Serum cytokeratin 18 as a biomarker for gastric cancer

Title: **Serum cytokeratin 18 as a biomarker for gastric cancer.**

Authors:

Katsunobu Oyama , Sachio Fushida , Jun Kinoshita , Koichi Okamoto , Isamu Makino , Keishi Nakamura , Hironori Hayashi , Masafumi Inokuchi , Hisatoshi Nakagawara , Hidehiro Tajima , Hideto Fujita , Hiroyuki Takamura , Itasu Ninomiya , Hirohisa Kitagawa , Takashi Fujimura , Tetsuo Ohta

Affiliation and address

Gastroenterological Surgery, Department of Oncology, Division of Cancer Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

13-1, Takara-machi, Kanazawa, Ishikawa 920-8641, Japan

Corresponding author: Katsunobu Oyama

E-mail:oya-ma@staff.kanazawa-u.ac.jp

Telephone number: +81-76-265-2000

Fax number: +81-76-234-4260

Conflict of interest: None.

Abstract

Cytokeratin 18 (CK18) fragments are released into circulation during epithelial cell death. M30 (reflect caspase cleaved CK18 fragment) and M65 (reflect total CK18 fragment) enzyme-linked immunosorbent assay (ELISA) detects circulating CK18 fragments released during caspase-dependent or total cell death, respectively; thus, CK18 has the potential of being a biomarker for epithelial cancers. In the present study, we investigated the serum levels of M30 and M65 in patients with gastric cancer, determined correlation of these levels with clinical features, and evaluated the usefulness of these enzymes as diagnostic and prognostic markers.

We enrolled 54 gastric cancer patients and 12 healthy volunteers in this study. We measured the serum levels of M30 and M65 by quantitative ELISA.

The levels of M30 and M65 in gastric cancer patients were significantly higher than those in healthy volunteers ($P = 0.001$, $P < 0.001$). The enzyme levels were elevated with the progress of gastric cancer. The sensitivity and specificity of M30 as a diagnostic marker were 67.5% and 90.9%, respectively, and those of M65 were 70.1% and 90.9%, respectively. The serum levels of M30 and M65 in patient with early gastric cancer were elevated in 38.1% and 66.7%, respectively. Further, increased serum level of M65 is an independent indicator of poor prognosis ($P = 0.036$).

The serum levels of M30 and M65 may be useful biomarkers for gastric cancer as diagnostic markers that can reflect the extent of cancer. Moreover, M65 levels can be

used as a prognostic indicator.

Keywords

gastric cancer, biomarker, cytokeratin 18, serum M30 level, serum M65 level

Introduction

Biomarkers are substances that are objectively measured and evaluated as indicators of biological and pathological processes or pharmacological responses to a therapeutic intervention. Biomarkers hold a great promise for the detection, diagnosis, and prognosis of cancer because of their ability in identifying unique molecular signatures detrimental to certain pathophysiological states [1]. An ideal marker has high sensitivity and specificity for diagnosis, its level should correlate with the disease status and response to treatment, and it should be easily and reproducibly measured. However, the biomarkers currently available for the management of solid tumors do not fulfill all the above criteria and, therefore, are not presently recommended for screening of the general population [2].

Gastric cancer is one of the leading causes of cancer-related death worldwide. The prognosis of metastatic gastric cancer remains poor despite the recent improvements in the therapeutic methods [3]. The diagnosis and treatment of gastric cancer requires further improvement; therefore, it is important to identify an ideal biomarker for diagnosis and prognosis. The level of the biomarker is expected to be measured using less invasive methods and monitored easily, for example, by a simple blood test. Several serum biomarkers have been used in gastric cancer. Carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 are established as biomarkers for advanced gastric cancer. However, these biomarkers were unable to detect early-stage gastric

cancer; some patients with advanced gastric cancer had normal serum levels of these biomarkers. Thus, development of novel biomarkers for gastric cancer that can be routinely used in clinical practice is required.

Circulating biomarkers of cell death have been proposed as useful biomarkers for patients with cancer and the other critical illness [4, 5]. The levels of nucleosomal DNA, Fas-ligand, cytochrome c, and a variety of cytoskeletal components have been measured as circulating biomarkers of cell death [6–9]. The products that are released into circulation by cancer cells upon their death can be measured. These biomarkers can be used clinically as diagnostic and prognostic factors of cancer and to predict the outcome of anticancer therapies. The biomarkers are increasingly being used in trials of new anticancer therapies.

Cytokeratin 18 (CK18) is a member of the intermediate filament family of cytoskeletal proteins, is widely expressed in epithelial and endothelial cells, and accounts for approximately 5% of the total cell protein [10]. CK18 is usually expressed during oncogenic transformation [11], and it is expressed in gastric cancer [12]. Moreover, CK18 expression correlated with lymph node metastases, tumor differentiation, and tumor invasion of gastric cancer [13].

CK18 may be a useful indicator of gastric cancer, because it is an accepted marker of cell death [14]. Several types of cell death such as apoptosis, necrosis, and autophagy have been described as a part of the ongoing disease process. ~~The possibility that death~~

~~of cancer cells may generate products into circulation, which can be detected in the serum, has an important diagnostic potential.~~ A novel method based on the measurement of different molecular forms of CK18 can be used to investigate the modes of epithelial cell death. CK18 fragments are released into the circulation during necrotic or apoptotic cell death. Enzyme-linked immunosorbent assays (ELISAs) have been developed to measure the circulating levels of ~~caspase-cleaved and total~~ soluble CK18 fragments. M30 and M65 antibodies can be used in ELISA to detect the different forms of CK18 released into circulation during caspase-dependent or total cell death, respectively, and these antibodies are potential biomarkers of epithelial cancers [15]. Activated caspases 3, 7, and 9 cleave CK18 during apoptosis, which leads to collapse of the cytoskeleton and ~~subsequent~~ formation of apoptotic bodies; subsequently, the proteolytic fragments of caspase-cleaved CK18 are released into circulation [16, 17]. M30-Apoptosense® ELISA detects a neoepitope specific to apoptosis caused by caspase-mediated cleavage of CK18 at aspartate 396 (CK18asp396), and thus, M30 is a selective biomarker of apoptosis. Conversely, necrosis of epithelial cells releases full-length CKs into circulation after breakdown of the cell membrane. Monoclonal antibody M6 and M5 detects total soluble CK18 fragments that contain full-length epitope of the protein; thus, M65 can be used to detect CK18 fragments released from necrotic cells in addition to those from apoptotic cells [16, 18]. Both assays may provide clinically useful information for the management of patients with epithelial

cancers. This assay can be used clinically for patients with several epithelial cancers such as head and neck tumors, endometrial carcinoma, testicular cancer, prostate cancer, breast cancer, lung cancer, colorectal cancer, and pancreatic cancer [18-24]. In addition, serum levels of M30 and M65 are increased in patients with advanced gastrointestinal adenocarcinoma [2, 25]. Recently, increased serum levels of M30 and M65 have been reported in patients with advanced gastric cancer [26].

The objectives of this study were to compare serum M30 (reflect caspase cleaved CK18 fragment) and M65 (reflect total CK18 fragment) levels between healthy volunteers and patients with gastric cancer and to determine whether the levels of these markers are correlated with the extent of cancer and prognosis. Here, we have carefully evaluated the correlation between serum M30 and M65 levels and clinical features and the diagnostic and prognostic significance of these levels in patients with gastric cancer to assess their clinical potential as biomarkers. To our knowledge, previous reports were concerned with advanced gastric cancer, this is the first report on increased serum both M30 and M65 levels in patients with gastric cancer even in an early stage and that these levels are closely correlated with extension of gastric cancer; further, serum level of M65 had an impact on survival.

Methods

Patients and serum collection

We enrolled 54 patients diagnosed with gastric cancer confirmed by histological examination at Kanazawa university hospital; blood samples of the patients were collected ~~before treatment~~. The disease status of patients were assessed according to the American Joint Commission on Cancer (AJCC)/International Union against Cancer (UICC) TNM classification [27]. Patients with a history of malignancies, diabetes mellitus, or uncontrolled infection were excluded from the study. Control blood samples were collected from 12 healthy volunteers (age; 28-72 (median; 58), 8 males and 4 females). Blood samples of patients were collected before any cancer therapy. Blood samples collected from patients and controls were stored into dry tubes, and the sera were centrifuged at 1000g for 10 min within 30 min of sampling. The samples were stored at −80°C until analysis. Written informed consent was obtained before starting the study from all patients and healthy volunteers for using data obtained from hematological and clinical examinations to be used for research purpose. The study was performed according to the guidelines of the Medical Ethics Committee of the Kanazawa university hospital in compliance with the Helsinki Declaration.

Assay procedure to determine the serum levels of CK18

Samples were assayed in duplicate for CK18asp196 using the M30-Apoptosense® ELISA (PEVIVA AB, Bromma, Sweden), which is a one-step in vitro immunoassay for

the quantitative determination of the apoptosis-associated CK18asp396 (M30) neoepitope in the serum and plasma. M65®-ELISA (PEVIVA) is a one-step in vitro immunoassay for the quantitative determination of total soluble CK18 in the serum and plasma. Both the procedures were performed according to the manufacturer's instructions. Mean of the duplicate values were used for analysis.

Detection of serum tumor markers

Serum levels of CEA and CA19-9 before treatment were routinely measured in patients with gastric cancer at our institute. Serum CEA levels less than 5 ng/mL and serum CA19-9 level less than 35 U/mL were considered to be normal values as per the manufacturer's protocol.

Statistical analysis

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) statistical software version 11.0. Continuous data were described using median values (± standard deviation) obtained by Mann–Whitney U-test or Kruskal–Wallis test for non-parametric comparisons of continuous data. In addition, receiver operator curve (ROC) analysis was performed; sensitivities, specificities, and area under curves (AUC) were calculated using ROC analysis for diagnosis and death. Survival data were analyzed using the Kaplan–Meier method and log-rank test was performed for the comparison of survival curves. Cox's proportional hazards regression was used for multivariate analysis. Prognostic variables of univariate significance were selected for

inclusion in the multivariate model. A P-value of 0.05 was considered significant.

Results

Serum levels of M30 and M65 in patients and controls

Patient demographics and tumor characteristics are summarized in Table 1. Patients with gastric cancer showed a wide range of serum levels of M30; reflect caspase-cleaved CK18 (111.4–1121.3 U/L); serum M30 levels were statistically higher in patients than in controls (184.1 ± 179.6 vs. 144.3 ± 10.6, P = 0.001, Fig. 1). Similarly, patients with gastric cancer showed a wide range of serum levels of M65; reflect total soluble CK18 (106.1–2290.0 U/L), and these values were statistically higher in patients than in controls (204.2 ± 392.9 vs 104.4 ± 36.1, P < 0.001, Fig. 1). Thus, serum M65 and M30 levels were positively correlated with the presence of gastric cancer. Furthermore, the serum levels of M30 and M65 statistically correlated with clinical factors, including depth of tumor invasion, lymph node metastasis, distant metastasis, and clinical stage; in addition, M65 levels correlated with the histological type (Table 1). The levels of both M30 and M65 significantly correlated with the extent of disease of the patient.

Usefulness of M30 and M65 levels as diagnostic markers of gastric cancer

To determine whether the serum levels of M30 and M65 could be used as diagnostic markers of gastric cancer, we measured the serum levels of each biomarker using ROC analyses for calculating the best cut-off value for diagnosis. The best cut-off value, AUC, and the range of 95% confidence interval (CI) for M30 were 155.0 U/L, 0.801,

and 0.810 to 0.914, respectively, and those for M65 were 142.2 U/L, 0.919, and 0.837 to 1.001, respectively. M30 has a sensitivity of 67.5% and a specificity of 90.9%, while M65 has a sensitivity of 70.1% and a specificity of 90.9% in distinguishing between patients and healthy volunteers. The sensitivities of M30 and M65, respectively, according to clinical stages were as follows: Stage I, 38.1% and 66.7%; Stage II, 66.7% and 33.3%; Stage III, 100% and 100%; and Stage IV, 82.6% and 95.7%. High values of CEA were found in 12 (22.2%) of the 54 patients (0% in Stage I and II, 50% in Stage III, and 39.1% in Stage IV), high values of CA19-9 were found in 12 (22.2%) of the 54 patients (8.3% in Stage I and II, 16.7% in Stage III, and 39.1% in Stage IV). These results suggest that the serum levels of M30 and M65 may be used as good diagnostic biomarkers. The sensitivity of serum M30 and M65 levels was superior to that of CEA and CA19-9 levels in all stages, including the early stage of gastric cancer, and thus, the former may be suitable for population screening.

Survival analysis

The median follow-up period was 26.5 months (range, 4.5–40.5 months), and the median overall survival was 20.4 months (range, 1.4–40.5 months). To evaluate the association between serum levels of M30, M65, CEA, and CA19-9 and patient survival, we measured the serum levels of each biomarker using ROC analyses for calculating the best cut-off value for prediction of death. The best cut-off value, AUC, and the range of 95% CI for M30 were 184.8 U/L, 0.705, and 0.558 to 0.852, respectively, and

those for M65 were 199.3 U/L, 0.829, and 0.712 to 0.947. The level of CEA was 3.1 ng/mL; AUC, 0.613 (95% CI, 0.459–0.767), and the level of CA19-9 was 21.5 mg/mL; AUC, 0.647 (95% CI, 0.492–0.802). Univariate analysis of overall survival was performed by Kaplan–Meier method and log-rank test was performed for clinical findings; the cut-off values of each biomarker were calculated using these tests. Clinical stage ($P < 0.001$), CEA level ($P = 0.015$), M65 level ($P = 0.017$), and histological type ($P = 0.034$) were significantly associated with survival (Table 2). The values of M30 were high in patients with poor prognosis, but these values were not statistically significant ($P = 0.061$). The above prognostic variables of univariate significance were selected for inclusion in the multivariate model. Multivariate analysis revealed that independent significant prognostic factors were clinical stage ($P = 0.033$) and serum M65 level ($P = 0.036$, Table 2). Increased serum M65 levels were associated with poorer survival on univariate analysis as dichotomized variable (log rank, $P = 0.017$; Fig. 3), and on multivariate analysis as a continuous data (Cox, $P = 0.036$; Table 2).

Discussion

Serum CK18 are increased in tumors such as head and neck tumors, endometrial carcinoma, testicular cancer, prostate cancer, breast cancer, lung cancer, colorectal cancer, and pancreatic cancer [18-24]. Increase in serum M30 levels in cancer were reported for the first time by Uneo et al. in breast cancer patients [28]. Uneo et al. showed that M30 levels were significantly higher in recurrent disease than in primary breast cancer and healthy subjects, and M30 levels correlated with tumor volume. M30 and M65 levels were also increased in patients with advanced gastrointestinal adenocarcinoma [2, 25]. Yaman E et al. reported that serum M30 and M65 levels were increased in patients with advanced gastric cancer, and only M30 levels reflected the tumor load [26]. There were no reports that indicate correlation between serum M30 and M65 levels and cancer extent. In the present study, we found that the serum levels of M30 and M65 were significantly higher in patients with gastric cancer than in healthy volunteers. ~~The levels of M30 and M65 statistically correlated with the extent of gastric cancer, reflecting high tumor burden and they were also elevated in early stage.~~

Classic tumor markers, CEA and CA19-9, are correlated with tumor load in advanced gastric cancer. However, these markers are not useful for the detection of early-stage gastric cancer. In addition, many patients with advanced gastric cancer have normal levels of these markers. Classical biomarkers of gastric cancer do not have sufficient

sensitivity for population screening. In the present study, diagnostic sensitivities of M30 (67.5%) and M65 (70.1%) were higher than those of classical tumor markers (CEA and CA19-9, both 22.2%). In addition, the levels of M30 and M65 are closely correlated with extent of cancer, depth, lymph node metastasis, distant metastasis, and clinical stage. In patient with early gastric cancer, they were elevated in 38.1% and 66.7%, respectively. These findings suggest that M30 and M65 were sensitive diagnostic markers of gastric cancer.

Serum M30 levels have been reported to be a prognostic marker in some tumor types such as endometrial carcinoma, testicular cancer, lung cancer, and colorectal cancer [19, 21-23]. Ueno et al. [28] reported that increased serum levels of M30 in breast cancer patients had no prognostic impact. In contrast, Ulukaya et al. showed that increased serum M30 levels in lung cancer patients were associated with shorter median survival time [19]. Serum levels of M30 and M65 significantly reflected the prognosis of testicular germ cell cancer [21]. Yaman E et al. reported that serum M30 level was an independent prognostic factor in patients with gastric cancer, but serum M65 level was not a prognostic factor [26]. In Bilici's study, univariate analysis showed that M30 and M65 levels were associated with progression-free survival in patients with advanced gastric cancer, but these findings could not be confirmed by multivariate analysis [29]. In our study, M30 levels were not statistically correlated with prognosis; in contrast, M65 levels were associated with a shorter survival time in

patients with gastric cancer. Increase of cell death, especially necrotic cell death might be reflecting aggressive behavior of cancer. In present study, serum M65 levels were correlated with cancer extent and histological type in either. M65 might reflect the cancer status including both the volume and the behavior of cancer cells, thus they associated with poorer outcome. And M65 might indicate the aggressive behavior of cancer cells than M30 in gastric cancer. The conflicting results obtained in different studies may be related to the low number patients examined in previous studies, and further studies with a greater number of patients are required to confirm the importance of serum M30 and M65 levels.

The source of serum CK18 fragments is not clear. The depth, status of lymph node metastasis and distant metastasis respectively correlated with serum M65 levels. They reflect the volume of cancer cells directly. These results indicate that serum CK18 fragments were mainly released from tumor cells; not from healthy cells affected by cancer.

Serum levels of M30 and M65 were investigated as a potential biomarker for treatment response. Fluctuations in serum M30 and M65 levels during chemotherapy were discussed as useful early indicators of response to chemotherapy. M30 levels had significant relationship with response to therapy in breast cancer and lung cancer [19, 30, 31]. Moreover, M65 and M30 levels reflect chemotherapy-induced changes in testicular germ cell cancer [21]. CK18 levels before initiation of chemotherapy may be

an indicator of tumor response to the chemotherapy in patients with advanced gastrointestinal adenocarcinomas [2]. On the other hand, the levels of M30 were increased during chemotherapy in patients with gastrointestinal cancer, which was also related with response to the therapy [25]. We conducted studies on changes in serum M30 and M65 levels as an early predictor for the effect of chemotherapy in gastric cancer.

The serum levels of M30 (reflect caspase cleaved CK18 fragment) and M65 (reflect total CK18 fragment) may be biomarkers for gastric cancer and highly sensitive diagnostic markers that can reflect the extent of cancer. Moreover, M65 levels can be used as a prognostic indicator. Further studies with a greater number of patients are required to confirm the importance of serum M30 and M65 levels.

References

1. Srivastava S, Srivastava RG (2005) Proteomics in the forefront of cancer biomarker discovery. J Proteome Res 13:1098-1103.
2. Scott LC, Evans TR, Cassidy J, Harden S, Paul J, Ullah R, et al. (2009) Cytokeratin 18 in plasma of patients with gastrointestinal adenocarcinoma as a biomarker of tumour response. Br J Cancer 101:410-417.
3. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. (2008) Cancer statistics. 2008. CA Cancer J Clin 58:71-96.
4. Holdenrieder S, Stieber P, Bodenmüller H, Busch M, Fertig G, Fürst H, et al. (2001) Nucleosomes in serum of patients with benign and malignant diseases. Int J Cancer 95:114-120.
5. Roth GA, Krenn C, Brunner M, Moser B, Ploder M, Spittler A, et al. (2004) Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients. Shock 22:218-220.
6. Holdenrieder S, Stieber P, VON Pawel J, Raith H, Nagel D, Feldmann K, et al. (2006) Early and specific prediction of the therapeutic efficacy in non-small cell lung cancer patients by nucleosomal DNA and cytokeratin-19 fragments. Ann N Y Acad Sci 1075:244-257.
7. Pichon MF, Labroquère M, Rezaï K, Lokiec F (2006) Variations of soluble fas and cytokeratin 18-Asp 396 neo-epitope in different cancers during

chemotherapy. Anticancer Res 26:2387-2392.

8. Barczyk K, Kreuter M, Pryjma J, Booy EP, Maddika S, Ghavami S, et al. (2005) Serum cytochrome c indicates in vivo apoptosis and can serve as a prognostic marker during cancer therapy. Int J Cancer 116:167-173.

9. Neuber K, Eidam B (2006) Expression of Fas ligand (CD95L) in primary malignant melanoma and melanoma metastases is associated with overall survival. Onkologie 29:361-365.

10. Chou CF, Riopel CL, Rott LS, Omary MB (1993) A significant soluble keratin fraction in 'simple' epithelial cells. Lack of an apparent phosphorylation and glycosylation role in keratin solubility. J Cell Sci 105:433-444.

11. Lane EB, Alexander CM (1990) Use of keratin antibodies in tumor diagnosis. Semin Cancer Biol 1:165-179.

12. Abe T, Fukumoto M, Tsuchiya K, Kuramochi K, Furuta T, Togoh S, et al. (1989) Human monoclonal antibodies against cytokeratin 18 generated from patients with gastric cancer. Jpn J Cancer Res 80:271-276.

13. Xu W, Zhang MW, Huang J, Wang X, Xu SF, Li Y, et al. (2005) Correlation between CK18 gene and gastric carcinoma micrometastasis. World J Gastroenterol 11:6530-6534.

14. Sheard MA, Vojtesek B, Simickova M, Valik D (2002) Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21-1) into the extracellular

space during apoptosis. J Cell Biochem 85:670-677.

15. Kramer G, Erdal H, Mertens HJ, Nap M, Mauermann J, Steiner G, et al. (2004) Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. Cancer Res 64:1751-1756.

16. Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L, et al. (2007) Cell death modalities: classification and pathophysiological implications. Cell Death Differ 14:1237-1243.

17. Caulín C, Salvesen GS, Oshima RG (1997) Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol. 138:1379-1394.

18. Kramer G, Schwarz S, Hägg M, Havelka AM, Linder S (2006) Docetaxel induces apoptosis in hormone refractory prostate carcinomas during multiple treatment cycles. Br J Cancer 94:1592-1598.

19. Ulukaya E, Yilmaztepe A, Akgoz S, Linder S, Karadag M (2007) The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. Lung Cancer 56:399-404.

20. Ueno T, Toi M, Bivén K, Bando H, Ogawa T, Linder S (2003) Measurement of an apoptotic product in the sera of breast cancer patients. Eur J Cancer 39:769-774.

21. de Haas EC, di Pietro A, Simpson KL, Meijer C, Suurmeijer AJ, Lancashire

LJ, et al. (2008) Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. Neoplasia 10:1041-1048.

22. Koelink PJ, Lamers CB, Hommes DW, Verspaget HW (2009) Circulating cell death products predict clinical outcome of colorectal cancer patients. BMC Cancer 9:88.

23. Y.X. Wu, J.H. Wang, H. Wang and X.Y. Yang (2003) Study on expression of Ki-67, early apoptotic protein M30 in endometrial carcinoma and their correlation with prognosis. Zhonghua Bing Li Xue Za Zhi 32:314–318.

24. Dive C, Smith RA, Garner E, Ward T, George-Smith SS, Campbell F, et al. (2010) Considerations for the use of plasma cytokeratin 18 as a biomarker in pancreatic cancer. Br J Cancer 102:577-582.

25. Brandt D, Volkmann X, Anstätt M, Länger F, Manns MP, Schulze-Osthoff K, et al. (2010) Serum biomarkers of cell death for monitoring therapy response of gastrointestinal carcinomas. Eur J Cancer 46:1464-1473.

26. Yaman E, Coskun U, Sancak B, Buyukberber S, Ozturk B, Benekli M (2010) Serum M30 levels are associated with survival in advanced gastric carcinoma patients. Int Immunopharmacol 10:719-722.

27. F.L.P.D. Greene and I.D. Fleming (2002) American Joint Committee on Cancer Staging Manual (6th ed). Philadelphia: Springer

28. Ueno T, Toi M, Bivén K, Bando H, Ogawa T, Linder S (2003) Measurement of an apoptotic product in the sera of breast cancer patients. Eur J Cancer 39:769-774.

29. Bilici A, Ustaalioglu BB, Ercan S, Orcun A, Seker M, et al. (2011) Is there any impact of plasma M30 and M65 levels on progression-free survival of patients with advanced gastric cancer? Cancer Chemother Pharmacol 68:309-316.

30. Demiray M, Ulukaya EE, Arslan M, Gokgoz S, Saraydaroglu O, et al. (2006) Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. Cancer Invest 24:669-676.

30. Olofsson MH, Ueno T, Pan Y, Xu R, Cai F, van der Kuip H, et al. (2007) Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. Clin Cancer Res 13:3198-3206.

Figures

Figure 1 - Serum M30 and M65 levels in healthy volunteers and patients.

Serum M30 and M65 levels were statistically higher in patients than in controls (P = 0.001). They were positively correlated with the presence of gastric cancer. Horizontal solid lines represent median values, boxes represent interquartile ranges, and whiskers represent ranges.

Figure2 - Overall survival rate of patients with gastric cancer, deference according to serum M65 level.

Increased serum M65 levels were associated with poorer survival on univariate analysis as dichotomized variable (log rank, P = 0.017; Fig. 3),

Tables

Table1 - Patient characteristics and serum M30 level, serum M65 level.

The serum levels of M30 and M65 statistically correlated with clinical factors, including depth of tumor invasion, lymph node metastasis, distant metastasis, and clinical stage; in addition, M65 levels correlated with the histological type.

Table 2 - Survival analysis.

Clinical stage (P < 0.001), CEA level (P = 0.015), M65 level (P = 0.017), and histological type (P = 0.034) were significantly associated with survival in univariate analysis. Multivariate analysis revealed that independent significant prognostic factors

were clinical stage (P = 0.033) and serum M65 level (P = 0.036).

Table Ⅰ. Patient characteristics and serum M30 level, serum M65 level.

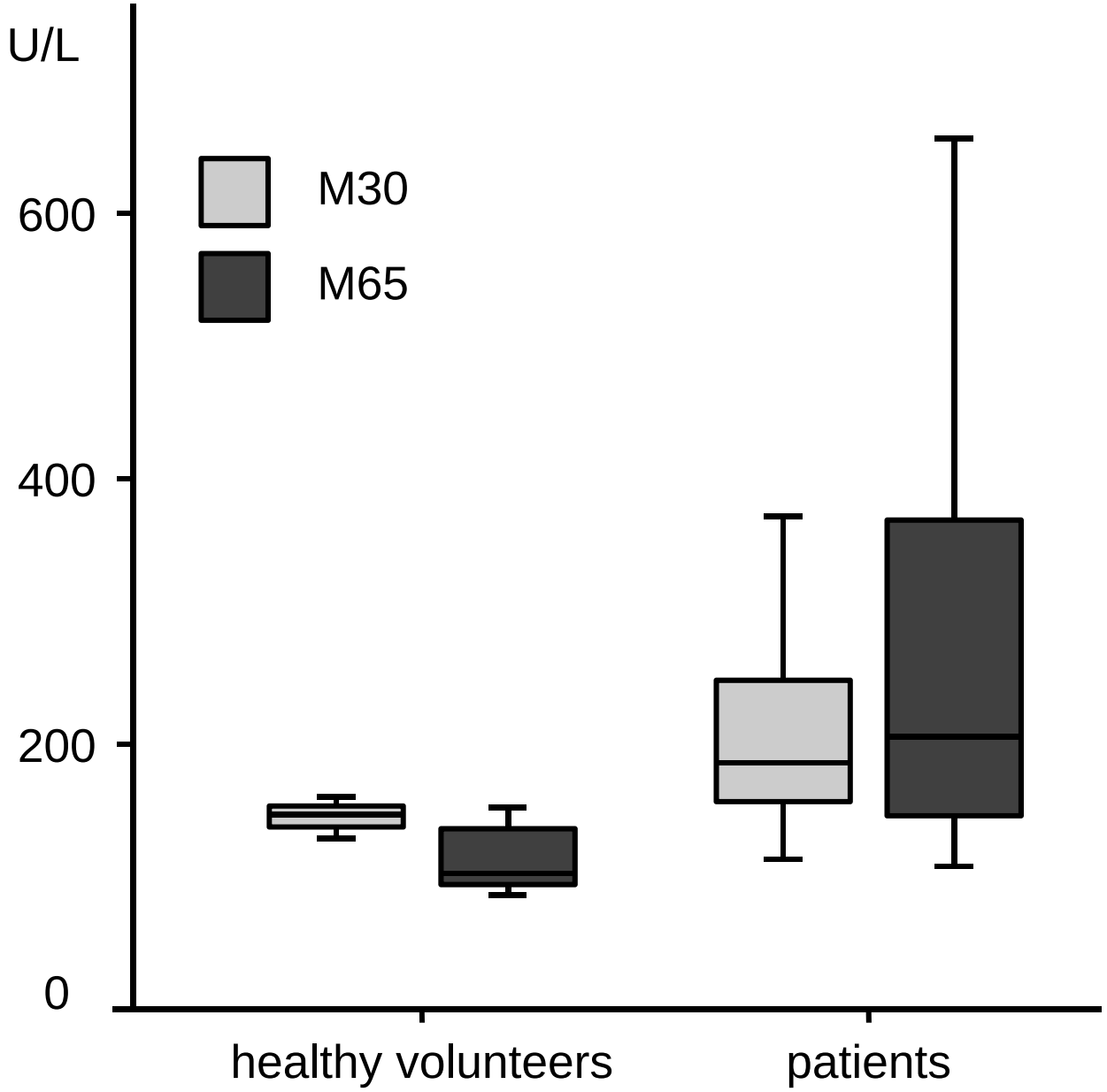
characteristics	n	serum M30 (U/L)	p-value	serum M65 (U/L)	P-value
All	54	184.1 ± 179.6		204.2 ± 392.9	
Age (31-80, median 68)			0.992		0.695
-68	26	174.1 ± 245.4		183.2 ± 527.9	
69-	28	188.4 ± 65.9		234.5 ± 187.3	
Sex			0.874		0.768
male	44	184.1 ± 141.4		204.2 ± 308.5	
female	10	188.6 ± 179.6		204.2 ± 392.9	
Histological type			0.295		0.027
intestinal type	25	182.4 ± 58.2		171.5 ± 121.6	
diffuse type	29	191.4 ± 252.4		305.9 ± 535.6	
Depth of tumor invasion			0.003		0.010
T1	23	154.6 ± 38.3		152.6 ± 134.8	
T2	12	190.0 ± 88.5		284.1 ± 174.5	
T3	15	211.0 ± 302.7		251.7 ± 657.7	
T4	4	183.4 ± 149.8		199.3 ± 342.8	
Lymph node metastasis			0.001		0.001
N0	25	154.9 ± 27.9		147.7 ± 62.8	
N1	6	213.7 ± 83.3		321.0 ± 138.5	
N2	8	196.2 ± 285.0		238.6 ± 590.7	
N3	15	249.4 ± 239.2		425.2 ± 530.4	
Distant metastasis			0.041		0.001
M0	41	182.6 ± 63.4		195.0 ± 150.4	
M1	13	249.4 ± 321.7		457.0 ± 668.3	
Clinical stage			0.001		0.001
Ⅰ	21	154.6 ± 25.9		213.7 ± 83.3	
Ⅱ	3	220.3 ± 198.8		139.1 ± 437.9	
Ⅲ	7	210.3 ± 42.3		299.9 ± 92.2	
Ⅳ	23	211.0 ± 250.9		311.0 ± 541.2	

Values are means ± SD.

Table Ⅱ. Survival analysis.

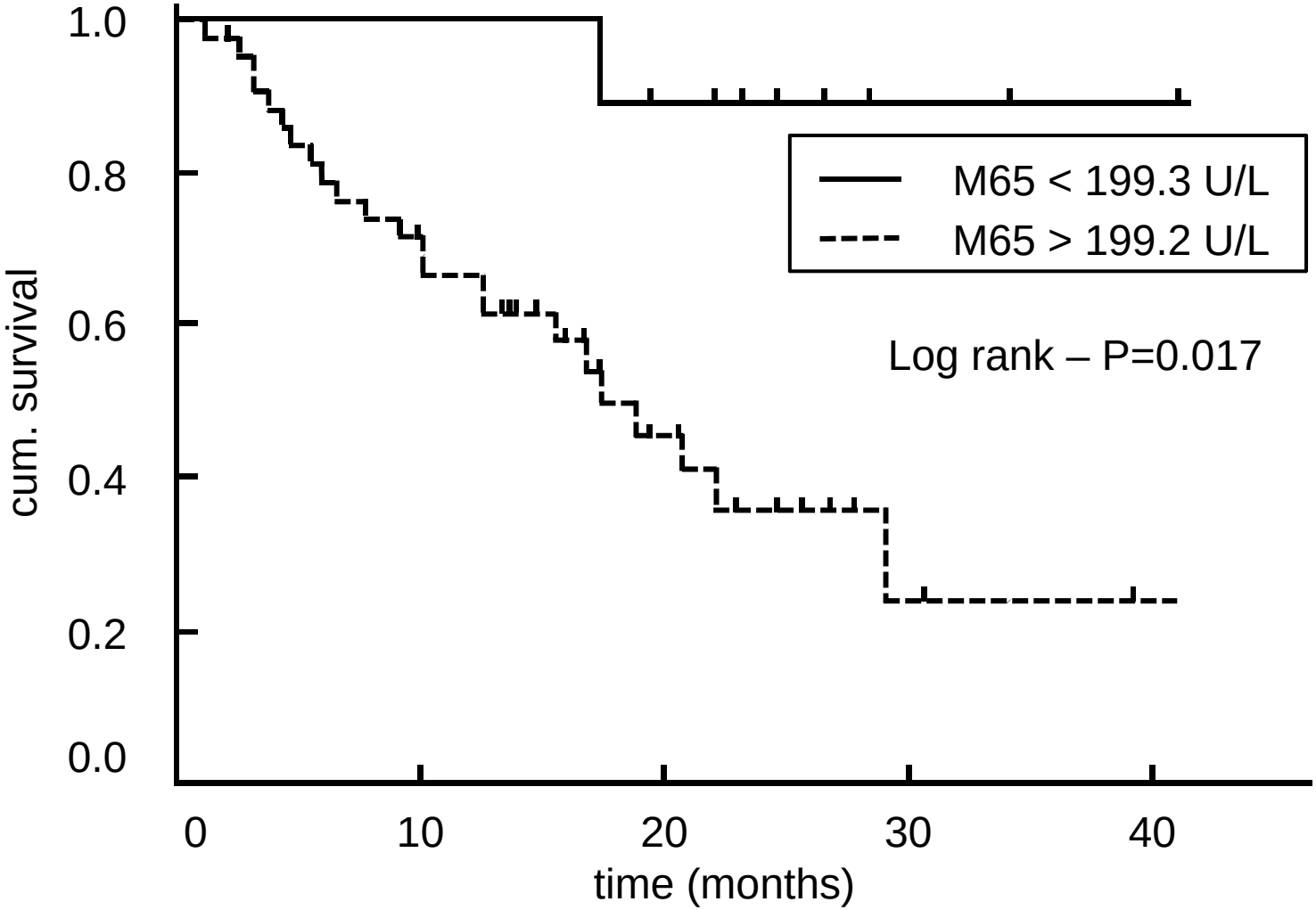
	overall survival		
	univariate analysis	multivariate analysis	
	P	Hazard ratio (95% CI)	P
age	0.548	-	-
gender	0.850	-	-
histological type	0.034	0.451 (0.123 – 1.651)	0.229
clinical stage	< 0.001	2.153 (1.107 – 4.189)	0.033
M30	0.061	-	-
M65	0.017	1.001 (1.000 – 1.002)	0.036
CEA	0.015	1.658 (0.443 – 6.213)	0.669
CA19-9	0.095	-	-

Figure 1. Serum M30 and M65 levels in healthy volunteers and patients



Katsunobu Oyama

Figure 2. Overall survival rate of patients with gastric cancer, deference according to serum M65 level.



Katsunobu Oyama