European Review for Medical and Pharmacological Sciences 2014; 18: 3870-3876

Clinical application and prognostic assessment of serum Tumor Associated Material (TAM) from esophageal cancer patients

K. ZHOU, Y. YAN[1], S. ZHAO, B. LI

Department of Thoracic Surgery, [1]Department of Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Abstract. – OBJECTIVE: To explore the correlation between serum levels of Tumor Associated Materials (TAM) and clinicopathological parameters and prognosis in patients with esophageal cancer (EC).

PATIENTS AND METHODS: The levels of TAM were determined by chemical colorimetry in 100 EC patients and 100 healthy controls.

RESULTS: Serum TAM levels were significantly higher in patients with esophageal carcinoma than in the control group ($p < 0.001$). High levels of TAM were associated with tumor size ($p = 0.004$), tumor depth ($p < 0.001$), stage ($p < 0.001$), lymph node metastases ($p < 0.001$), tumor differentiation ($p = 0.001$), tumor respectability ($p = 0.002$) and disease progression ($p < 0.001$). The poor prognostic outcomes were correlated with an elevated level of TAM ($p = 0.001$). Kaplan-Meier analysis showed patients with increased levels of TAM after operation had a lower overall survival ($p < 0.001$) and disease-free survival ($p < 0.001$). In addition, multivariate Cox proportional hazard analyses revealed that TAM may be an independent factor affecting the overall survival and disease-free survival ($p < 0.001$).

CONCLUSIONS: The detection of TAM could be used to screen for tumor and assess unfavorable prognosis in patients with EC.

Key Words:
Tumor associated materials (TAM), Esophageal cancer, Prognosis.

Introduction

Esophageal carcinoma is one of the most tedious and fatal malignancies in the world at present[1]. Although there are multidisciplinary approaches for the treatment of esophageal cancer (EC), such as surgical resection, chemotherapy and radiotherapy. But results of the treatment are not satisfactory. The overall 5-year survival rate is less than 10%, largely due to the tumor's clinical and biological characteristics. Early lymphatic spread into the regional lymph node and recurrence after surgical resection are special features of EC. Because of EC cells' ability to degrade the extracellular matrix and components of the basement membrane, the malignant tumors can grow rapidly and invade lymphatics and blood vessels. Then, it often presents lymph node metastasis and vascular invasion[2,3]. The metastasis of malignant tumors is a complex process. Structural alterations in the extracellular matrix are the most important step for cell migrations which lead to tissue remodeling and tumor invasion[4,5]. Someone described altered glycosylation patterns were a symbol of tumor phenotype and demonstrated that healthy fibroblasts had smaller membrane glycoproteins than their transformed counterparts several years ago. Since then, people began to investigate the relationship between glycosylation and malignant tumor. They found that glycan changes occurred upon cell malignant transformation, and further to confirm that changes of glycosyltransferases in the Golgi compartment of cancerous cells lead to an increase in size and branching of N-linked glycan in the core structure. The changed branching increases activity of N-acetylglucosaminyltransferase V which creates additional sites for terminal sialic acid residues. In tumor cells, we can often find these materials[6-9].

In addition, glycosyltransferases overexpressed terminating residues in tumor tissue, which leads to overexpression of certain terminal glycan. For instance, Globo H, sialyl Lewis x(sLex), Lexis y(Ley), sialyl Tn(sTn) and polysialic acid (prostate-specific antigen, PSA). Most of these epitopes have been found in many malignant tumors of different parts of the body, such as bone, lung, brain, esophageal and prostate[10-14].

Corresponding Author: Yan Yan, MD; e-mail: yanyancn@yeah.net

The mass-production of certain glycoproteins and glycolipids is another common feature of therioma. Some people have detected the glycoproteins and glycolipids from tumor tissue. For example, mucin glycoproteins are found overproducted in epithelial tumors and complex ganglioside are found excess in small cell lung carcinomas, neuroblastomas and melanomas[8,15,16]. At present, there are many markers for detection of tumors. For example, CEA for colorectal malignancy, CA125 for ovarian tumor, prostate specific antigen for prostate tumor tissue, CA19-9 for pancreas cancer. Most of them find the evidence of tumor by detecting changes of glycosyltransferases. However, many studies showed that detection efficiency of them was not high. Subsequent research found that CEA, CA125, AFP, CA19-9 were also positive in healthy people. The reason may be that the detection was interfered by macromolecular protein complex[17-20].

Fortunately, a new reagent named serum tumor associated materials (TAM) can remove macromolecular protein complex and other impurities (at 450 nm can color in the non-tumor-related material). Its principle is to find the tumor tissue by detecting a specific oligosaccharide sequence through a special polymer carrier. Because TAM polymer carrier can be identified by the sequence of these oligosaccharides with a variety of markers and it can remove most of the macromolecular protein complex that could affect the test results by sedimentation centrifuge, the kit makes the test results more accurate than other tumor markers. It could be quickly and easily used for cancer early detection, screening and monitoring the efficacy of cancer treatment[21-23]. The purpose of this study is to assess the value of serum TAM in predicting the clinical therapeutic effect and prognosis in EC patients.

Patients and Methods

Patients

Serum specimens were obtained from 100 patients who were histopathologically confirmed EC treated in our hospital. Among the cancer patients, there were 21 females and 79 males, median age 60 years (range: 45-89 years). 85 patients were pathological diagnosed as squamous cell carcinoma and 15 patients were confirmed adenocarcinoma. All primary tumor cases were staged clinically according to the American Joint Committee on Cancer Staging Manual (7th edition). The final diagnosis was confirmed by microscopic examination of the material obtained during biopsy or surgery. In this study, the following physical and pathological factors were evaluated: age, sex, tumor location, tumor size, histological type, histological grading, the depth of tumor invasion (T factor), lymph node metastases (N factor), distant metastasis (M factor), stage, resectability and disease progression. 85 EC patients underwent surgical tumor resection with lymphadenectomy. We excluded the patients who received chemotherapy or radiotherapy before operation. Other 15 cases didn't undergo esophagectomy because of the direct infiltration of adjacent vital viscera by local tumor diffusion or distant organ metastasis. During the treatment, we recorded the morbidity and mortality. A follow-up survey was generally scheduled every 3 months in the first year, every 4 months in the second year and every 6 months after that. The periodic assessment projects included physical examination, tumor marker (TAM), thoracic and abdominal computed tomography (CT), contrast radiographic studies of the upper digestive tract with barium, ultrasound examination of the abdominal cavity and cerbical nodes, endoscopy examination and chest radiography. Nevertheless, examinations were arranged whenever patients had any uncomfortable symptoms. The median follow-up period was 35 months (range: 1-72 months). A total of 71 of the patients died of cancer during the 6-year follow-up.

The control group consisted of 100 healthy volunteers (70 men and 30 women). The median age of the controls was 62 years (range: 37-80 years). There were no significant differences in age or gender between the patients and controls ($p = 0.56$ and $p = 0.33$, respectively). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Serum TAM Analysis

Venous blood samples in EC patients were drawn into sterile vacuum tubes before surgery and after therapy. Measured by fasting blood 2 ml. All sera were separated within 1 h after blood collection, packaged and saved at –20°C until assayed. Strict accordance with the clinical serum TAM detection kit operating guides. In the colorimetric under 450 nm, calculated measured

value. Criterion of detection: TAM detection value ≥ 95 U/ml indicates positive, < 95 U/ml means negative (Serum TAM testing kit, Boxing Biotechnology Co., Ltd, Qingdao, China).

Statistical Analysis

All the data were analyzed through the SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). Continuous variables were described by median values and an interquartile range because the distributions of TAM showed no normal distributions (Shapiro-Wilk test). The Mann-Whitney's U-test was used to determine the significance of statistical differences for two groups and the Kruskal-Wallis one-way analysis of variance (ANOVA) for three or more groups. Categorical variables were summarized by frequency. Count data were analyzed by Chi-square test. We defined the overall survival (OS) as the time from surgical resection to the date of death or the date of last follow-up. Disease-free survival (DFS) meant the days from surgical resection to the date of death or the last known follow-up date. Survival was calculated by the Kaplan-Meier method, and the log-rank test was used to evaluate differences in survival of the subgroup of patients. All p values were based on two-tailed statistical analysis, and $p < 0.05$ was considered statistically significant.

Results

A total of 100 patients and 100 healthy people were included in the study. The associations between the TAM levels in patients with EC and the clinicopathological characteristics are summarized in Table I. The serum levels of TAM in EC patients were significantly higher than the control group ($p < 0.001$). It showed that TAM levels were not related to age ($p = 0.28$), gender ($p = 0.21$), location ($p = 0.67$) (Table I), but had a significant association with Tumor size ($p = 0.004$), tumor depth ($p < 0.001$), stage ($p < 0.001$), lymph node metastases ($p < 0.001$), tumor differentiation ($p = 0.001$), tumor respectability ($p = 0.002$) and disease progression ($p < 0.001$) (Table I).

In order to evaluate the prognostic value of TAM, we observed postoperative patients with the levels of TAM increased and decreased. The median follow-up period of operated patients was 40 months. Patients with TAM levels increased were assigned to the positive group, whereas those with levels decreased were assigned to the negative group. The median OS of patients in the positive group was 32 months (95% confidence interval (CI): 27 -36), and patients in the negative group was 59 months (95% CI: 53-65). The OS rates of patients in positive group were significantly lower than those in negative group ($p < 0.001$; Figure 1). The median DFS of patients in positive group was 31 months (95% CI: 27-35), and patients in the negative group was 49 months (95% CI: 41-56). The DFS rates of patients in positive group were distinctly lower than those in negative group ($p < 0.001$; Figure 2).

Multivariate Cox proportional hazard analyses revealed tumor length, pathologic T stage TAM were significant prognostic factors for OS and DFS (all $p < 0.05$). Age, gender, lymph node metastasis didn't have the significant difference (all $p>0.05$) (Table II). Chi-square test showed postoperative patients with TAM level increased had poor prognosis ($p = 0.001$; Table III).

Discussion

In the present study, we measured the serum contents of TAM in EC patients, and compared with healthy controls. Based on these data, we explored the relationship between serum levels of TAM and the clinicopathological parameters and prognostic significance in patients with EC. In our study, the serum levels of TAM in EC patients showed a significant elevation compared with healthy controls. These findings were in accordance with the findings in prostate cancer of Peracaula et al[20], which showed that patients with cancer had higher concentrations of serum TAM than healthy people. Many studies provided evidence that patients with colonic carcinomata had higher serum TAM levels than healthy people, and similar results were reported for patients with various types of cancer[24-26].

In our investigation the high levels of TAM in EC occurred in tumors longer than 4 cm, deeper invaded lesions, the presence of lymph node metastases, distant metastases, advanced stages and non-resectable cases. Our findings are in agreement with those obtained by Liu et al[27], who showed a significant correlation of TAM with the tumor size, the depth of tumor invasion, N factor, M factor and the stage of the disease. Multivariate logistic regression analysis suggested age, gender, status of lymph node had no rela-

Table I. The correlation between serum TAM levels and clinicopathologic characteristics of EC.

Variables	n	TAM (U/ml) Median, IQ_1-IQ_3	*p* value
Esophageal cancer	100	112 (99-125)	< 0.00[a]
Healthy	100	78 (66-90)	
Age			
< 60	33	109 (95-123)	0.28[a]
> 60	67	113 (99-127)	
Sex			
F	21	119 (97-125)	0.21[a]
M	79	117 (96-127)	
Location			
Upper	5	97 (86-108)	0.67[b]
Midthoracic	67	127 (112-137)	
Lower	28	121 (105-133)	
Tumor size			
< 4 cm	30	100 (92-108)	0.004[a]
> 4 cm	70	118 (107-129)	
Histological type			
Sqcc	85	112 (99-125)	0.47[a]
Ade	15	110 (97-123)	
Histological grading			
G1	7	94 (86-102)	0.001[b]
G2	80	110 (101-118)	
G3	13	132 (122-140)	
Tumor depth			
$T_1 + T_2$	25	102 (90-115)	< 0.001[a]
$T_3 + T_4$	75	115 (103-127)	
Stage			
I+II	21	97 (91-103)	< 0.001[a]
III+IV	79	116 (105-127)	
N_0	37	102 (92-112)	< 0.001[a]
N_1	63	118 (107-129)	
M_0	87	109 (98-120)	< 0.001[a]
M_1	13	130 (122-138)	
Resectable	85	109 (97-121)	0.002[a]
Nonresectable	15	132 (127-137)	
Disease progression	17	129 (118-139)	< 0.001[a]
Disease control	83	108 (96-122)	

Note: [a]Mann-whitney U-test; [b]Kruskal-Wallis test; IQ_1-IQ_3-interquartile range.

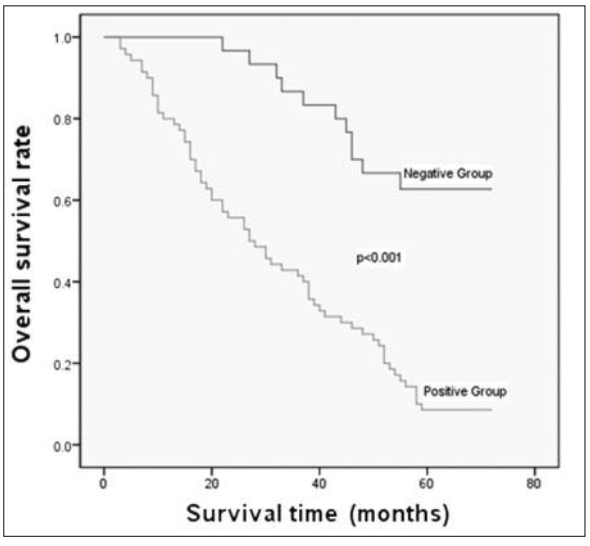

Figure 1. Kaplan-Meier estimates show for overall survival of EC patients for the positive and negative groups.

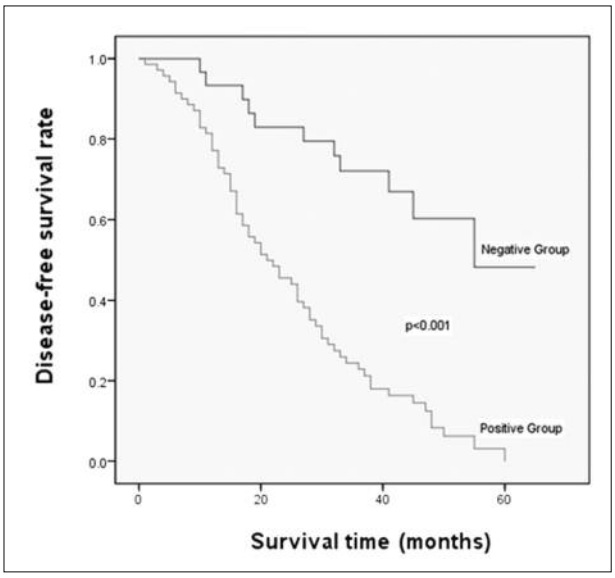

Figure 2. Kaplan-Meier estimates show for disease-free survival of EC patients for the positive and negative groups.

tionship with patients' survival and tumor length, pathologic T stage had relationship with postoperative survival. But some scholars reported lymph node metastasis was also related to survival[28-30].

We confirmed a significant correlation between high serum levels of TAM before operation and high postoperative recurrence rate. Patients with high serum TAM levels in the peripheral vein had a higher rate of recurrence than patients with low serum concentration. Hakomori et al[7] showed a significant correlation between high TAM levels with more recurrence cases in many kinds of cancer. Postoperative patients with elevated level of TAM had a higher recrudescence and the transfer rate, this outcome was in agreement with those obtained by Shu et al[22] in non-small cell lung cancer. For this study, patients with serum TAM levels increased after esophagectomy had a shorter overall survival and disease-free survival than those with TAM contents decreased. Hakomoris et al[8] revealed that elevated TAM expression levels were associated with poorer outcomes of EC patients.

Owing to TAM testing kit can remove macromolecular protein complex and other impurities and can detect more glycan terminal epitopes, it has a higher test efficiency than the traditional tumor marker. Many authors have found elevated TAM levels occurred in the weeks before the clinical diagnosis[21], which is critical for early detection and promptly treatment. In our study, we found that growth of TAM levels exhibited a significant correlation with early relapse and poor prognosis. Although it's reckless to use pretherapeutic TAM levels as the standard of judging whether or not tumor can be resected. We can regard it as a prognostic factor or perhaps a part of a prognostic factor for treatment of EC.

Table II. Logistic regression analysis of factors correlated to the levels of TAM.

Factors	Disease-free survival		Overall survival	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.330 (1.149-1.436)	0.17	1.366 (1.170-1.629)	0.327
Sex	1.210 (1.067-1.357)	0.326	1.232 (1.490-2.190)	0.926
Tumor length	1.300 (1.093-1.649)	0.003	1.375 (1.163-1.779)	0.027
Pathologic T stage	1.750 (1.280-2.896)	0.018	1.843 (0.631-2.098)	0.012
Lymph node metastasis	1.370 (1.153-2.368)	0.538	1.464 (0.874-2.987)	0.092
TAM	2.370 (2.153-4.368)	< 0.001	1.290 (0.120-1.497)	< 0.001

CI = confidence interval; OR = odds ratio.

Table III. The correlation between changes of TAM after operation and disease prognosis.

Group	TAM		*p* value (Chi-square)
	Positive (%)[a]	Negative (%)[b]	
Disease progress	11 (64.7)	6 (35.3)	0.001
Disease control	17 (20.5)	66 (79.5)	

[a]Patients with TAM levels increased after operation; [b]Patients with TAM levels decreased after operation.

Conclusions

Our results indicate that TAM play an important role in prognosis of patients with EC. Longer tumor length, higher pathologic T stage, lymph node metastases are associated with high TAM levels. Elevated levels of TAM in patients after esophagectomy indicate a worse prognosis and higher recurrence rate for EC patients. Nowadays, the treatment effect of esophageal cancer is not ideal. Therefore, the evaluation of serum TAM maybe play an important role in selecting EC patients for multimodality therapy and predicting the efficacy of treatment. Of course, this conclusion requires further investigation.

Acknowledgements

The work was supported by Youth Foundation from the First Affiliated Hospital of Zhengzhou University. Thanks to the guidance of Professor Zhao Song.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Crew KD, Neugut A. Epidemiology of upper gastrointestinal malignancies. Semin Oncol 2004; 31: 450-464.
2) Daly JM, Fry WA, Little AG, Winchester DP, McKee RF, Stewart AK, Fremgen AM. Esophageal cancer: results of an American College of Surgeons Patient Care Evaluation Study. J Am Coll Surg 2000; 190: 562-573.
3) Shimada Y, Imamura M, Watanabe G, Uchida S, Harada H, Makino T, Kano M. Prognostic factors of oesophageal squamous cell carcinoma from the perspective of molecular biology. Br J Cancer 1999; 80: 1281-1288.
4) Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161-174.
5) Hsu CP, Hsu NY, Shai SE, Hsia JY, Chen CY. Pre-tracheal thoracic esophagus. Eur J Surg Oncol 2005; 31: 749-754.
6) Dennis JW, Laferté S, Waghorne C, Breitman ML, Kerbel RS. β1-6 branching of asn-linked oligosaccharides is directly associated with metastasis. Science 1987; 236: 582-585.
7) Hakomori S. Traveling for the glycosphingolipid path. Glycoconj J 2000; 17: 627-647.
8) Hakomori S, Zhang Y. Glycosphingolipid antigens and cancer therapy. Chem Biol 1997; 4: 97-104.
9) Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology 2002; 62 Suppl 1: 57-63.
10) Yu X, Feizpour A, Ramirez NG, Wu L, Akiyama H, Xu F, Gummuluru S, Reinhard BM. Glycosphingolipid-functionalized nanoparticles recapitulate CD169-dependent HIV-1 uptake and trafficking in dendritic cells. Nat Commun 2014; 5: 4136.
11) Boucher D, Cournoyer D, Stanners CP, Fuks A. Studies on the control of gene expression of the carcinoembryonic antigen family in human tissue. Cancer Res 1989; 49: 847-852.
12) Duffy MJ, Sturgeon C, Lamerz R, Haglund C, Holubec VL, Klapdor R, Nicolini A, Topolcan O, Heinemann V. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. Ann Oncol 2010; 21: 441-447.
13) Taylor-Papadimitriou J, Epenetos AA. Exploiting altered glycosylation patterns in cancer- progress and challenges in diagnosis and therapy. Trends Biotechnol 1994; 12: 227-233.
14) Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. Physiol Rev 2014; 94: 461-518.
15) Hollingsworth MA, Swanson BJ. Mucins in cancer: Protection and control of the cell surface. Nat Rev Cancer 2004; 4: 45-60.
16) Saussez S, Marchant H, Nagy N, Decaestecker C, Hassid S, Jortay A, Schüring MP, Gabius HJ, Danguy A, Salmon I, Kiss R. Quantitative glycohistochemistry defines new prognostic markers for cancers of the oral cavity. Cancer 1998; 82: 252-260.

17) Kui Wong N, Easton RL, Panico M, Sutton-Smith M, Morrison JC, Lattanzio FA, Morris HR, Clark GF, Dell A, Patankar MS. Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. J Biol Chem 2003; 278: 28619-28634.

18) Babcan A, Kizilaslan C, Gun L, Muhcu M, Mungen E, Atay V. CA 125 and other tumor markers in uterine leiomyomas and their association with lesion characteristics. Int J Clin Exp Med 2014; 7: 1078-1083.

19) Wu CS, Lee TY, Chou RH, Yen CJ, Huang WC, Wu CY, Yu YL. Development of a highly sensitive glycan microarray for quantifying AFP-L3 for early prediction of hepatitis B virus-related hepatocellular carcinoma. PLOS One 2014; 9: e99959.

20) Peracaula R, Tabarés G, Royle L, Harvey DJ, Dwek RA, Rudd PM, de Llorens R. Altered glycosylation pattern allows the distinction between prostate-specifc antigen (PSA) from normal and tumor origins. Glycobiology 2003; 13: 457-470.

21) Li CG, Huang XE, Xu L, Li Y, Lu YY. Clinical application of serum Tumor Associated Material (TAM) from non-small cell lung cancer patients. Asian Pacific J Cancer Prev 2012; 13: 301-304.

22) Shu J, Li CG, Liu YC, Yan XC, Xu X, Huang XE, Cao J, Li Y, Lu YY, Wu XY, Liu J, Xiang J. Comparison of serum Tumor Associated Material (TAM) with Conventional Biomarkers in Cancer Patients. Asian Pacific J Cancer Prev 2012; 13: 2399-2403.

23) Miller MC, DiSilvestro P, Brown AK, DiSilvestro P, Miller MC, Allard WJ, Gajewski W, Kurman R, Bast RC Jr, Skates SJ. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of overian cancer in patients with a pelvic mass. Gynecol Oncol 2009; 112: 40-46.

24) Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting recommendations for tumor marker prognostic studies (Remark): explanation and elaboration. BMC Med 2012; 10: 51.

25) de Haas RJ, Wicherts DA, Flores E, Ducreux M, Lévi F, Paule B, Azoulay D, Castaing D, Lemoine A, Adam R. Tumor marker evolution: comparison with imaging for assessment of response to chemotherapy in patients with colorectal liver metastases. Ann Surg Oncol 2010; 17: 1010-1023.

26) He CZ, Zhang KH, Li Q, Liu XH, Hong Y, Lv NH. Combined use of AFP, CEA, CA125 and CA19-9 improves the sensitivity for the diagnosis of gastric cancer. BMC Gastroenterol 2014, 13: 87.

27) Liu ZM. Serum free proline and free hydroxyproline and its peptide-binding determination of hydroxyproline in the evaluation of bone tumors. Chin J Lab Med 1986; 9: 129-133.

28) Stein HJ, Feith M, Bruecher BL, Naehrig J, Sarbia M, Siewert JR. Early esophageal cancer: pattern of lymphatic spread and prognostic factors for long-term survival after surgical resection. Ann Surg 2005; 242: 566-575.

29) Bollschweiler E, Besch S, Drebber U, Schröder W, Mönig SP, Vallböhmer D, Baldus SE, Metzger R, Hölscher AH. Influence of neoadjuvant chemoradiation on the number and size of analyzed lymph nodes in esophageal cancer. Ann Surg Oncol 2010; 17: 3187-3194.

30) Mariette C, Piessen G, Briez N, Triboulet JP. The number of metastatic lymph nodes and the ratio between metastatic and examined lymph nodes are independent prognostic factors in esophageal cancer regardless of neoadjuvant chemoradiation or lymphadenectomy extent. Ann Surg 2008; 247: 365-371.