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The Relationship Between the Stromal Mast Cell Number, Microvessel Density, C-erbB-2 Staining and Survival and Prognostic Factors in Colorectal Carcinoma

Kolorektal Adenokarsinomlarda Stromal Mast Hücre Sayısı, Mikrodamar Yoğunluğu ve C-erbB-2 Boyanmasının Sağkalım ve Prognostik Faktörler ile İlişkisi

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ABSTRACT

Objective: Colorectal adenocarcinomas take second place among the causes of death from carcinoma, and account for 98% of colorectal carcinomas. There is a need to determine new prognostic factors because of high frequency and significance.

Material and Method: 204 colorectal carcinomas diagnosed between 01.01.2005 - 31.12.2008 at Uludağ University Medical Faculty Pathology Department were studied for Factor VIII and c-erbB-2 immunohistochemically and with toluidine blue stain histochemically. Association of mast cell number, microvessel density, and c-erbB-2 staining pattern with survival and known prognostic factors was evaluated.

Results: Follow-up period was 4-60 months. A total of 111 cases were alive, and 65 had died. The mean number of mast cells was 8.00 (1-21) and the mean density of microvessels was 10.00 (2-21). Five-year survival rate of the mast cell group was 48.3% for values under 10 and 57.9% for values of 10 and higher. Five-year survival rate was 58.2% in the group with a microvessel density of 10 and above and 45.9% for values under 10. Five-year survival rate was 53.9% for the group with c-erbB-2 cytoplasmic staining and 48.2% for the group with membranous staining.

Conclusion: The grade increased with the number of mast cells, while survival decreased with an increase in the number of mast cells. The ratio of c-erbB-2 staining increased as the grade and stage increased. There was an association between mast cell number and microvessel density. We found no relationship between prognosis and c-erbB-2, mast cell number, and microvessel density.

Key Words: Prognosis, Colorectal carcinoma, Mast cells, Human, ERBB2 protein

ÖZ

Amaç: Kolorektal adenokarsinomlar kolorektal karsinomların %98'ini oluşturmakta ve tüm karsinomlardan ölüm sebepleri arasında 2. sırada yer almaktadır. Sık karşılaşıldığı ve insan sağlığı açısından en önemli tümörlerden biri olduğu için prognozu belirlemede bilinen faktörlerin yanı sıra yeni yardımcı yöntemlerin belirlenmesine ihtiyaç vardır.

Gereç ve Yöntem: 01.01.2005 - 31.12.2008 tarihleri arasında Uludağ Üniversitesi Tıp Fakültesi Patoloji Anabilim Dalı'nda kolorektal adenokarsinom tanısı almış 204 olgu çalışma kapsamına alındı. Olgulara ait bloklardan elde edilen kesitlere immünohistokimyasal olarak Faktör VIII, c-erbB-2 ve histokimyasal olarak toluidin blue boyamaları uygulandı. Mast hücre sayısının, mikrodamar yoğunluğunun, c-erbB-2 boyanma paterninin sağkalım ile ve bilinen diğer prognostik faktörler ile ilişkisi değerlendirildi.

Bulgular: Olguların takip süreleri 4-60 ay arasında değişmekte idi. Olguların 111'i hayatta iken 65'i hayatını kaybetmişti. Ortalama mast hücre sayısı 8,00 (1-21), ortalama mikrodamar yoğunluğu 10,00 (2-21) idi. Mast hücre sayısı 10'un altında olan olguların 5 yıllık sağkalım oranı % 48,3 olup 10 ve üzerinde olan olguların 5 yıllık sağkalım oranı %57,9'du. Mikrodamar yoğunluğu 10 ve üzerinde olan olguların 5 yıllık sağkalım oranı %58,2 olup 10'un altında olan olguların 5 yıllık sağkalım oranı % 45,9 idi. C-erbB-2 sitoplazmik boyanma gösteren olguların 5 yıllık sağkalım oranı %53,9, membranöz boyanma gösteren olguların 5 yıllık sağkalım oranı %48,2 idi.

Sonuç: Derece arttıkça mast hücre sayısı artmakta ve mast hücre sayısı arttıkça sağkalım azalmaktadır. Evre ve derece arttıkça c-erbB-2 boyanma oranı artmaktadır. Mast hücre sayısı artışına mikrodamar yoğunluğu artışı eşlik etmektedir. Mast hücre sayısı, mikrodamar yoğunluğu ve c-erbB-2'nin kolorektal karsinomlarda prognostik etkisinin olmadığı saptanmıştır.

Anahtar Sözcükler: Prognoz, Kolorektal karsinom, Mast hücreleri, İnsan, ERBB2 proteini

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INTRODUCTION

Colorectal carcinomas constitute 98% of all colorectal carcinomas and are important malignancies as they cause approximately 450.000 mortalities per year and take second place among causes of death from carcinomas (1). The cancer institute of our country provides data showing these carcinomas took 4th place among males and 2nd place among females between 2004 and 2006. Colorectal carcinomas are seen with equal incidence in males and females and the mean age is 62 years. The most common location is the rectosigmoid region (2).

Angiogenesis is the development of new capillaries from existing vessels and is required for tumor growth and metastases. Microvessel density (MVD) is important in evaluating angiogenesis. C-erbB-2, a member of the tyrosine kinase receptor family, is an oncogene that resembles the epidermal growth factor receptor and is located in the cell membrane. Its increased expression is associated with malignant transformation, increased mitotic activity, invasion and metastases (3). Mast cells (MC) play a role in the immune system.

The aim of our study was to investigate markers that could become new prognostic indicators for colorectal carcinomas as these tumors are common and have high mortality.

MATERIAL and METHODS

This study received the approval of the Uludag University Medical Faculty Medical Investigations Ethic Committee on April 21, 2009 with number 2009-7/27. A total of 204 cases that have been diagnosed with colorectal adenocarcinoma at the Uludag University Medical Faculty Department of Pathology between January 2005 and December 2008 were included in the study. The immunohistochemical Factor VIII and c-erbB-2 and histochemical toluidine blue dyes were applied on the sections obtained from paraffin blocks. Immunohistochemical streptoavidin-biotin staining technique was used (c-erbB-2 Ab 17, Lot: 730p504A and Related antigen, Factor VIII 186, Lot:722p 602A). Histochemically, NovaUltra Toluidine Blue Stain kit was used. Regarding c-erbB-2 staining, a score of 0 was the lack of staining on tumor cells or less than 10% incomplete membrane staining, score 1 was more than 10% weak incomplete membrane staining, score 2 was more than 10% weak or moderate complete membrane staining and score 3 was more than 10% strong complete membrane staining (Figure 1) (4). The MVD and MC counting procedure was performed on Olympus BX50 microscope. Ten peritumoral areas that showed the highest density vascularization on low magnification (40x) were chosen. Microvessels that stained positively with Factor VIII were counted on high magnification (400x) and the mean value was calculated (Figure 2). These were divided into two groups as <10 and ≥10 (5). The peritumoral areas where the staining was highest density on low magnification (40x) were chosen. The number of MC stained with toluidine blue in 10 areas with dense staining was counted and the mean value calculated. The mean MC number was divided into two groups as <10 and ≥10 (Figure 3) (6).

The statistical analysis of the data was performed with SPSS for Windows 13.0 statistical package software. The Pearson chi-square, Mann-Whitney U test, T test, and Spearman correlation tests were used to compare the categorical groups. Kaplan-Meier analysis was used for survival. A p value ≤0.05 was accepted as statistically significant.

RESULTS

The cases' age ranged between 32 and 93 years (mean 65.00 years). There was no correlation between age and MC, MVD, or c-erbB-2 values (p=0.092, p=0.956, p=0.096, respectively). There were 109 (53.4%) males and 95 (46.6%) females. Tumor diameter was 1-13 cm and the mean diameter was 4.14 cm. The tumor was less than 5 cm in 118 cases (57.8%) and was 5 cm or more in 86 cases (42.2%). There were 12 (5.9%) grade 1, 171 (83.8%) grade 2, and 21 (10.3%) grade 3 cases (Table I). A statistically significant relationship was found between grade and number of MC (p=0.018). The number of mast cells increased as the grade increased. The survival rate increased as the mast cell number increased (p=0.035). An increased mast cell number was associated by increased microvessel density (r=0.082). We found no statistically significant relationship between grade and MVD of the cases (p=0.110). There was an almost significant statistical relationship between grade and c-erbB-2 staining (p=0.052) (Table II). We found a statistical correlation between MC number and MVD (p=0.04, r=0.201). The follow-up duration of the cases varied between 4-60 months. The mean follow-up duration was 38.50 months. The number of survivors was 111 while 65 had died. We were unable to access the chart of 28 cases. The mean MC number was 8.00 (1-21) while the mean MVD value was 10.00 (2-21). The 5-year survival was 57.9% for cases with an MC number of 10 or more. The 5-year survival rate was 48.3% for cases with MC number less than 10, 58.2% for cases with an MVD value of 10 or more, 45.9% for cases with an MVD value of 10 or less, 53.9% for cases with c-erbB-2 cytoplasmic staining, and 48.2% for cases with c-erbB-2 membrane staining. We found a statistically significant relationship between MC number and survival (p=0.035) (Figures 4-6). Cytoplasmic c-erbB-2

staining was found in 15 cases in stage 1, 51 cases in stage 2, 45 cases in stage 3, and 6 cases in stage 4. Membranous c-erbB-2 staining was found in 16 cases in stage 1, 29 cases in stage 2, 29 cases in stage 3, and 13 cases in stage 4. There was a statistically significant relationship between c-erbB-2 staining and stage (p=0.049).

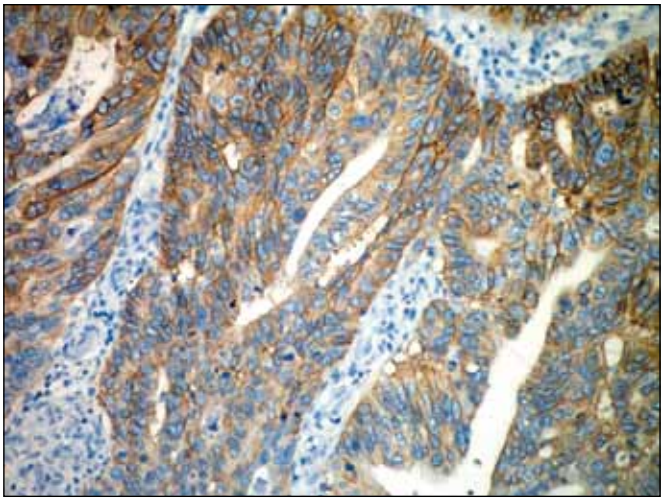

Figure 1: C-erbB-2 membranous staining (x200).

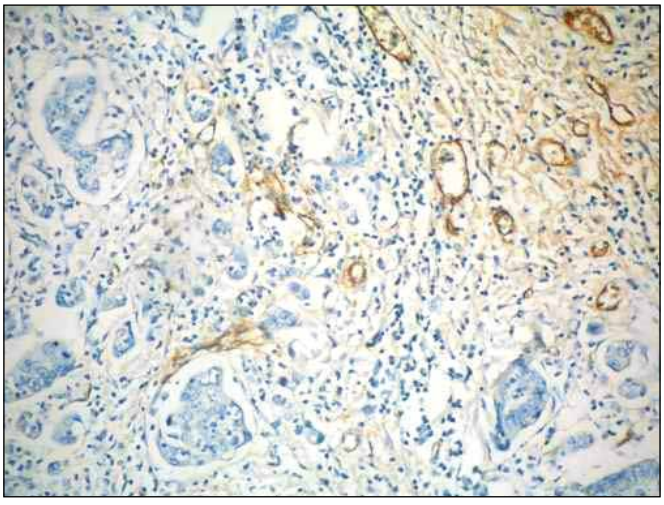

Figure 2: Microvessels stained with Factor VIII (x100).

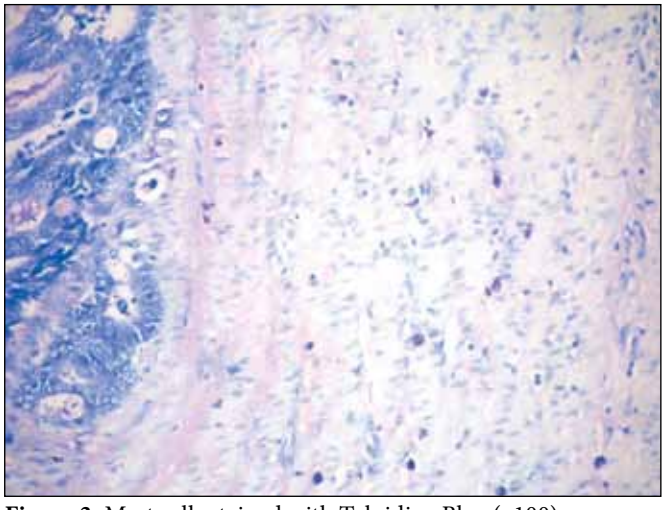

Figure 3: Mast cells stained with Toluidine Blue (x100).

Table I: Histopathological features of the cases

Variables	Number of cases
Vascular invasion	
Yes	28
No	176
Lymphatic invasion	
Yes	52
No	152
Perineural invasion	
Yes	44
No	160
Lymph node metastases	
Yes	88
No	116
Grade	
1	12
2	171
3	21
Desmoplastic reaction	
None	2
Mild	28
Moderate	96
Dense	78
Lymphocytic infiltration	
None	2
Mild	72
Moderate	90
Dense	40
Stage	
1	31
2	80
3	74
4	19
C-erbB-2	
Membranous	87
Cytoplasmic	117
Microvessel density	
<10	96
≥10	108
Mast cell number	
<10	125
≥10	79

Table II: C-erbB-2 staining and histopathological variables

Variables	Cytoplasmic C-erbB-2	Membranous C-erbB-2	Total number of cases(n)	P value
Vascular invasion				
Yes	16	12	28	0.981
No	101	75	176	
Lymphatic invasion				
Yes	31	21	52	0.702
No	86	66	152	
Perineural invasion				
Yes	27	17	44	0.544
No	90	70	160	
Lymph node metastasis				
Yes	49	39	88	0.674
No	68	48	116	
Grade				
1	4	8	12	0.052
2	97	74	171	
3	16	5	21	
Stage				
1	15	16	31	0.049
2	51	29	80	
3	45	29	74	
4	6	13	19	

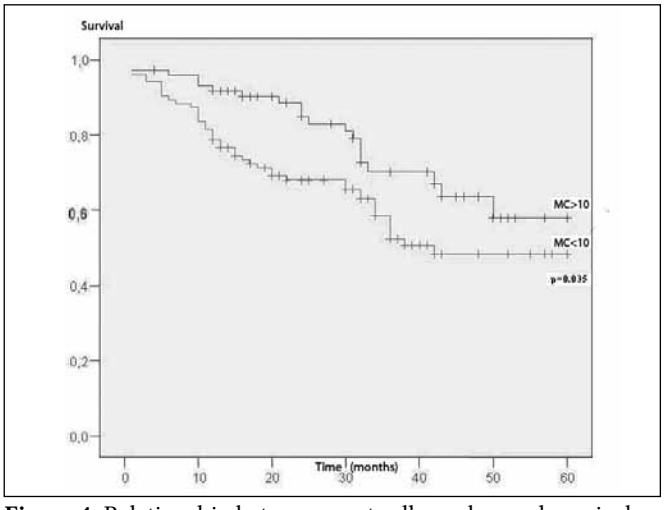

Figure 4: Relationship between mast cell number and survival.

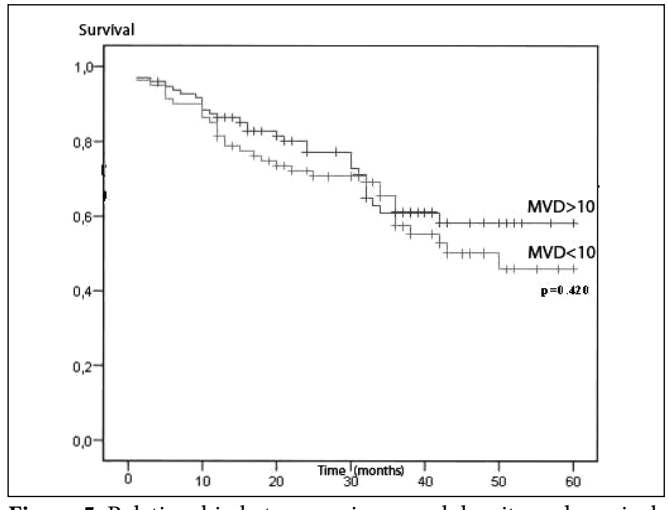

Figure 5: Relationship between microvessel density and survival.

DISCUSSION

Colorectal carcinomas constitute 10% of all carcinomas and take 2nd place among the causes of carcinoma-related death. Colorectal adenocarcinomas make up 98% of all colorectal carcinomas. Markers that can be used as prognostic indicators to define prognosis are required besides the standard criteria as these disorders are encountered commonly and have a high mortality.

MC accumulation is seen in many carcinomas. This accumulation is because of the active migration of MCs

or the accumulation of MC precursors at tumor region in response to the chemoattractants secreted from the tumor. Kessler et al. have shown MC accumulation before the start of angiogenesis in experimentally induced tumors (7). There are various studies to determine the relationship between the prognosis and MC in bladder, larynx, breast, lung and renal cell carcinomas. Serel et al. have shown that MCs are beneficial prognostic factors in bladder tumors in their 56 case series (8).

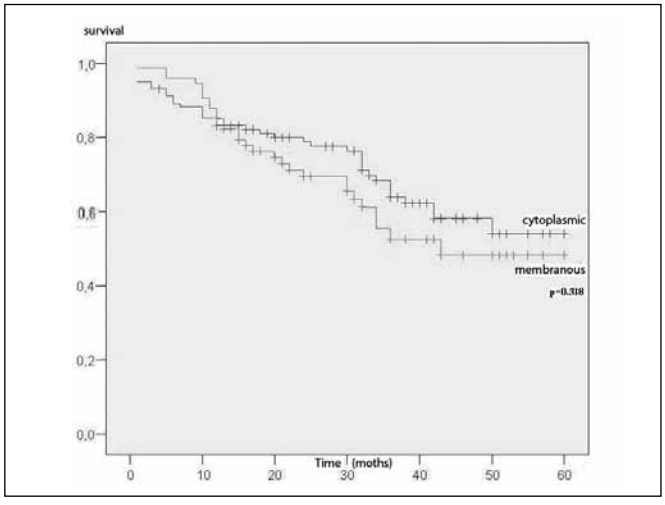

Figure 6: Relationship between c-erbB-2 and survival.

Many studies have reported better survival in colorectal carcinomas with higher numbers of MC (9-10). Lachter et al. have reported that MC is a beneficial prognostic factor (11). However Pretlow et al. have not been able to demonstrate a relationship between MC and the prognosis (12). Fisher et al. have found that cases with 0-3 MC in each high-power field have markedly higher survival rates than cases with 4 or more MC (13). Taweevisit et al. have found the number of MC to be higher in poorly differentiated tumors than in well-differentiated tumors (14).

We found a statistically significant relationship between the MC number and survival and the grade (p=0.035, p=0.018). Increased grade and decreased survival correlated with increased mast cell number (Table III).

The reason for the varying results in studies on MC in the literature could be the method used to demonstrate the MC and inflammatory cells, the type and stage of the tumor study, the various periods for the biopsy, and the location of the evaluated area within the tumoral tissue (normal tissue border or at the center of the tumor).

Table III: Mast cell count and histopathological variables

Variables	MC<10	MC≥10	Total number of cases (n)	P value
Vascular invasion				
Yes	21	7	28	0.108
No	104	12	116	
Lymphatic invasion				
Yes	32	20	52	0.964
No	72	80	152	
Perineural invasion				
Yes	27	10	37	0.989
No	98	62		
Lymph node metastasis				
Yes	55	33	88	0.754
No	70	46	116	
Grade				
1	12	0	12	0.018
2	101	70	171	
3	21	9	30	
Stage				
1	16	15	31	0.636
2	50	30	80	
3	46	28	74	
4	13	6	19	

There are many studies on angiogenesis-stimulating feature of MCs (3,15). They cause activation of matrix metallopeptidase-9 that has a direct effect on metastasis and on tumor distribution in the premalignant regions and initiate the angiogenic process. Activated MCs lead to the secretion of factors such as VEGF, b-FGF, IL-8, and TNF alpha that stimulate angiogenesis (16). The relationship between MC and MVD has been shown in many studies. A relationship between MC number and MVD has been found in renal carcinoma by Tuna et al. and in breast carcinoma by Kwon et al. (17, 18). A relationship between MC number and MVD has also been shown in laryngeal carcinomas by Balica and primary non small cell carcinomas of the lung by Dundar (19, 20). Mohseni has not found a relationship between MC and MVD in a study on 40 renal cell carcinoma cases (21). We found a correlation between the MC number and MVD in our study.

There are studies on angiogenesis in many different carcinoma types. These studies have shown MVD, which was found to be an indicator of tumor aggressiveness in breast, prostate, lung, endometrium, cervix, oral cavity, and bladder carcinomas and malignant melanoma, to be a prognostic factor (22-24).

Some studies have reported longer survival periods for patients with higher MVD (25,26). Many studies report a relationship between increased MVD and decreased survival (27-38). Some investigators have reported that MVD is not a prognostic factor in colorectal carcinomas (39,40).

We were unable to find a relationship between MVD and survival or other prognostic factors (Table IV). The reason may be the non-standard MVD counting procedure in different articles (using various modifications and different numbers of microscopic fields), the use of different antibodies for immunohistochemistry, the retrospective nature of our study, the decreased tissue antigenicity over time and the difficulty in evaluating immunohistochemical stains accordingly.

Although there are many reports on breast carcinoma and c-erbB-2 staining, only a few articles are available on this subject in colorectal carcinoma. C-erbB-2 membranous staining in breast carcinoma patients is associated with shorter survival periods (41). Looking at the results of studies on colorectal carcinoma, we can see that various results have been found and most patients show cytoplasmic

Table IV: Microvessel density and histophatological variables

Variables	MVD<10	MVD≥10	Total number of cases (n)	P value
Vascular invasion				
Yes	16	12	28	0.250
No	80	96	176	
Lymphatic invasion				
Yes	24	28	52	0.9880
No	72	80	152	
Perineural invasion				
Yes	20	24	44	0.473
No	76	84	160	
Lymph node metastasis				
Yes	36	52	88	0.125
No	60	56	116	
Grade				
1	8	4	12	0.110
2	75	96	171	
3	13	8	21	
Stage				
1	13	18	31	0.311
2	44	36	80	
3	32	42	74	
4	7	12	19	

staining (42-45). The rate was 54% in the Essapen's study, 38% in the Kluftinger's study, and 63.5% in the Half's study (42, 43, 45). Many studies have not found a relationship between survival period, stage, grade and c-erbB-2 staining in colorectal carcinomas (46-50). Kountourakis et al. agree with these views but report that c-erbB-2 is expressed in early stage colorectal carcinomas (51). They found membranous staining at a rate of 47.16% and cytoplasmic staining at a rate of 30.19% in their study.

Tavangar et al. found a significant relationship between c-erbB-2 and stage and grade in their study on colorectal carcinoma cases (52). Park et al. and Osako et al. have reported that c-erbB-2 is an independent prognostic factor, related to survival in colorectal carcinomas and recurrence increases while the survival decreases as the expression is increased (53, 54). Kapitanovich et al. have studied samples of benign lesions and adenocarcinomas in 221 colons and have found increased c-erbB-2 staining in adenocarcinomas compared to benign lesions and reported a relationship between c-erbB-2, and stage, grade and survival in colorectal carcinomas (55).

We found a significant relationship between c-erbB-2 staining and stage and an almost significant relationship between c-erbB-2 staining and grade in our study. We did not find a statistically significant relationship c-erbB-2 staining and other prognostic factors or survival.

Our study was limited by our inability to access the clinical records of some of our cases and limited follow-up duration of most of the cases.

In conclusion, we found a significant relationship between MC number and survival and grade. An increased grade meant increased number of mast cells. An increased number of MCs meant increased MVD. There was a correlation between MC number and MVD. An increased MC number was associated by increased MVD. MC number may be used as an alternative criteria to the standard criteria used to determine the prognosis of colorectal carcinoma. We found a significant relationship between c-erbB-2 and stage and an almost significant relationship between c-erbB-2 and grade. We did not find a relationship between c-erbB-2 and survival. Studies on larger series and with longer follow-up periods are needed to determine any significance regarding the prognosis.

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