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• *BRIEF REPORTS* •

Preoperative sorting of circulating T lymphocytes in patients with esophageal squamous cell carcinoma: Its prognostic significance

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Abstract

AIM: To elucidate the immunologic parameters for the outcome of patients with malignant tumors, especially esophageal squamous cell carcinoma (ESCC) associated with high malignant potential.

METHODS: Clinicopathologic features were compared between patients with lower and higher CD4 and CD8 values as well as CD4/CD8 ratio in peripheral blood.

RESULTS: The survival rate of patients with higher CD4 value was significantly better than that in patients with lower CD4 value (P = 0.039). The survival rate of patients with higher CD8 value was significantly worse than that of patients with lower CD8 value (P = 0.026). Similarly, the survival rate of patients with higher CD4/CD8 ratio was significantly better than that of patients with lower CD4/CD8 ratio (P = 0.042). Additionally, multivariate analysis demonstrated that lower CD8 and lower CD4/CD8 ratio were factors independently associated with worse prognosis of patients.

CONCLUSION: All the immunologic parameters can predict the outcome of patients with ESCC.

Key words: Lymphocyte sub-population; Esophagus; Squamous cell carcinoma; Prognostic indicator

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INTRODUCTION

Impaired immunity is well known to be correlated with the tumorigenesis and/or progressive behavior of human tumors[1-3]. Therefore, it is important to assess the immunologic dynamics of patients with malignant tumors, especially esophageal carcinoma.

We have reported the significance of preoperative assessment of such immunological parameters as serum C-reactive protein concentration[4], prognostic nutritional index[5], and phytohemagglutinin (PHA) response test[6] as a prognostic indicator in esophageal carcinoma.

CD8+, cytotoxic T lymphocytes, plays an immunologic role as the specific tumor terminator and CD4+, helper T lymphocyte, serves the function of controlling CD8+ T-cell-dependent tumor termination[7]. However, only a few investigations are available on the clinicopathologic significance of these lymphocytes in controlling esophageal carcinoma[8-10].

It was reported that lower CD4/CD8 ratio in peripheral blood can be used as an indicator for worse prognosis of patients with esophageal carcinoma[11]. In the current study, we investigated the clinical significance of the serum values of CD4 and CD8, and the CD4/CD8 ratio in patients with esophageal squamous cell carcinoma (ESCC).

MATERIALS AND METHODS

One hundred and thirty-four patients (118 men and 16 women) with ESCC, who underwent esophageal resection and reconstruction of the digestive tract in our institute between 1990 and 1997, were enrolled in this study. The patients had a median age of 62 years (range, 41-82 years).

Follow-up was continued until their death. The interval of follow-up ranged from 29 d to 8 years and 9 mo averaged 2 years and 11 mo. Serum values of lymphocyte sub-populations, CD4 and CD8, were measured as previously described[11].

Pathological features were presented according to the guidelines for clinical and pathologic studies on carcinoma of the esophagus established by the Japanese Society for Esophageal Diseases[12], and clinical stages were determined by the TNM classification of malignant tumors approved by the International Union Against Cancer[13]. Clinicopathologic features were compared between patients with lower and higher values of CD4 and

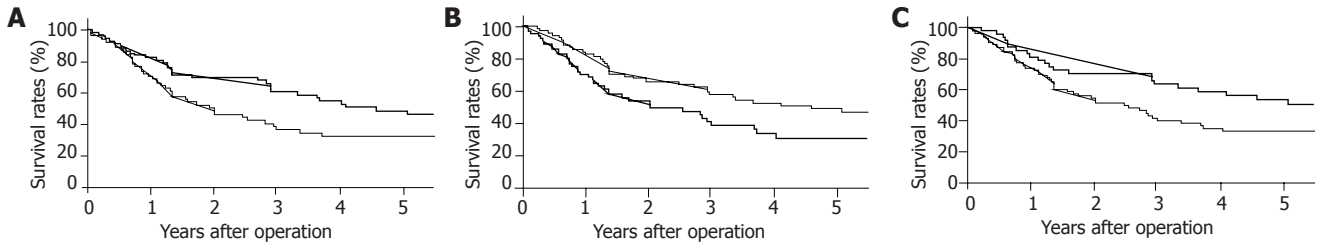

Figure 1 **A:** Survival rate in group H-CD4 (thick line) and group L-CD4 (thin line, *P*=0.039); **B:** Survival rate in group H-CD8 (thick line) and group L-CD8 (thin line, *P*=0.026); **C:** Survival rate in group H-CD4/8 (thick line) and group L-CD4/8 (thin line, *P*=0.042).

Table 1 Relationship between serum CD4 value and clinicopathological features (mean±SD), *n* (%)

Variables	Group H-CD4 (*n* = 68)	Group L-CD4 (*n* = 66)	*P*
Gender			
Male	57 (83.8)	61 (92.4)	0.205
Female	11 (16.2)	5 (7.6)	
Age	60.9±8.8	64.1+9.6	0.046
Location of tumor			
Upper	12 (17.6)	11 (16.7)	0.845
Middle	38 (55.9)	40 (60.6)	
Lower	18 (26.5)	15 (22.7)	
Degree of differentiation			
Well	4 (5.9)	6 (9.1)	0.620
Moderately	57 (83.8)	51 (77.3)	
Poorly	7 (10.3)	9 (13.6)	
Tumor size (cm)	5.3+2.7	5.4+2.5	0.970
Depth of tumor			
Tis	2 (2.9)	2 (3.0)	0.051
T1a	6 (8.8)	5 (7.6)	
T1b	15 (22.1)	9 (13.6)	
T2	14 (20.6)	9 (13.6)	
T3	15 (22.1)	33 (50.0)	
T4	16 (23.5)	8 (12.2)	
Lymph nodes metastasis			
Positive	24 (35.3)	31 (53.0)	0.231
Negative	44 (64.7)	35 (47.0)	
Lymphatic invasion			
Positive	12 (17.6)	18 (27.3)	0.259
Negative	56 (82.4)	48 (72.7)	
Venous invasion			
Positive	5 (7.4)	11 (16.7)	0.163
Negative	63 (92.6)	55 (83.3)	
TNM stage			
0	2 (2.9)	2 (3.0)	0.749
I	17 (25.0)	11 (16.7)	
IIA	16 (23.5)	18 (27.3)	
IIB	10 (14.7)	8 (12.2)	
III	23 (33.8)	27 (40.8)	
Curability			
Curative resection	52 (76.5)	56 (84.8)	0.314
Non curative resection	16 (23.5)	10 (15.2)	

Well = well differentiated squamous cell carcinoma; Moderately = moderately differentiated squamous cell carcinoma; Poorly = poorly differentiated squamous cell carcinoma.

CD8 as well as CD4/CD8 ratio.

Chi-square test and Student's *t* test were used to compare the clinicopathologic data. The cumulative survival rates were calculated by the Kaplan-Meier method and the survival curves were tested by the Mantel-Cox method. $P<0.05$ was considered statistically significant.

RESULTS

Among the clinicopathologic factors, the mean age of patients with higher CD4 value (group H-CD4) was significantly lower than that of patients with lower CD4 value (group L-CD4, $P = 0.046$). However, no significant difference was observed in other factors including tumor-related factors (Table 1). The 1-, 3-, and 5-year survival rates were 82.2%, 60.4% and 48.4%, respectively, in group H-CD4 and 70.8%, 36.9% and 32.7%, respectively, in group L-CD4 ($P = 0.039$, Figure 1A).

No significant difference was found in the clinicopathologic factors between patients with higher (group H-CD8) and lower CD8 value (group L-CD8, Table 2). The 1-, 3-, and 5-year survival rates were 69.7%, 38.5% and 30.0%, respectively, in group H-CD8 and 82.7%, 57.7%, and 49.0%, respectively, in group L-CD8 ($P = 0.026$, Figure 1B).

Significant difference between patients with higher (group H-CD4/8) and lower CD4/CD8 ratio (group L-CD4/8) was observed only in gender proportion ($P = 0.036$, Table 3). The 1-, 3-, and 5-year survival rates were 80.9%, 62.9%, and 52.9%, respectively, in group H-CD4/8 and 74.0%, 40.3% and 33.0%, respectively, in group L-CD4/8 ($P = 0.042$, Figure 1C).

Multivariate analysis demonstrated that lower CD8 (95%CI, 2.07, 1.26–3.38; $P = 0.004$) and lower CD4/CD8 ratio (95%CI, 1.73, 1.02-2.93; $P = 0.043$) were factors independently associated with worse prognosis of patients.

DISCUSSION

With the development of monoclonal antibodies in detecting lymphocytes subpopulation[14], lymphocyte subtypes in peripheral blood were examined to investigate their functions in immune-surveillance. Among the subpopulations of lymphocytes, investigations of cancer immunology have been focused on CD8, suppressor/

Table 2 Relationship between serum CD8 value and clinico-pathological features (mean±SD), *n* (%)

Variables	Group H-CD8 (*n* = 64)	Group L-CD8 (*n* = 70)	*P*
Gender			
Male	58 (90.6)	60 (85.7)	0.543
Female	6 (9.4)	10 (14.3)	
Age	63.4+9.6	61.6+9.1	0.265
Location of tumor			
Upper	9 (14.1)	14 (20.0)	0.635
Middle	38 (59.4)	40 (57.1)	
Lower	17 (26.5)	16 (22.9)	
Degree of differentiation			
Well	4 (6.3)	6 (8.6)	0.810
Moderately	53 (82.8)	55 (78.6)	
Poorly	7 (10.9)	9 (12.8)	
Tumor size (cm)	5.4+2.4	5.3+2.8	0.930
Depth of tumor			
Tis	2 (3.1)	2 (2.8)	0.899
T1a	4 (6.3)	7 (10.0)	
T1b	11 (17.2)	13 (18.6)	
T2	10 (15.6)	13 (18.6)	
T3	26 (40.6)	22 (31.4)	
T4	11 (17.2)	13 (18.6)	
Lymph nodes metastasis			
Positive	29 (45.3)	26 (37.1)	0.382
Negative	35 (54.7)	44 (62.9)	
Lymphatic invasion			
Positive	11 (17.2)	19 (27.1)	0.241
Negative	53 (82.8)	51 (72.9)	
Venous invasion			
Positive	9 (14.1)	7 (10.0)	0.647
Negative	55 (85.9)	63 (90.0)	
TNM stage			
0	2 (3.1)	2 (2.8)	0.858
I	12 (18.8)	16 (22.9)	
IIA	15 (23.4)	19 (27.1)	
IIB	8 (12.5)	10 (14.3)	
III	27 (42.2)	23 (32.9)	
Curability			
Curative resection	51 (79.7)	57 (81.4)	0.830
Non curative resection	13 (20.3)	13 (18.6)	

Well = well differentiated squamous cell carcinoma; Moderately = moderately differentiated squamous cell carcinoma; Poorly = poorly differentiated squamous cell carcinoma.

Table 3 Relationship between serum CD4/CD8 ratio and clinico-pathological features (mean±SD), *n* (%)

Variables	Group H-CD4/8 (*n* = 48)	Group L-CD4/8 (*n* = 86)	*P*
Gender			
Male	38 (79.2)	80 (93.0)	0.036
Female	10 (20.8)	6 (7.0)	
Age	61.4+8.9	63.0+9.6	0.322
Location of tumor			
Upper	10 (20.8)	13 (15.1)	0.675
Middle	26 (54.2)	52 (60.5)	
Lower	12 (25.0)	21 (24.4)	
Degree of differentiation			
Well	5 (10.4)	5 (5.8)	0.459
Moderately	36 (75.0)	72 (83.7)	
Poorly	7 (14.6)	9 (10.5)	
Tumor size (cm)	4.9+2.5	5.6+2.7	0.125
Depth of tumor			
Tis	2 (4.2)	2 (2.3)	0.213
T1a	4 (8.3)	7 (8.1)	
T1b	11 (22.9)	13 (15.1)	
T2	8 (16.7)	15 (17.5)	
T3	11 (22.9)	37 (43.0)	
T4	12 (25.0)	12 (14.0)	
Lymph nodes metastasis			
Positive	16 (33.3)	39 (34.9)	0.241
Negative	32 (66.7)	47 (65.1)	
Lymphatic invasion			
Positive	12 (25.0)	18 (20.9)	0.745
Negative	36 (75.0)	68 (79.1)	
Venous invasion			
Positive	5 (10.4)	11 (12.8)	0.647
Negative	43 (89.6)	75 (87.2)	
TNM stage			
0	2 (4.2)	2 (2.3)	0.858
I	13 (27.1)	15 (17.5)	
IIA	11 (22.9)	23 (26.7)	
IIB	6 (12.5)	12 (14.0)	
III	16 (33.3)	34 (39.5)	
Curability			
Curative resection	36 (75.0)	72 (83.7)	0.830
Non curative resection	12 (25.0)	14 (16.3)	

Well = well differentiated squamous cell carcinoma; Moderately = moderately differentiated squamous cell carcinoma; Poorly = poorly differentiated squamous cell carcinoma.

cytotoxic T lymphocyte responses. Attention has also been paid to CD4, helper/inducer T lymphocytes, as a critical component of the anti-tumor immune response[15].

Tumor-specific immune response depends on the function of activated CD4 cells[16], and therefore the deficiency in the function of activated CD4 cells might be directly correlated with the immune-deficiency of the host. CD4 helper/inducer T lymphocytes produce lymphokines, thus promoting the cytotoxic activity of CD8 T lymphocytes[17,18]. Therefore, activation of both CD4 and CD8 can exert a synergistic immune response to the termination of tumor cells.

Though some investigations have demonstrated an immunologic anti-tumor effect of CD4 and CD8[8], the clinical significance of CD4/CD8 ratio in tumor infiltrating lymphocytes and/or in peripheral blood as an indicator of progressive gastrointestinal tumor and/or worse prognosis of patients has been occasionally reported[19-21]. Diederichsen *et al*[19] reported that low CD4/CD8 ratio in tumor infiltrating lymphocytes is an independent prognostic indicator in patients with colorectal carcinoma. Decrease of the CD4/CD8 ratio is correlated with progressive behavior of the tumor indicated by such tumor-related factors as stage of the tumor, tumor invasion, lymph node metastasis, and size of the tumor in gastric cancer[20]. Moreover, severe pre-

operative cellular immune-suppression, where CD4/CD8 ratio was less than 1.0, is a predictive parameter for mortality in patients with gastric cancer[21].

CD8 expression in TIL in tumor tissue can serve the function of suppressing the proliferation of ESCC[9], and similarly CD8 infiltration into the tumor is an independent prognostic indicator for ESCC[10]. Recently, increase of the number of CD4 and CD8 T lymphocytes in tumor nests and stroma has been found to be an independent indicator of favorable prognosis of patients with ESCC[8].

These results suggest that CD8 T-lymphocyte infiltration, as have been investigated in some other tumors[22,23], plays a pivotal role in immune-potential against ESCCs.

However, it was reported that the prognosis of patients with lung carcinoma associated with more CD8 expressing T cells within cancer nests is significantly worse than that of patients with tumors of fewer CD8 expressing T cells[24]. High percentage of activated CD8-positive cells in postoperative peripheral blood is an indicator of worse prognosis for renal cell carcinoma[25].

The different methods used to evaluate the value or expression of CD8, histological type of the tumor, or balance between immunologic dynamics of the tumor and the host might explain this possible discrepancy in the significance of CD8 T lymphocytes in anti-tumor immune.

In the current study, the decreased CD4/CD8 ratio as well as the increased CD8 and the decreased CD4 in peripheral blood could predict the worse prognosis in patients with ESCCs. Preoperative coexistence of impaired immunity could influence the postoperative complications[5]. The incidence of postoperative complications is an independent indicator of worse prognosis in patients with esophageal carcinoma[26]. Therefore, preoperative impaired immunity seems not to be negligible as the cause of death, other than esophageal carcinoma.

Assessment of preoperative immunity in patients seems to be of great importance in predicting the subsequent outcome of patients with ESCCs.

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