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Original Article

Comparison of peripheral blood T lymphocyte immune function among venous thromboembolism patients with and without infection and patients with simple infection

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Abstract: Objective: To investigate the differences of T lymphocyte subgroups and high-sensitivity C reactive protein (HsCRP) levels among patients with venous thromboembolism (VTE), VTE patients with infection, simple infection patients and the normal controls. Method: 289 patients were enrolled in this study and divided into control group, VTE group, VTE with infection group and simple infection group. Result: Compared with the control group, the serum levels of $CD3^+$, $CD4^+$, $CD8^+$ T lymphocytes significantly decreased and $CD4^+/CD8^+$ ratio significantly increased in simple infection group ($P < 0.05$); $CD3^+$ and $CD8^+$ T lymphocytes significantly decreased and $CD4^+/CD8^+$ ratio significantly increased in VTE and VTE with infection group ($P < 0.05$); the proportion of declined $CD3^+$ and $CD8^+$ T lymphocytes increased, and the proportion of increased $CD4^+/CD8^+$ ratio statistically elevated in three disease groups. As an important inflammatory factor, all HsCRP levels in three disease groups significantly increased when compared with the control group. Conclusion: Immune dysfunction exists in both of VTE and infection patients, while VTE patients tend to be accompanied with infections. The changes of T lymphocyte subgroups in VTE patients, who were independent from infection, could cause T lymphocyte immune dysfunction, suggesting that there were abnormalities of T lymphocyte immune function in VTE itself. The overall T lymphocyte functions of recognizing antigens and transducing activation signals decline in VTE patients. Besides, the function of T lymphocyte of directly killing virus microbes declines significantly and the inflammatory mechanisms are involved in the occurrence and development of venous thrombosis.

Keywords: T lymphocyte, venous thromboembolism, immune function

Introduction

Venous thromboembolism (VTE) is a severe life-threatening disease, including pulmonary thromboembolism (PE) and deep venous thrombosis (DVT), which are two different stages of a same disease process. Sudden death is the initial clinical manifestation in almost 25% of PE patients [1]. According to the American epidemiological statistics, PE is the third leading cause of death ranking after coronary heart disease and stroke [2], and it is also common in China and other Asian countries. So far, the etiology and pathogenesis of VTE are still not clear. In recent years, researches believe that venous thrombosis was associated with infections, and inflammation and immunologic mechanisms were involved in the occurrence and development of VTE. Thus, this study aimed to explore the immune function changes in patients with VTE and infection, and to seek the possible pathogenesis of VTE.

Materials and methods

Patient samples

From January 2008 to August 2012, 68 patients with VTE (VTE group), 44 VTE patients with infections (VTE with infection group) and 95 patients with simple infection (simple infection group) from the hospitalized patients, and 82 healthy outpatients (control group) from Tongji Hospital affiliated to Tongji University were enrolled in this study. The diagnosis of PE patients is in accordance with any two of the following principles: 1) selective pulmonary artery angiography showed pulmonary arterial obstruction or filling defect; 2) lung ventilation-perfusion scan showed single or multiple blood

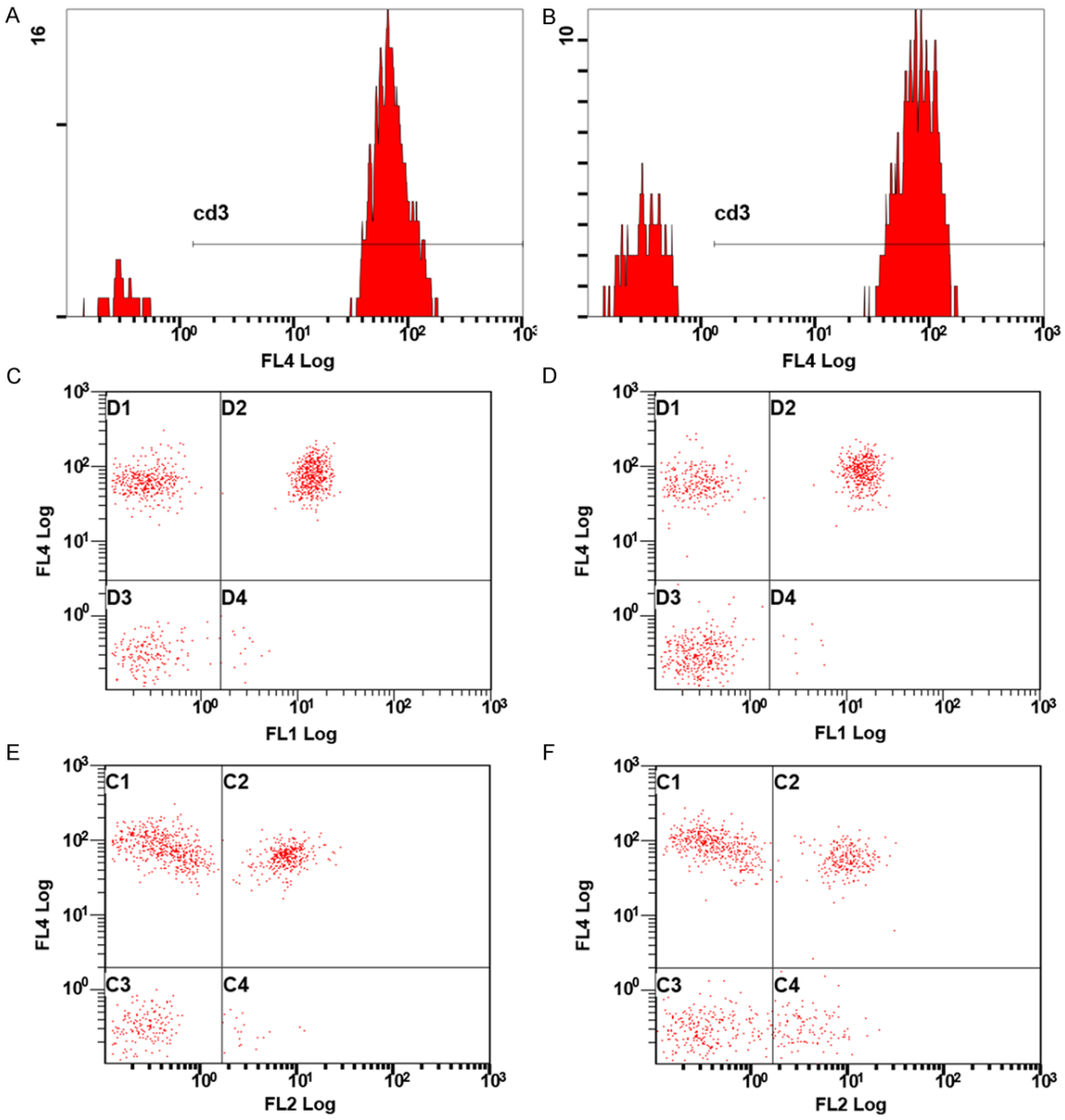

Figure 1. Flow cytometry was used to detect the differentiation antigens on the surface of T lymphocytes in control and VTE group. CD3 (A), CD4 (C) and CD8 (E) in control group; CD3 (B), CD4 (D) and CD8 (F) in VTE group.

perfusion defects, normal or abnormal ventilation and mismatched ventilation perfusion ratio; 3) clinical diagnosis: risk factors of pulmonary embolism, clinical manifestations, echocardiography, chest CT, arterial blood gas analysis, D dimer detection and Wells' score supported diagnosis of PE, while other cardiopulmonary diseases were ruled out by electrocardiogram and chest X-ray. DVT is diagnosed with color Doppler flow imaging of lower limb vessels. The patients with malignant tumor, autoimmune disease, immunosuppressant usage or arterial thrombosis were excluded from this study. The controls were selected from the healthy outpatients.

Methods

Indexes of patients in this study were measured immediately after hospitalization. Fasting cubital veins blood samples in the morning were tanken from all the subjects. Hitachi 7170 automatic biochemical analyzer was applied to detect high-sensitivity C reactive protein (HsCRP), FBG, total cholesterin, triglyceride, high-density lipoprotein cholesterol and low-

Table 1. Baseline data of study population and CD values

Variable	Control group (1)	Simple infection group (2)	VTE group (3)	VTE with infection group (4)	P (1 *vs.* 2, 3 and 4)	P (2 *vs.* 3)	P (3 *vs.* 4)	P (2 *vs.* 4)
Case number	82	95	68	44				
Gender (male) (n/%)	27/32.9	54/56.8	27/39.7	22/50.0	0.010	0.031	0.283	0.451
Age	64.2 ± 15.3	69.7 ± 17.2	64.9 ± 14.6	66.8 ± 18.5	0.124	0.061	0.559	0.367
Smoking (Yes, n/%)	36/43.9	39/41.1	10/14.7	10/22.7	0.012	< 0.001	0.279	0.035
Alcohol (Yes, n/%)	13/15.9	13/13.7	5/7.4	7/15.9	0.392	0.203	0.153	0.728
History of HBP (Yes, n/%)	0	32/33.7	28/41.2	16/36.4	< 0.001	0.328	0.611	0.757
History of DM (Yes, n/%)	0	22/23.2	10/14.7	8/18.2	< 0.001	0.180	0.625	0.507
SBP	122.6 ± 7.8	128.8 ± 16.4	130.5 ± 19.9	128.5 ± 19.1	0.001	0.564	0.596	0.913
DBP	73.0 ± 7.9	74.7 ± 9.3	79.0 ± 11.4	76.9 ± 9.0	0.002	0.008	0.299	0.187
HR	78.7 ± 11.4	82.3 ± 13.9	84.1 ± 13.5	89.3 ± 16.7	0.001	0.407	0.089	0.018
BMI	24.9 ± 3.7	24.0 ± 3.0	24.8 ± 2.4	24.1 ± 2.8	0.243	0.055	0.119	0.949
CRP	3.2 ± 2.6	18.1 ± 13.7	12.6 ± 7.5	14.7 ± 12.9	< 0.001	0.001	0.307	0.109
$CD3^+$	71.0 ± 7.2	61.6 ± 12.6	63.6 ± 13.5	64.6 ± 13.8	< 0.001	0.343	0.700	0.212
$CD4^+$	39.9 ± 7.2	34.6 ± 12.5	37.1 ± 11.0	34.1 ± 10.4	< 0.001	0.182	0.155	0.828
$CD8^+$	28.0 ± 6.1	24.4 ± 8.9	22.4 ± 9.6	23.3 ± 8.6	< 0.001	0.180	0.621	0.504
$CD4^+/CD8^+$	1.49 ± 0.48	1.70 ± 1.03	2.15 ± 2.01	1.81 ± 1.73	0.003	0.061	0.367	0.615

Footnotes: HBP = high blood pressure; DM = diabetes mellitus; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; BMI = body mass index; CRP = C-reaction protein; VTE = venous thromboembolism.

density lipoprotein cholesterol. EPICS XL-II flow cytometry (America BECKMANCOULTER Company) was applied to detect differentiation antigens on the surface of T lymphocytes (**Figure 1**).

Statistical analysis

Epidata 3.1 was applied for double entries. All analysis was carried out with SPSS14.0 software (IBM corporation). Normally distributed continuous data was presented as mean ± standard deviation. Comparison among groups was done with one-way analysis of variance, while comparison between two groups was done with LSD test. Abnormal distributed data was compared by nonparametric Kruskal-Wallis test. According to the normal range, the values of $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ were divided into high, low and normal groups. The differences between groups were compared by x^2 test. All the statistics was taken by bilateral tests, *P* value less than 0.05 was considered significant.

Result

Comparison of CD values and baseline data among four groups

The baseline data and CD values of 289 objects are shown in **Table 1**. There were no significant differences of age, BIM and drinking among four groups (P > 0.05). Male rates in control group and VTE group were lower than those in VTE with infection and simple infection groups. Smoking rates in control group and simple infection group were higher than that in VTE group. There was no history of high blood pressure and diabetes mellitus in control group. Among VTE group, VTE with infection group and simple infection group, there were no differences in the rates of hypertension and diabetes mellitus. However, systolic pressure, diastolic pressure and heart rate level in these three groups were higher than that in control group. The levels of HsCRP in VTE group, VTE with infection group and simple infection group were significantly higher than that in control group. There were no differences of the levels of HsCRP between VTE group and VTE with infection group (P = 0.320), as well as between VTE with infection group and simple infection group (P = 0.078).

Results of comparison of T lymphocyte subgroups among four groups showed that compared with control group, the levels of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes declined significantly (P < 0.005), while there was no significant change about $CD4^+/CD8^+$ ratio (P = 0.335) in simple infection group. Compared with control group, the levels of $CD3^+$ and $CD8^+$ T lym-

Table 2. x^2 value of pairwise comparison among four groups

Variable	Control group (1)	Simple infection group (2)	VTE group (3)	VTE with infection group (4)	P (1 *vs.* 2, 3 and 4)	P (2 *vs.* 3)	P (3 *vs.* 4)	P (2 *vs.* 4)
$CD3^+$ (n/%)					< 0.001	0.166	0.894	0.089
Low	3/3.7	40/42.1	23/34.3	10/39.5				
Normal	77/93.9	55/57.9	42/62.7	26/60.5				
High	2/2.4	NA	2/3.0	2/4.7				
$CD4^+$ (n/%)					< 0.001	0.135	0.413	0.233
Low	1/1.2	21/22.1%	7/10.4	7/16.3				
Normal	77/93.9	64/67.4%	50/74.6	27/62.8				
High	4/4.9	10/10.5%	10/14.9	9/20.9				
$CD8^+$ (n/%)					< 0.001	0.404	0.847	0.807
Low	3/3.7	26/27.4%	25/37.3	14/32.6				
Normal	78/95.1	66/69.5%	40/59.7	28/65.1				
High	1/1.2	3/3.2%	2/3.0	1/2.3				
$CD4^+/CD8^+$ (n/%)					< 0.001	0.165	0.473	0.502
Low	7/8.5	24/25.3	12/17.9	7/16.3				
Normal	69/84.1	42/44.2	25/37.3	21/48.8				
High	6/7.3	29/30.5	30/44.8	15/34.9				

phocytes decreased significantly ($P < 0.001$), the level of $CD4^+$ T lymphocyte did not change ($P = 0.110$), and $CD4^+/CD8^+$ ratio increased significantly ($P = 0.003$) in VTE group. Compared with control group, the levels of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes decreased significantly ($P < 0.005$), but there was no significant change of $CD4^+/CD8^+$ ratio ($P = 0.213$) in VTE with infection group. Compared with simple infection group, there were no significant differences of levels of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes and $CD4^+/CD8^+$ ratios in both VTE group and VTE with infection group ($P > 0.05$).

x^2 value of pairwise comparison among four groups

The normal range of $CD3^+$ T lymphocyte is 60-85%; $CD4^+$ T lymphocyte 24.5-48.8%; $CD8^+$ T lymphocyte 18.5-42.1%; and $CD4^+/CD8^+$ ratio 1.02-1.94%. Results of comparisons of the proportions of low, normal and high CD values between three disease groups and control group showed significant differences. Compared with control group, the proportion of declined $CD3^+$ and $CD8^+$ T lymphocytes increased, and the proportion of elevated $CD4^+/CD8^+$ ratio significantly increased ($P < 0.01$) in three disease groups. However, there were no significant differences of the proportions of low, normal and high CD values among VTE group, VTE with infection group and simple infection group ($P > 0.05$). In VTE group, there were 25 cases with abnormal $CD3^+$ values (23 cases increased and 2 cases declined), 17 cases with abnormal $CD4^+$ values (10 cases increased and 7 cases declined), 27 cases with abnormal $CD8^+$ values (2 cases increased and 25 cases declined) and 42 cases with abnormal $CD4^+/CD8^+$ ratio (30 cases increased and 12 cases declined), and the increased rate reached 44.8%. In VTE with infection group, there were 12 cases with abnormal $CD3^+$ values (2 cases increased and 10 cases declined), 16 cases with abnormal $CD4^+$ values (9 cases increased and 7 cases declined), 15 cases with abnormal $CD8^+$ values (1 cases increased and 14 cases declined) and 22 cases with abnormal $CD4^+/CD8^+$ ratio (15 cases increased and 7 cases declined) and the increased rate reached 34.9%. In simple infection group, the values of $CD3^+$, $CD4^+$ and $CD8^+$ mainly showed declining trends, however the percentage of increased $CD4^+/CD8^+$ ratio was similar to the percentage of declined $CD4^+/CD8^+$ ratio (**Table 2**). As shown in **Figure 2**, compared with control group, the proportion of increased $CD4^+/CD8^+$ ratios elevated significantly in VTE group and VTE with infection group.

Discussion

DVT and PE have the characteristics of high morbidity, mortality, misdiagnosis rate and

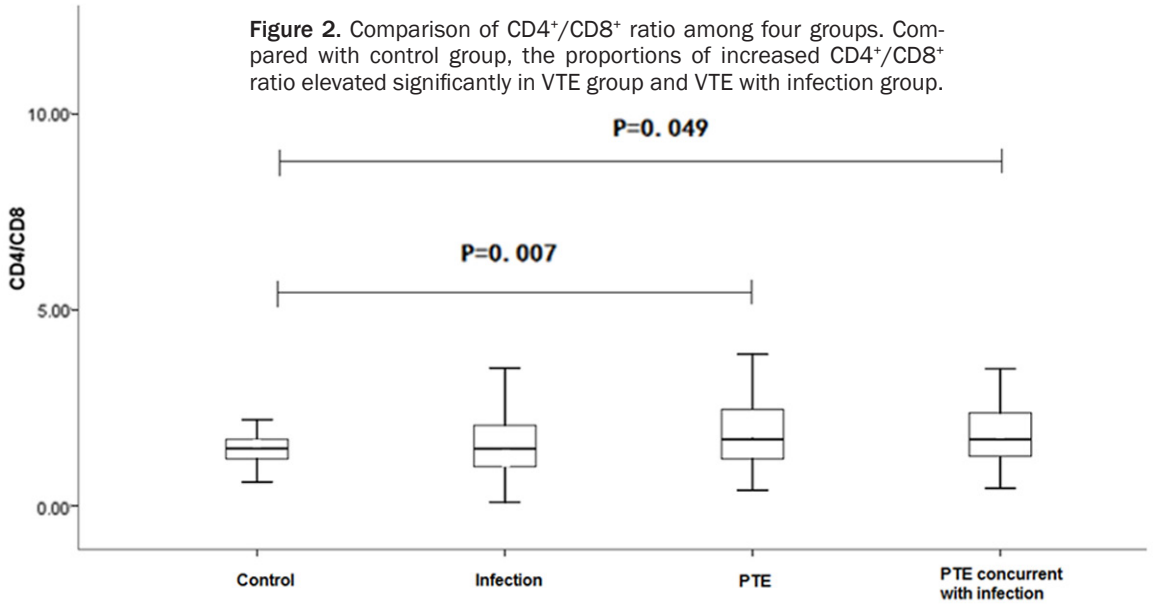

Figure 2. Comparison of $CD4^+/CD8^+$ ratio among four groups. Compared with control group, the proportions of increased $CD4^+/CD8^+$ ratio elevated significantly in VTE group and VTE with infection group.

missed diagnosis rate. According to an American report, there are 0.5 out of one thousand patients developing DVT every year [3]. In recent 20 years, clinical reports of PE increased sharply in Asia, including China, Japan, Thailand, Singapore, Taiwan and other places year by year [2]. American College of Chest Physicians have been formulating several thrombus prevention guidelines about risk stratification of patients with surgical operations and applications of anticoagulants to treat or prevent VTE for many years, but the incidence of VTE still goes up. It is suggested that there might be a deviation in current VTE preventions and the traditional theory of thrombosis.

Recently, the roles of inflammation and immune reactions in the pathogenesis of VTE attract the attention of researchers and clinicians. In 2006, Smeeth et al. reported that the occurrence of VTE was associated with infection, and VTE often occurred within 2 weeks after infections [4]. Besides, M. Schmidt et al. found that patients with infection had a twofold increased risk of VTE through a retrospectively study of 15000 VTE patients in 2012 [5]. A variety of inflammatory markers have been proved to promote venous thrombosis. The occurrence and reoccurrence of VTE and post-thrombotic syndrome can be reduced by inhibiting interleukin (IL)-6, IL-8, tumor necrosis factor-α or soluble P-selectin directly [6, 7]. These evidences suggest that the increase of inflammatory markers is not the performance of systemic inflammatory responses, but directly involved in the thrombosis.

The author reported venous thrombosis in multiple organs in a biopsy of SARS patient, and virus like structures in the lymph node of a chronic thromboembolic pulmonary hypertension (CTEPH) patient, suggesting that virus attacked T lymphocytes, and lead to serious damages of T lymphocyte immune function [8, 9]. The author also reported T lymphocyte dysfunction in CTEPH and VTE patients, which was that the level of $CD3^+$ and $CD8^+$ T lymphocytes decreased with a significant increase of $CD4^+/CD8^+$ ratio [10]. The authors' team found that the expression of T lymphocytes related mRNAs changed significantly with the imbalance of Th1/Th2 in VTE patients [11]. This study suggested that the abnormal immune function of T lymphocytes was involved in venous thrombosis, however the mechanism remains unclear.

As for now, it is known that cellular immune dysfunction exists in patients with infection, showing immunosuppression characterized of reduced reactivity or no response status of T lymphocytes. Immune defense plays a vital role in the process of host against infections. Immunomodulatory therapy can play a role of assisting anti-infections. The subgroups of T lymphocytes play the most important role in immune system. In this study, $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes represent three kinds of

monoclonal antibodies of total T lymphocytes, T-helper lymphocytes and suppressive T lymphocytes respectively. T-helper lymphocyte has the ability of enhancing humoral and cellular immune response, while suppressive T lymphocytes can inhibit humoral and cellular immunity. Once T-helper lymphocytes or suppressive T lymphocytes are inadequate or dysfunctional in human body, T lymphocytes regulation network will lose balance, leading to a variety of diseases. Therefore, through the detection of T lymphocytes monoclonal antibodies, particularly $CD4^+/CD8^+$ ratio, we can recognize the variation of T lymphocytes network between physiological and pathological conditions, and then assess immune status of patients accurately.

In patients with infections, the levels of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes reduce; the overall T lymphocytes functions of recognizing antigen, transducing activated signal and directly killing virus microbial decline. These are consistent with the results of this study. T lymphocytes immunity disorders in VTE patients and VTE patients with infection were similar with but not identical to the patients with simple infection. The level of $CD4^+$ T lymphocytes did not reduce significantly, while the levels of $CD3^+$ and $CD8^+$ T lymphocytes declined, and $CD4^+/CD8^+$ ratio increased in VTE patients and VTE patients with infection. However, this cannot be explained with infections, because the decrease of $CD8^+$ T lymphocytes and the increase of $CD4^+/CD8^+$ ratio in VTE group were significant when compared with simple infection group and VTE with infection group. Therefore, immune dysfunction, which is independent from abnormal immune function caused by infections, exists in patients with VTE.

Furthermore, there were almost 50% of VTE patients accompanied with infections and VTE occurred in nearly 16% patients within 2 weeks after fractures in this study. So it can be speculated that acute inflammation, such as infection and some autoantibodies stimulated T lymphocytes function disorders, is one of the main causes of the occurrence and development of VTE. The limitation of this study is the simple research method, and further clinical trials as well as animal experiments are needed to certify the correlation and mechanism between VTE symptoms and immune inflammatory response. But the proposition of VTE immune inflammation theory can provide researchers with new enlightenment, new research directions and approaches, which is of great significance.

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Disclosure of conflict of interest

None.

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