European Review for Medical and Pharmacological Sciences 2014; 18: 509-515

Clinical significance of serum matrix metalloproteinase-13 levels in patients with esophageal squamous cell carcinoma (ESCC)

X.-L. JIAO, D. CHEN, J.-G. WANG[1], K.-J. ZHANG

Department of Surgery, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, China
[1]Department of Pathology, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, China

Abstract. – OBJECTIVES: Matrix metalloproteinase-13 (MMP13) is a highly regulated zinc-dependent endopeptidase and has been reported to be associated with vascular invasion and lymph node metastasis and predicts poor outcome for relatively early stage in esophageal squamous cell carcinoma (ESCC) patients. However, the role of the serum MMP-13 levels in ESCC is still unknown. In the present study, we investigate the clinical significance of MMP-13 levels in patients with ESCC

PATIENTS AND METHODS: The serum level of MMP-13 was measured with commercially available ELISA kit in 112 healthy controls and 141 ESCC patients prior to surgical resection. Statistical associations between clinicopathological observations and MMP-13 levels were determined using the Mann-Whitney U test. The clinical value of MMP-13 level as a prognostic parameter was evaluated using the Cox's proportional hazards model.

RESULTS: The results showed compared with the healthy control group (74.5±12.3 ng/ml), ESCC patients tended to have significantly higher serum MMP-13 concentrations (86.2 ± 14.6 ng/ml) ($p < 0.05$). Elevation of MMP-13 levels (≥ 76.4 ng/ml) was observed in 61.7% (87/141) of patients with ESCC, and 18.4% (26/141) in healthy controls. MMP-13 levels were associated with lymph node metastasis ($p < 0.001$), distant metastasis ($p < 0.001$), histological differentiation ($p = 0.026$), T classification ($p < 0.005$), but not with the tumor size, clinical stage, age and gender of the patients or tumor location. Multivariate analysis revealed that patients with an elevated level of MMP-13 (≥ 76.4 ng/ml) had significantly lower 5 year survival rate than those with non-elevated MMP-13 (< 76.4 ng/ml, log-rank $p < 0.001$).

CONCLUSIONS: It is suggested that the elevated level of preoperative MMP-13 was found to associate with tumor progression and poor survival in patients with ESCC.

Key Words:
Serum Matrix metalloproteinase-13, Esophageal squamous cell carcinoma, Prognosis.

Introduction

There is remarkable geographic variation in both the incidence and the constituents of esophageal cancer. In Asian countries,where esophageal cancer is common, more than 90% of esophageal cancers are squamous cell carcinomas[1-3]. In Western countries, where the incidence of esophageal cancer is relatively low, the incidence rate of esophageal adenocarcinoma has been increasing in the last few decades, whereas that of esophageal squamous cell carcinoma (ESCC) remains constant[4-5]. As a result, ESCC constitutes 50-60% of all esophageal cancers in Western countries at present[4-5].

ESCC shows a poor prognosis because of the occurrence of systemic metastasis, mainly via lymphatic vessels[1-6]. Detection of ESCC at an early stage is possible with the use of X-ray and endoscopic examinations[7-8], but there might be occult micrometastases at the time of surgery even in such cases[9-10]. In this respect, an assessment of metastatic potential is important to establish appropriate therapeutic modalities for ESCC. It is of great clinical value to find sensitive and specific early biomarkers for the diagnosis and prognosis of this malignancy, as well as novel therapeutic strategies.

Matrix metalloproteinases are important enzymes involved in the breakdown of various extracellular matrix (ECM) components.Matrix metalloproteinases can be divided into subgroups, including collagenases, stromelysins, stromelysins-like MMPs, gelatinases, membrane-type MMPs and others. Matrix metalloproteinase-13 is a member of the collagenase family,which degrades fibrillar collagens of types I, II, III, IV, X and XIV, tenascin, fibronectin, aggrecan, versican and fibrillin-1[11]. It is now accepted that MMP-13 plays a key role in the MMP activation cascade, both acti-

Corresponding Author: Kejun Zhang, MD; e-mail: qyfyzhang@126.com

vating and being activated by several MMPs. The expression of MMP-13 has been well studied in cancer diseases. Elevated expression of MMP-13 has been documented in numerous malignancies, including colorectal cancer[12-13]. epithelial cancers of the eyelids[14], non-small cell lung cancer[15], cutaneous malignant melanoma[16], prostate cancer[17], breast cancer[18], renal cell carcinoma bone metastasis[19], head and neck squamous cell carcinomas[20] and esophageal cancer[21] and its association with tumour behaviour and patient prognosis has been reported[11-21]. Significant expression of MMP-13 is observed in highly invasive tumours, suggesting that MMP-13 probably plays a role in regulating tumour invasion, which needs remodeling of ECM.

Serum levels of MMPs are known to be altered in many human pathologic conditions. Increased serum levels of MMPs have been reported in, for example, polycystic kidney disease[22], systemic sclerosis[23], rheumatoid arthritis[24-25], and scleroderma[26]. In comparison with healthy controls, the serum levels of MMP-9,13 are elevated in head and neck squamous cell carcinoma[27]. Furthermore, elevated MMP-9,13 serum levels have been shown to correlate with metastasis and poor survival in hepatocellular carcinoma[28] and head and neck squamous cell carcinoma[27].

Although increased expression of MMP-13 was found in many cancers including esophageal cancer tissues[21] and its association with tumour behaviour and patient prognosis, there are few studies analyzing the serum levels of MMP-13 in patients with metastatic diseases. In the present work, we have determined the serum levels of MMP-13 in 141 patients with ESCC with a special interest to determine the levels and prognostic value of their serum levels.

Patients and Methods

Patients

From Oct. 1998 to Oct. 2009, 146 consecutive patients with histopathologically proven ESCC were enrolled for study. The average age was 62.7 ± 11.3 years, and the ratio of male: female was 40:3. Tumor stage was classified according to the TNM system[29]. Medical Ethical Committee (Tianjin University) approved the protocol, and the written informed consent was obtained from every patient before surgery. Extensive preoperative measures including esophagoscopy with biopsy, esophagogram, chest radiography, sonograms of abdomen and neck, computed tomography of the chest, and radionuclide bone scanning were followed to determine the need of surgery. Patients with resectable tumor (n = 100) underwent en bloc esophagectomy with locoregional lymphadenectomy through right thoracotomy, laparotomy with reconstruction using the stomach through a retrosternal route, and cervical esophagogastrostomy. For patients at stage IIb or beyond, concurrent chemoradiotherapy was applied after surgery. Patients with unresectable tumor (n = 46) received chemoradiotherapy after the installation of feeding jejunostomy or bypass procedure. None of these patients received neoadjuvant therapy. After treatment, all patients were followed regularly. Five patients died of cardiopulmonary or COPD complication after surgery and were excluded from the prognosis analysis. Serum samples were obtained from each patient at the time of diagnosis. Serum samples from 112 healthy individuals with equivalent distribution of age and sex were used as normal controls. The healthy individuals were negative for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. Abdominal ultrasound examination, routine blood tests, and biochemistry tests were performed for the healthy controls, and the results were within normal ranges. After centrifugation of the peripheral blood, serum samples were stored at −20°C until assayed. This study was approved by the medical Ethics Committee of Cancer Center at Tianjin University.

The Measurement of Serum MMP-13 Levels

According to the manufacturer's instructions, specific kits were used for the measurement of serum MMP-13 levels (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Briefly, 96-well ELISA microplates were coated overnight with 100 µL MMP-13 antibody (Santa Cruz, Shanghai, China) at a final concentration of 0.25 mg/L in phoshate buffered saline (PBS). After washing with PBS/0.05% (w/v) Tween-20 (PBST, pH 7.4), the wells were blocked with blocking buffer at room temperature for 1 h. Then, 100 µL diluted serum samples (at 1:40 dilution) were added and incubated at room temperature for 2 h. Similarly, 100 µL PBST lacking antibody was used as a negative control. Following three washes with PBST, 100 µL antibody diluted to a concentration of 0.25 mg/L was added. After incubation at room temperature for 2 h, 100 µL avidin-horseradish peroxidase conjugated secondary antibody (at 1:1500 dilution) was added, and plates

were incubated at room temperature for 30 min. The excess conjugate was removed by washing the plates three times with PBST. The amount of bound conjugate was determined by adding 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) liquid substrate solution to each well, and plates were incubated at room temperature for color development. The absorbance was measured at 405 nm using a Model 680 microplate reader (Bio-Rad Lab. Inc., Hercules, CA, USA). All analyses were performed in triplicate. The coefficient of variation was lower than 15% between analyses. Concentrations of MMP-13 are presented in ng/mL.

Statistical Analysis

The results are expressed as mean ± SD. The relationship between serum MMP-13 level and each of the clinicopathological parameters was analyzed by χ^2 analysis. When any analysis cell had fewer than five cases, the Fisher's exact test was used. The statistical difference between groups A and B in each clinicopathological category was determined by Student's *t* test (two-tailed) or ANOVA. Survival curves were plotted with method of Kaplan-Meier. The statistical difference in survival between different groups was compared by the log-rank test. Survival correlation with the prognostic factors was further investigated by multivariate analysis using Cox proportional hazards model with backward stepwise likelihood ratio. Statistical analysis was performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed for $p < 0.05$.

Results

Serum Levels of MMP-13

The serum MMP-13 levels were significantly higher in ESCC patients than in healthy controls (66.2 ± 10.2 ng/ml vs 74.5±12.3 ng/ml, $p < 0.05$). The cut-off value was set at 76.4 ng/ml, based on the data of the 112 healthy control sera. Increased serum MMP-13 levels were found in 61.7% (87/141) of patients with ESCC and 38.3% (54/141) was found less 76.4 ng/ml.

Serum MMP-13 Level and Clinicopathological Features in ESCC Patients

Using 76.4 ng /ml as the cutoff value, these ESCC patients were then divided into group A (n = 87) as those with the higher level (≥ 76.4 ng /ml;) and group B (n = 54) as those with lower level (< 76.4 ng /ml). χ^2 analysis showed that the preoperative serum MMP-13 levels correlated well with lymph node involvement (N status), distant metastasis (M status), histological differentiation, T classification, but not with the tumor size, clinical stage, age and gender (Table I). Serum levels of MMP-3 were significantly higher in patients with lymph node metastasis or distant metastasis than in those without lymph node involvement or distant metastasis. There was no significant difference between these groups in terms of sex or age of onset and lymphovascular invasion. Spearman analysis also revealed a correlation between serum MMP-13 level and the T classification ($r = 0.314$, $p < 0.0001$), N classification ($r = 0.482$, $p < 0.0001$), lymph node involvement ($r = 0.246$, $p = 0.002$), distant metastasis ($r = 0.328$, $p < 0.001$) and histological differentiation ($r = 0.274$, $p < 0.001$) (Table II). These observations support the hypothesis that the progression of ESCC is associated with increased serum MMP-13 levels.

Association Between Serum Levels of MMP-13 and Prognosis,And Survival Rates for ESCC Patients

Statistical analysis revealed an positive correlation between the serum levels of MMP-13 and clinical pathologic factors ($r = 0.273$, $p < 0.001$; Table II). Furthermore, a log-rank test and Kaplan-Meier analysis were used to calculate the effect of serum levels of MMP-13 on survival. The log-rank test showed that the serum levels of MMP-13 attested remarkably to patients' survival time ($p < 0.0001$; Figure 1). More specifically, the median survival time of patients with high serum levels of MMP-13 was only 13 months, whereas the median survival time of those with low levels of MMP-13 was 47 months. The cumulative 5-year survival rate was 42.6% (95%CI, 0.1782-0.5237) in the low serum levels of MMP-13 group (n= 54), whereas in the high serum levels of MMP-13 group (n= 87; Figure 1), the survival rate was only 7.13% (95% CI, 0.0076-0.1237). Clinical stage, metastasis, serum levels of MMP-13, TNM classification, histological differentiation were analyzed using univariable and multivariable Cox regression analyses. Univariable analyses revealed that clinical stage, serum levels of MMP-13, TNM classification, and histological differentiation were significant predictors of ESCC (Table III). Multivariable analysis

Table I. Relationship between serum levels of MMP-13 and clinicopathological factors.

Clinicopathological factors	Groups[a] A (n)	B (n)	p^b
Age (yr)			0.372
< 65 (n=72)	47 (65.3%)	25 (34.7%)	
> 65 (n=69)	40 (60%)	29 (40%)	
Tumor status			0.017
T1 (n=16)	4 (25%)	12 (75%)	
T2 (n=14)	5 (35.7%)	9 (64.3%)	
T3 (n=75)	51 (68%)	24 (32%)	
T4 (n=36)	27 (75%)	9 (25%)	
Lymph node involvement			0.005
Positive (n=90)	67 (74%)	23 (26%)	
Negative (51)	20 (39.2%)	31 (60.8%)	
Distant metastasis			0.016
Positive (n=32)	27 (84.4%)	5 (15.6%)	
Negative (n=109)	60 (55%)	49 (45%)	
Lymphovascular invasion			
Positive (n=42)	20 (47.6%)	22 (52.4%)	0.114
Negative (n=99)	67 (67.7%)	32 (32.3%)	
Stage (TNM)			0.026
I (n=17)	7 (41.2%)	10 (58.8%)	
II (n=37)	20 (54%)	17 (46%)	
III (n=53)	36 (68%)	17 (32%)	
IV (n=34)	24 (70.6%)	10 (76%)	
Cell differentiation			0.02
Well (n=23)	7 (30.4%)	16 (69.6%)	
Moderate (n=90)	60 (66.7%)	30 (33.3%)	
Poor (n=28)	20 (71.4%)	19 (28.6%)	

[a]Patients were grouped by preoperative serum levels of MMP-13. In group A (n= 87), serum MMP-13 levels were > 76.4 ng/ml, and in group B (n = 54), MMP-13 levels were 76.4 ng/ml. [b]*p* were determined by χ^2 test.

showed that clinical stage, serum levels of MMP-13, TNM classification, and histological differentiation were independent predictors for ESCC on the basis of changes in likelihood interactions between the parameters listed in univariable regression analyses (Table III).

Discussion

Identification of targets for early detection of ESCC is important to improve the prognosis of the patients with this pernicious disease. Currently, carcinoembryonic antigen, cytokeratin-19

Table II. Univariable and Multivariable analysis various prognosic parameters in patients with ESCC.

Characteristic	Univariable analysis		Multivariable analysis		
	Regression coefficient (SE)	*p* value	Hazard ratio	95% CI	*p* value
Serum MMP-13 levels	0.296 (0.082)	0.0003	2.456	1.547-5.832	0.002
T classification	0.640 (0.174)	< 0.0001	2.964	1.76-6.24	0.0016
Lymph node involvement	0.532 (0.13)	0.018	3.244	1.58-6.83	0.003
Distant metastasis	0.743 (0.367)	0.0002	2.366	1.420-5.183	0.012
pTNM	0.325 (0.076)	0.036	0.56	0.342-0.843	0.023
Lymphovascular invasion	0.0902 (0.025)	0.074	0.42	0.13-1.56	0.104
Differentiation	0.467 (0.163)	0.026	1.42	1.25-1.93	0.014

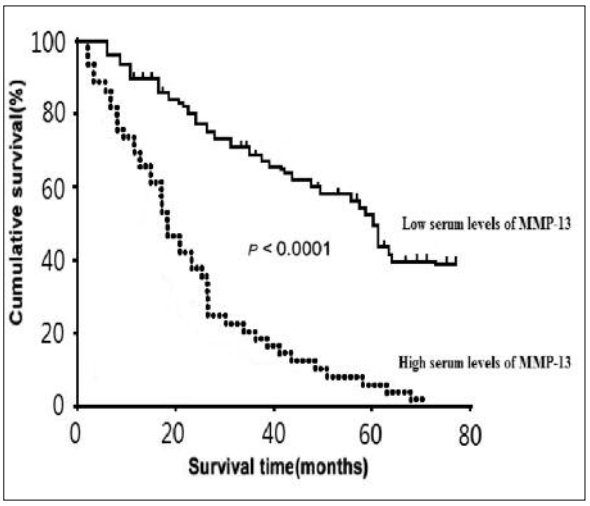

Figure 1. The serum MMP-13 level corresponded with the progression of ESCC.

fragments, and squamous cell carcinoma-associated antigen are routinely used as serum markers for detection of ESCC. Due to the low sensitivity and specificity of detection of these markers[30], additional serum markers must be established for early detection and diagnosis of ESCC.

MMP-13 is characterized by wide substrate specificity and restricted expression. Previous studies have reported that it was expressed tumor cells in human melanoma metastases[16], breast cancer[18] and esophageal cancer[24]. In the present study, we detected MMP-13 levels in the serum of patients are significantly higher than in a control population of healthy blood donors. Notably, serum MMP-13 levels were higher in patients with metastatic disease compared to those without known metastases. Thus, MMP-13 concentrations may be as the suitability as a routine serum marker for the detection of metastatic disease in ESCC. In addition, serum MMP-13 levels was much higher in ESCC patients with advanced stages and poor differentiation. However, no significant differences were found in the serum levels of MMP-13 in ESCC patients with, ages germ and tumor size. Serum levels of MMP-13 is useful for predicting the prognosis of patients with ESCC.

Evaluation of the survival data showed that the survival was significantly shorter in patients with MMP-13 concentrations > 76.4 ng/ml, and high MMP-13 concentrations predict poor prognosis for patients with ESCC, especially in those presenting with distant and lymph node metastasis. In addition, the uni- and multivariate analyses in the present study showed the prognostic significance of clinicopathologic factors such as depth of the tumor, lymph node metastasis, TNM and differentiation, as reported previously from Western countries[31,32]. These findings indicate that the results obtained from the present series of cases are applicable to ESCC in other counties.

In addition, Cox regression analysis showed that MMP-13 was an independent variable.

Several studies have been done to identify different easily assayed quantitative markers that could serve as prognostic indicators for patients with ESCC. One of the most established is the level of the carcinoembryonic antigen (CEA) and squamous cell cancer antigen (SCC-Ag) assessed as a marker for cancer[33-34]. However, there is a need for new easily measurable markers, which could predict disease progression or therapeutic response. In the present study, we have assessed the prognostic value of serum MMP-13 in ESCC patients and identified MMP-13 as an independent prognostic factor. Studies with serum samples obtained at different phases of ESCC progression could further elucidate the role of MMP-13 in growth and metastasis of ESCC.

Table III. Univariable and Multivariable analysis various prognosic parameters in patients with ESCC.

Variable	Univariate analysis		Multivariate analysis	
	SE	p	RR (95% CIa)	p
Serum MMP-13	0.296 (0.082)	< 0.001	2.456 (1.547-5.832)	0.002
T classification	0.640 (0.174)	< 0.0001	2.964 (1.76-6.240	0.0016
Lymph node status	0.532 (0.13)	0.018	3.244 (1.58-6.83)	0.003
Distant metastasis	0.743 (0.367)	< 0.001	2.366 (1.42-5.183)	0.012
pTNM	0.325 (0.076)	0.036	0.56 (0.342-0.843)	0.023
Lymphovascular	0.092 (0.025)	0.074	0.42 (0.13-1.56)	0.104
Differentiation	0.467 (0.163)	0.026	1.42 (1.25-1.93)	0.014

Conclusions

Our findings provide evidence, that MMP-13 have important roles at different phases of metastatic spread and that measurement of serum MMP-13, in particular, could be of clinical value when identifying patients at high risk for progression.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Dawsey SM, Wang GQ, Weinstein WM. Squamous dysplasia and early esophageal cancer in the Linxian region of China: distinctive endoscopic lesions. Gastroenterology 1993; 105: 1333-1340.
2) Kakizoe T, Yamaguchi N, Mitsuhashi F. Cancer incidence in Japan, 2001. The editorial board of Cancer Statistics in Japan. Tokyo:Foundation for Promotion of Cancer Research, 2001.
3) Shiozaki H, Doki Y, Kawanishi K. Clinical application of malignancy potential grading as a prognostic factor of human esophageal cancers. Surgery 2000; 127: 552-561.
4) Faivre J, Forman D, Esteve J, Gatta G. Survival of patients with oesophageal and gastric cancers in Europe. Eur J Cancer 1998; 34: 2167-2175.
5) Eloubeidi MA, Desmond R, Arguedas MR, Reed CE, Wilcox CM. Prognostic factors for the survival of patients with esophageal carcinoma in the U.S.: the importance of tumor length and lymph node status. Cancer (Phila) 2002; 95: 1434-1443.
6) Greenlee RT, Hill HM, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001; 51: 15-36.
7) Ueyama T, Kawamoto K, Yamada Y, Masuda K. Early esophageal carcinoma. Evaluation of the depth of invasion based on double-contrast esophagography. Acta Radiol 1998; 39: 133-137.
8) Lambert R. Diagnosis of esophagogastric tumors. Endoscopy 2002; 34: 129-138.
9) Vazquez SE, Wang L, Burgart L. Occult lymph node metastases as a predictor of tumor relapse in patients with node-negative esophageal carcinoma. Gastroenterology 2002; 122: 1815-1821.
10) Nozoe T, Saeki H, Ohga T, Sugimachi K. Clinicopathological features of early esophageal squamous cell carcinoma with subsequent recurrence. Dis Esophagus 2002; 15: 145-148.
11) Leeman MF, Curran S, Murray GI. The structure, regulation, and function of human matrix metalloproteinase-13. Crit Rev Biochem Mol Biol 2002; 37: 149-166.
12) Yang B, Gao J, Rao Z, Shen Q. Clinicopathological significance and prognostic value of MMP-13 expression in colorectal cancer.Scand J Clin Lab Invest 2012; 72: 501-505.
13) Yamada T, Oshima T, Yoshihara K, Tamura S, Kanazawa A, Inagaki D, Yamamoto N, Sato T, Fujii S, Numata K, Kunisaki C, Shiozawa M, Morinaga S, Akaike M, Rino Y, Tanaka K, Masuda M, Imada T. Overexpression of MMP-13 gene in colorectal cancer with liver metastasis. Anticancer Res 2010; 30: 2693-2699.
14) Alvarez Suárez ML, González Vázquez LO, Barbón García JJ, Vázquez Rojo J, Lamelas Suárez-Pola ML, Vizoso Piñeiro FJ. Collagenase-3 (MMP-13) expression in epithelial cancers of the eyelids. Arch Soc Esp Oftalmol 2004; 79: 281-288.
15) Lin TS, Chiou SH, Wang LS, Huang HH, Chiang SF, Shih AY, Chen YL, Chen CY, Hsu CP, Hsu NY, Chou MC, Kuo SJ, Chow KC. Expression spectra of matrix metalloproteinases in metastatic non-small cell lung cancer. Oncol Rep 2004; 12: 717-723.
16) Corte MD, Gonzalez LO, Corte MG, Quintela I, Pidal I, Bongera M, Vizoso F. Collagenase-3 (MMP-13) expression in cutaneous malignant melanoma. Int J Biol Markers 2005; 20: 242-248.
17) Morgia G, Falsaperla M, Malaponte G, Madonia M, Indelicato M, Travali S, Mazzarino MC. Matrix metalloproteinases as diagnostic (MMP-13) and prognostic (MMP-2, MMP-9) markers of prostate cancer. Urol Res 2005; 33: 44-50.
18) Zhang B, Cao X, Liu Y, Cao W, Zhang F, Zhang S, Li H, Ning L, Fu L, Niu Y, Niu R, Sun B, Hao X. Tumor-derived matrix metalloproteinase-13 (MMP-13) correlates with poor prognoses of invasive breast cancer. BMC Cancer 2008; 8: 83.
19) Kominsky SL, Doucet M, Thorpe M, Weber KL. MMP-13 is over-expressed in renal cell carcinoma bone metastasis and is induced by TGF-beta1. Clin Exp Metastasis 2008; 25: 865-870.
20) Culhaci N, Metin K, Copcu E, Dikicioglu E. Elevated expression of MMP-13 and TIMP-1 in head and neck squamous cell carcinomas may reflect increased tumor invasiveness. BMC Cancer 2004; 4: 42.
21) Etoh T, Inoue H, Yoshikawa Y, et al. Increased expression of collagenase-3 (MMP-13) and MT1-MMP in esophageal cancer is related to cancer aggressiveness. Gut 2000; 47: 50-56.
22) Nakamura T, Ushiyama C, Suzuki S, Ebihara I, Shimada N, Koide H. Elevation of serum levels of metalloproteinase-1, tissue inhibitor of metalloproteinase-1 and type IV collagen, and plasma levels of metalloproteinase-9 in polycystic kidney disease. Am J Nephrol 2000; 20: 32-36.
23) Young-Min SA, Beeton C, Laughton R. Serum TIMP-1, TIMP-2 and MMP-1 in patients with systemic sclerosis, primary Raynaud's phenomenon, and in normal controls. Ann Rheum Dis 2001; 60: 846-851.
24) Young-Min S, Cawston T, Marshall N, Coady D, Christgau S, Saxne T, Robins S, Griffiths I. Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. Arthritis Rheum 2007; 56: 3236-3247.

25) Fiedorczyk M, Klimiuk PA, Sierakowski S, Domysławska I, Chwie ko J. Correlations between serum matrix metalloproteinase (MMP-1, MMP-3, MMP-9, MMP-13) concentrations and markers of disease activity in early rheumatoid arthritis. Przegl Lek 2005; 62: 1321-1324.

26) Asano Y, Ihn H, Kubo M, Jinnin M, Mimura Y, Ashida R, Tamaki K. Clinical significance of serum matrix metalloproteinase-13 levels in patients with localized scleroderma. Clin Exp Rheumatol 2006; 24: 394-399.

27) Marcos CA, Martínez DA, de Los Toyos JR, Domínguez Iglesias F, Hermsen M, Guervós MA, Pendás JL. The usefulness of new serum tumor markers in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg 2009; 140: 375-380.

28) Yeh HC, Lin SM, Chen MF, Pan TL, Wang PW, Yeh CT. Evaluation of serum matrix metalloproteinase (MMP)-9 to MMP-2 ratio as a biomarker in hepatocellular carcinoma. Hepatogastroenterology 2010; 57: 98-102

29) International Union Against Cancer. TNM Classification of Malignant Tumors, revised edition. New York: Springer-Verlag, Inc., 1987.

30) Saeki H, Watanabe M, Sugimachi K. Markers determination in patients with esophageal squamous cell carcinoma: clinical utility for detection of recurrences. Cancer 2000; 89: 1413-1417.

31) Eloubeidi MA, Desmond R, Arguedas MR, Reed CE, Wilcox CM. Prognostic factors for the survival of patients with esophageal carcinoma in the U.S.: the importance of tumor length and lymph node status.Cancer (Phila) 2002; 95: 1434-1443.

32) Vazquez SE, Wang L, Burgart L. Occult lymph node metastases as a predictor of tumor relapse in patients with node-negative esophageal carcinoma. Gastroenterology 2002; 122: 1815-1821.

33) Nawata S, Sugino N, Kato H. Squamous cell carcinoma antigen. Nihon Rinsho 2005; 63(Suppl 8): 684-686.

34) Nakashima S, Natsugoe S, Matsumoto M, Miyazono F, Nakajo A, Uchikura K, Tokuda K, Ishigami S, Baba M, Takao S, Aikou T. Clinical significance of circulating tumor cells in blood by molecular detection and tumor markers in esophageal cancer. Surgery 2003; 133: 162-169.