical neck dissection.

By gross examination, 53 cases (77.9%) showed a single nodule and 15 cases (22.1%) presented as a dominant nodule in a multinodular goiter. The nodules in 39 cases (57.3%) were encapsulated, while in the remaining 29 cases (42.65%) they were non- or partially encapsulated. The size of the nodules (largest dimension) ranged from 0.5 to 5 cm with a mean of 2.75 cm ±SD = 0.33. Cut sections were grayish white in 52 cases (76.47%) and glistening brown in 16 cases (23.53%). Areas of hemorrhage and necrosis were seen in six cases (8.8%). Cystic degeneration was noted in six cases (8.8%), and two cases (2.9%) showed protruding papillae from the cut surface.
Microscopically, the studied cases included 30 cases (44.1%) of follicular thyroid lesions with benign behavior (15 cases [22%] of HN, and 15 cases [22%] of FA), and 33 cases (48.5%) of malignant tumors (18 cases [26.6%] of PTC [including 5 cases [7.4%] of conventional PTC and 13 cases [19.2%] of FVPC] and 15 cases [22%] of minimally invasive FC). In addition, 5 cases (7.4%) of well-differentiated tumor of uncertain malignant potential (WDT-UMP) were included, in which questionable capsular invasion and incomplete nuclear features of PTC were detected.

Results of Immunohistochemistry

Overall, CD56 was expressed in 31 cases (45.6%). The overall total score ranged from 0 to 6 with a mean of 1.78. CD56 showed a tendency towards a positive correlation with patients’ age ($\rho = 0.239, P = 0.050$) and was significantly higher expressed in male patients ($U = 50.5, P = 0.04$). The correlation between CD56 expression and nodule size was statistically insignificant ($\rho = 0.039, P = 0.750$).

The results of CD56 expression in the studied histopathological types are summarized in Table 1 and illustrated in Figure 1.

Total score of CD56 expression was statistically significant among the studied groups of follicular thyroid lesions ($H = 41.865, P \leq 0.001$). Pair-wise comparison showed that CD56 expression was significantly higher in PTC (conventional and FVPC) compared to FC, FA and HN ($P = 0.008$, $P < 0.001$ and $P < 0.001$ respectively). In contrast, no statistically significant difference in CD56 expression was found between HN and FA ($P = 1.000$), HN and FC ($P = 0.917$), as well as between FA and FC ($P = 0.157$). Also, no statistically significant difference was found between WDT-UMP and any of the studied types.

### Table 1: CD56 expression in the different histopathological types of follicular thyroid lesions studied

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>No. (%)</th>
<th>Frequency of CD56 positivity (% of No.)</th>
<th>Pattern of staining</th>
<th>Total staining score $M \pm SD$, $Mdn \pm range$ (Test $P$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN</td>
<td>15 (22)</td>
<td>3 (20)</td>
<td>3 (20)</td>
<td>0.53 (1.1), 0 (0-3) ($H = 41.87$, $P \leq 0.001$)</td>
</tr>
<tr>
<td>FA</td>
<td>15 (22)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PTC</td>
<td>18 (26.6)</td>
<td>18 (100)</td>
<td>11 (61.1)</td>
<td>4.5 (1.2), 5 (2-6) ($H = 41.87$, $P \leq 0.001$)</td>
</tr>
<tr>
<td>FC</td>
<td>15 (22)</td>
<td>7 (46.6)</td>
<td>3 (42.9)</td>
<td>1.9 (2.2), 0 (0-5) (n.s.)</td>
</tr>
<tr>
<td>WDT-UMP</td>
<td>5 (7.4)</td>
<td>3 (60)</td>
<td>3 (60)</td>
<td>1.8 (1.7), 2 (0-4) (n.s.)</td>
</tr>
</tbody>
</table>


![Figure 1](image-url): Immunohistochemical expression of CD56 in follicular thyroid lesions: (a) Focal weak cytoplasmic staining in a case of hyperplastic nodule ($\times 200$) (b) Negative staining in a case of follicular adenoma ($\times 40$) (c) A case of well-differentiated tumor of uncertain malignant potential showing cytoplasmic immunopositivity of moderate intensity ($\times 400$) (d) A case of conventional papillary thyroid carcinoma showing diffuse cytoplasmic staining of moderate intensity ($\times 200$) (e) A case of follicular variant of papillary thyroid carcinoma showing cytoplasmic positive staining with membranous accentuation of strong intensity ($\times 200$), (f) A case of follicular carcinoma showing cytoplasmic staining of strong intensity ($\times 400$)
On considering conventional PTC and FVPC as separate groups, there was a statistical significance in the total score of CD56 expression among the studied groups ($H = 42.150$, $P \leq 0.001$). Pair-wise comparison showed that CD56 expression was significantly higher in FVPC compared to FA and HN ($P < 0.001$ and $P < 0.001$ respectively). Similarly, CD56 expression was significantly higher in conventional PTC compared to FA and HN ($P < 0.001$ and $P = 0.002$ respectively). However, CD56 expression showed no statistically significant difference between PTC and FVPC on one hand and FC on the other hand ($P = 0.119$ and $P = 0.061$ respectively). Also, no statistically significant difference in CD56 expression was found between HN and FA ($P = 1.000$), PTC and FVPC ($P = 1.000$), HN and FC ($P = 0.235$). In addition, no statistically significant difference was found between WDT-UMP and any of the studied types.

In order to assess the relation between CD56 expression and other clinicopathological factors on one hand and tumor’s behavior on the other hand, a bivariate analysis was done and showed that malignancy was significantly associated with higher CD56 expression ($U = 140.5$, $P < 0.001$) and older patients’ age ($t = 2.72$, $P = 0.008$).

Subsequently, a logistic regression model [Table 2] and equation were built to predict malignancy in follicular thyroid lesions:

\[
\text{Probability of malignancy} = \frac{e^Y}{1+e^Y}
\]

(e) is a natural number, it is approximately equal to 2.71828

Given that $Y = (-0.027) \times \text{Age in years} + 0.942 \times \text{Total CD56 score}$

The diagnostic accuracy of CD56 expression, patients’ age and the developed model was further tested by ROC curve analysis in order to detect a cut-off point above which malignancy is predicted.

**Table 2: The logistic regression model for prediction of malignancy in the studied cases of follicular thyroid lesions**

<table>
<thead>
<tr>
<th>Independent predictors</th>
<th>$\chi^2$ (P-value)</th>
<th>Nagelkerke</th>
<th>Model equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and total CD56 staining score</td>
<td>32.4 ($&lt;0.001$)</td>
<td>0.536</td>
<td>$(-0.027) \times \text{Age} + 0.942 \times \text{Total CD56 score}$</td>
</tr>
</tbody>
</table>

**Table 3: Diagnostic accuracy of CD56 total score, patients’ age, and the developed model in prediction of malignancy in the studied cases**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC (95% CI)</th>
<th>P value</th>
<th>Cut-off value</th>
<th>Sn (95% CI) (%)</th>
<th>Sp (95% CI) (%)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CD56 score</td>
<td>0.86</td>
<td>$&lt;0.001$</td>
<td>2</td>
<td>72.73-93.33</td>
<td>54.5-56.7</td>
<td>54.5-59.2</td>
<td>75.7</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.68</td>
<td>0.010</td>
<td>36</td>
<td>75.76-93.33</td>
<td>55.4-58.7</td>
<td>54.5-74.5</td>
<td>55.4-68.8</td>
</tr>
<tr>
<td>The model</td>
<td>0.80</td>
<td>$&lt;0.001$</td>
<td>0.598</td>
<td>75.76-93.33</td>
<td>55.4-58.7</td>
<td>54.5-74.5</td>
<td>55.4-68.8</td>
</tr>
</tbody>
</table>

AUC: Area under the curve, 95% CI: 95% Confidence interval, Sn: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NPV: Negative predictive value

ROC curve analyses showed that at a cut-off point of score 2 for CD56 expression, 36 years for age, and 0.598 for the developed model, sensitivity and specificity were at their highest levels.

On comparing the diagnostic performance of patients’ age, total CD56 score, and the developed model, it was found that the developed model was better than the other two parameters in prediction of malignancy among the studied cases of follicular thyroid lesions [Table 3, Figure 2].

On the application of the built equation to the studied cases, it was found to conform to their diagnostic nature.

**DISCUSSION**

Follicular thyroid lesions represent a heterogeneous group that comprises both non-neoplastic and neoplastic lesions with varying biological behavior [19].

Follicular-patterned thyroid lesions represent a diagnostic grey zone even to experienced pathologists with a significant interobserver variability [5,19]. Accurate pathological diagnosis is essential for optimal clinical management [20].

Most of the patients in our study were adult females, a finding that coincides with the study of Mokhtari et al. [21] who reported that the mean age of their patients was 42.4 years.
with a female preponderance and a male to female ratio of 1:2.9.

We investigated the immunohistochemical expression of CD56 in follicular thyroid lesions. CD56 is cell surface adhesion glycoprotein, of the immunoglobulin gene superfamily [13,14]. It has three isoforms that vary in their cytoplasmic domains. The first (120 kDa) is cell membrane anchored, the second (140 kDa) has a short cytoplasmic domain, and the third (180 kDa) has a long cytoplasmic domain [22]. The used primary antibody in this study was CD56 (Clone 123C3), that recognizes a protein consisting of at least two isoforms [23].

The pattern of CD56 expression varied from cytoplasmic to cytoplasmic with membranous accentuation. Different previous studies [18,24-33], have reported different staining patterns for CD56 that could be attributed to the different antibody clones, and thus isoforms, used.

There was a tendency towards a positive correlation between patients’ age and CD56 expression. This might be due to the higher incidence of malignancy with advanced age. In addition, there was a statistically significant association between CD56 expression and male gender. However, this result should be interpreted cautiously due to the small number of male patients.

Among benign lesions, CD56 was weakly positive in 20% of HN cases and was negative in FA cases. This contrasts with previous studies that have reported higher frequencies of CD56 expression in HN and FA [18,26,27,29,30].

Shin et al. [26] reported that 58.3% of HN and 100% of FA cases showed immunopositivity for CD56. Park et al. [18] found that 90.5% of HN cases and 91.7% of FA cases were positive for CD56. Abd El Atti and Shash [27] reported that 87.5% of cases of HN and 91.7% of FA cases showed CD56 expression. The studies of both El Demellawy et al. [29] and Migita et al. [30] mentioned that CD56 expression was found in 100% of the studied cases of HN and FA.

In the current study, 60% of WDT-UMP cases were positive for CD56. Abd El Atti and Shash [27] recorded a lower frequency (30%) of CD56 expression in WDT-UMP.

As regards FC, the present study revealed positivity for CD56 in 46.6% of the studied cases. In contrast, more frequent CD56 expression in FC cases was documented by both Park et al. [18] and El Demellawy et al. [29] who reported CD56 positive staining in 100% and 82.6% of FC cases respectively.

All cases (100%) of PTC in our study (conventional PTC and FVPC) showed positive CD56 expression. These results are in general agreement with those of Scarpino et al. [25] who found that 70.4% of the studied PTC cases were positively stained for CD56.

On the other hand, Migita et al. [30] reported that only 33% of PTC cases were positively stained for CD56. Less frequent CD56 expressions in papillary carcinomas were reported by other investigators. The studies of Park et al. [18] and Abd El Atti and Shash [27] revealed that only 7.5% and 17.2% of PTC cases (including its follicular variant) were positively stained for CD56 respectively. In addition, Ozolins et al. [28] reported that CD56 expression was detected in only 4% of papillary carcinoma cases.

Considering the total score of CD56 expression, the results of our study showed a significantly higher expression of CD56 in PTC (including its FVPC) compared to the other studied categories. This suggests that CD56 expression could be used as a good positive marker distinguishing PTC from other follicular thyroid lesions.

Park et al. [18] reported that CD56 expression showed a statistically significant difference between papillary carcinoma cases (as a negative marker) when compared to HN, FA and FC.

On the other hand, Etem et al. [34] reported near equal percentages of CD56 positivity in PTC and other follicular lesions including HN, FA and FC, thus concluded that CD56 does not discriminate between these categories.

On considering conventional PTC and FVPC as separate groups, our results showed that CD56 expression was significantly higher in each of them when compared to FA and HN. However, CD56 expression showed no statistically significant difference between PTC, FVPC as separate groups when compared to FC. Thus, CD56 proved to be of no value in the discrimination between FVPC and PC.

Abd El Atti and Shash [27] reported that CD56 distinguished FVPCs (as a negative marker) from other follicular patterned nodules (FA, HN and WDT-UMP) with a statistically significant difference.

Similarly, other studies [18,21,26,27,29,31] have reported that CD56 could serve as a good negative marker for PTC and FVPC distinguishing them from other CD56-positive thyroid lesions with a high sensitivity and specificity.

Needless to say, the discrimination between benign and malignant follicular thyroid lesions has a major impact on patients’ management. However, the histological similarities between these lesions blur the distinction. Moreover, the unusual histologic criteria for diagnosing thyroid malignancy, namely capsular and/or vascular invasion for minimally invasive FC and nuclear characteristics for papillary carcinoma, render the discrimination more challenging [5,19].

Much attention has been directed towards the identification of molecular or immunohistochemical markers that can help distinguish follicular thyroid lesions of benign behavior from malignant lesions [35].
In our study, CD56 total score was significantly higher in malignant tumors compared to lesions with benign behavior.

Several previous studies, on tissues other than thyroid, revealed that CD56 was exclusively or predominantly expressed in malignant tumors compared to their benign counterparts or normal tissues [36-42].

CD56 was significantly expressed in small cell carcinoma of the lung [36], chronic myeloid leukemia [37], multiple myeloma [38], ovarian serous neoplasms [39], malignant gastrointestinal stromal tumors [40], malignant mesothelioma [41], and colorectal carcinoma [42], compared to their benign counterparts.

The findings of our study together with the aforementioned studies, suggest that CD56 plays a role in malignant transformation, a finding that mandates further investigation to clarify the exact role of CD56 in the process of tumorgenesis.

In stark contrast to our findings, previous studies employing CD56 on thyroid lesions have shown higher expression of CD56 in benign cases as compared to malignant cases [27,31-33].

The noted discrepancy in the results of CD56 expression in follicular thyroid lesions among different studies could be attributed to the different number of the studied cases, the utilization of different antibody clones with different antibody dilution, and different cut-off values of positive cells used to establish positivity. For example, Shin et al. [26] and Park et al. [18], utilized (Ncl-CD56-1B6), moreover, Shin et al. [26] used a 1:300 dilution. Abd El Atti and Shash [27] used CD56 (MS-204-R7). Migita et al. [30] performed immunostaining on frozen sections, considered 5% as a cut-off value of positivity, and cases were recognized as positive if they demonstrated cytoplasmic and membranous staining. Ozolins et al. [28] performed immunostaining on FNA smears and considered cases as positive if they showed membranous staining pattern. In addition, one should consider the geographical, environmental, and racial differences.

In this study, a bivariate and ROC curve analyses showed that CD56 expressions (at score >2) and patients’ age (>36 years) were significant independent predictors of malignancy in thyroid lesions with a high sensitivity and specificity.

A logistic regression model was developed to predict malignancy in follicular thyroid lesions using CD56 expression and patients’ age. It was found that the sensitivity and capability of the developed model to predict malignancy was higher than that of CD56 expression and patients’ age.

Subsequently, an equation was built to detect the probability of malignancy in thyroid lesions. If the probability of malignancy in a given case of follicular thyroid lesions was >0.598, this favors malignancy. Conversely, if the resultant value was ≤0.598, it is more likely for this lesion to be of a benign nature. On the application of the equation to the studied cases, it was found to conform to their diagnostic nature.

All the studied cases of FA and HN had a probability of malignancy below 0.598, a result that favors benignity, and this conforms to the benign nature of FA and HN.

For example, in a case of HN from our studied group, the patient’s age was 24 years and the total score of CD56 expression was 0. On the calculation of the Y value, it was found to be −0.648, and the probability of malignancy was 0.343 which is <0.598, a result that favored benignity. This conforms to the benign nature of HN.

In addition, in a case of FA from our studied group, the patient’s age was 25 years and the total score of CD56 expression was 0. On the calculation of the Y value, it was found to be −0.675, and the probability of malignancy was 0.337 which is <0.598. The resultant value favored benignity that was supported by the benign nature of FA.

On the other hand, the malignant cases (PTC, FVPC, and FC) in our study had a probability of malignancy >0.598. Such a result favors malignancy that is supported by the malignant nature of PTC, FVPC, and FC.

For example, in a case of conventional papillary carcinoma from our study, the age of the patient was 52 years and the CD56 total score was 5. On the calculation of the Y value, it was equal to 3.306 and the probability of malignancy in this case was equal to 0.965 which is >0.598. Such a result favored malignancy that is supported by the malignant nature of PTC.

In addition, in a case FVPC from our study, the age of the patient was 40 years and the CD56 total score was 5. On the calculation of the Y value, it was equal to 3.62 and the probability of malignancy in this case was equal to 0.974 which is >0.598. Such a result favored malignancy that is supported by the malignant nature of FVPC.

In a case FC from our study, the age of the patient was 54 years and the CD56 total score was 3. On the calculation of the Y value, it was equal to 0.362 and the probability of malignancy in this case was equal to 0.796 which is >0.598. Such a result favored malignancy that is supported by the malignant nature of FC.

The formulated equation was also applied to all the studied cases of WDT-UMP; and it was found that three cases out of the studied 5 cases (60%) had a probability of malignancy >0.598. Our result suggests that these cases might pursue a malignant course. While the other 2 cases (40%) had a probability of malignancy below 0.598 suggesting that these cases might be of a benign nature. Confirmation of our results requires follow-up of these cases.

For example, in a case diagnosed as WDT-UMP, the age of the patient was 42 years, and the total score of CD56 expression was 4. On the calculation of the Y value, it was equal to 2.634 and the probability of malignancy was equal to 0.933 which is >0.598. This result suggests that this case might pursue a malignant course. Follow-up of this case may disclose its nature.
From the aforementioned results, it could be suggested that the developed equation may play an important role in the prediction of malignancy of equivocal cases of follicular thyroid lesions especially cases of WD'T-UMP.

From the results of the present study it can be concluded that CD56 could be utilized as a good positive marker favoring the diagnosis of PTC (including its FVPC) over other categories of follicular thyroid lesions (HN, FA, and FC). It also could be of value in differentiating FVPC from other lesions of benign nature including FA and HN.

CD56 expression is significantly associated with malignant behavior in thyroid follicular lesions, which suggests a role for CD56 in tumorgenesis.

More importantly, the developed model and equation based on CD56 expression and patient’s age might be of value in the prediction of malignancy in follicular thyroid lesions, but it needs to be validated on a large scale accompanied by follow-up.

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