Impact of Adjuvant Immunochemotherapy Using Protein-bound Polysaccharide-K on Overall Survival of Patients with Gastric Cancer

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Abstract. *Protein-bound polysaccharide K (PSK) is an anticancer agent used for adjuvant therapy against gastric cancer. The aim of this study was to evaluate the effect of PSK on the overall survival of patients with gastric cancer. A total of 254 patients who underwent surgical curative resection were included in this retrospective study. We identified 138 patients who received antimetabolites alone (control group) and 115 patients who received antimetabolites plus PSK (PSK group). In patients with early tumor recurrence, overall survival was significantly better in the PSK group (p=0.023). In patients with pN3 lymph node metastasis, median overall survival was better in the PSK group compared with the control group (p=0.032). Our results suggest that adjuvant immunochemotherapy with PSK increased the overall survival for patients with pN3 and early tumor recurrence. Thus, the combination of PSK with oral chemotherapeutic agents might be suitable for postoperative adjuvant therapy against gastric cancer in patients with lymph node metastases.*

Protein-bound polysaccharide K (PSK) is derived from the CM-101 strain of the fungus *Coriolus versicolor* and is believed to act as a biological response modifier (1). Multiple mechanisms of antitumor action have been reported for PSK, including augmentation of depressed natural killer cell activity, inhibition of immunosuppressive cytokine production, up-regulation of human leukocyte antigen (HLA) class I expression on tumor cells, and induction of apoptosis through inhibition of nuclear factor kappa beta (NF-kB) (2-5).

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In Japan, PSK is considered well-suited for concurrent use along with cytotoxic agents, as postoperative adjuvant treatment against colorectal and gastric cancer. Several studies have reported good results from adjuvant treatment with a combination of PSK and chemotherapeutic drugs. Owada *et al*. reported that adjuvant chemotherapy of tegafur/uracil (UFT) with PSK reduced the risk of recurrence by 43.6% in patients with stage II or III colorectal cancer (6). Nakazato *et al*. demonstrated the efficacy of PSK combined with mitomycin C and oral fluorouracil as adjuvant immunochemotherapy for stage II/III gastric cancer after curative resection (7). To date, this is the only report demonstrating the efficacy of PSK in patients with gastric cancer (8). A means of predicting which patients will respond to PSK therapy is necessary.

In this study, we analyzed the clinicopathological features of patients with stage II/III gastric cancer to clarify the impact of PSK on their survival, in order to predict the outcome of postoperative adjuvant immunochemotherapy using PSK.

Patients and Methods

This is a retrospective analysis of 254 patients who were presented with gastric carcinoma at the Department of Surgical Oncology in Osaka City University Hospital between January 1999 and 2008. Inclusion was limited to patients, to whom curative surgery with postoperative adjuvant treatment with clinical stage II/III (UICC International Union Against Cancer TNM classification of malignant tumor) was offered. Pathological staging was based on the pathological TNM classification (9). Patients receiving only oral fluoropyrimidine agents [*e.g*. 5-fluorouracil (5-FU), tegafur/uracil (UFT), 5'-deoxy-5-fluorouridine (5'DFFR), and S-1 [TS-1 (Taiho Pharmaceutical, Tokyo, Japan) is a dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine preparation combining tegafur, gimeracil, and oteracil potassium in a molar ratio of 1:0.4:1] were assigned to the control group. Patients treated with oral fluoropyrimidine agents combined with PSK were assigned to the PSK group. This study was approved by the Osaka City University Ethics Committee. Informed consent was obtained from all patients.

Statistical analysis. All statistical analyses were performed using SPSS II for Windows (SPSS Inc., Chicago, IL, USA). Univariate

Table I. *Clinical characteristics of patients according to treatment group.*

		Control group (n=139)	PSK group (n=115)	*p*-value
Age, year	(Mean±SD)	64±12	65±9.5	0.398
Gender	Male/female	99/40	83/32	0.805
Tumor size (mm)	(Mean±SD)	60±37	64±38	0.805
Surgery	Distal/total	85/54	65/50	0.301
Reconstruction	B-I/B-II/R-Y	50/26/63	41/11/63	0.334
Removed LN	(Mean±SD)	37±15	38±15	0.655
Metastatic LN	(Mean±SD)	4.9±5.6	4.8±5.5	0.968
Tumor differentiation	Well, mod/poor	63/75	49/67	0.586
pT	1a/1b/2/3/4a/4b	0/4/26/20/86/3	1/5/13/20/73/3	0.653
pN	0/1/2/3a/3b	27/41/31/31/9	19/29/39/19/9	0.615
pStage	Ib/IIa/IIb/IIIa/IIIb/IIIc	2/19/41/24/25/28	1/6/37/24/26/21	0.277
Infiltrating growth	a/b/c	5/74/59	9/64/43	0.180
Venous invasion	0/1/2/3	18/37/57/26	16/37/39/24	0.691
lymphatic invasion	0/1/2/3	103/25/8/2	62/24/15/5	0.051
Chemotherapeutic drug	5-FU/UFT/5'DFFR/S1	5/75/9/50	5/67/14/29	0.093

LN: Lymph node; 5-FU: 5-fluorouracil; UFT: tegafur/uracil; 5'DFFR: 5'-deoxy-5-fluorouridine; S1: TS-1(Taiho Pharmaceutical, Tokyo, Japan); B-I: Billroth-I; B-II: Billroth-II; R-Y: Roux-en Y.

logistic regression was used to test for heterogeneity among the individual hazard ratios. Cox regression models were used to evaluate the association between clinicopathological features and recurrence-free survival (RFS). Any variable attaining a significance of $p<0.05$ was entered into multivariate analysis. Survival was calculated from the date of surgical operation by the Kaplan Meier method, and comparisons between groups were made by log-rank test. Significance was defined as $p<0.05$ throughout the study.

Results

Patients' characteristics. In this retrospective analysis, we compared two groups of previously treated patients with gastric cancer. These were sequential and non-overlapping groups. The first group received oral antimetabolite agents alone (control group). The second group was treated with antimetabolites plus PSK (PSK group). Out of a total of 254 patients with pathological stage II/III gastric cancer, 139 were allocated to the control group and 115 to the PSK group. The median administration period was 16 months in the control group and 15 months in the PSK group. The subsequent administration period was one year or longer, in 101 patients (72.6%) of the control group and in 73 patients (63.4%) of the PSK group. As shown in Table I, there were no significant differences between the two groups with respect to baseline characteristics, including pathological lymph node metastasis, tumor invasion, histological differentiation, venous invasion and lymphatic invasion. Surgical procedures were similar in both groups. No significant differences were found in the type of orally administered chemotherapeutic agents, which included 5-FU, UFT, 5'DFFR and S-1. Although patients who developed tumor recurrence underwent secondary chemotherapy such as TS-1/cisplatin, Taxol and Taxotel, there were no differences in regimens between the control group and the PSK group.

Survival rates. Out of the 254 patients, 139 had post-surgical recurrences: 52 (37.4%) in the control group and 48 (41.3%) in the PSK group. There was no significant difference in the pattern of recurrences (data not shown). The mean RFS of the control group and the PSK group was 63 and 57 months, respectively. The 5-year RFS rate was 54.7% in the control group and 52.7% in the PSK group ($p=0.541$) (Figure 1A). The mean survival was 76 months in the control group *versus* 67 months in the PSK group. The 5-year overall survival rate was 58.3% in the control group and 57.1% in the PSK group ($p=0.685$) (Figure 1B). In the analysis of all patients, no significant differences were found in relapse-free or overall survival.

Patients in whom there was no recurrence had remarkably good prognosis regardless of PSK treatment (Figure 2A). As reported elsewhere, patients who developed recurrent disease had an extremely poor prognosis: the 5-year overall survival rate for these patients was 12.5% in the control group and 19.6% in the PSK group. However, the median survival was 26 months in the control group *versus* 33 months in the PSK group, indicating a tendency towards better prognosis with PSK treatment ($p=0.062$) (Figure 2B). With particular reference to time to relapse, the survival improvement following PSK treatment was significantly better in patients with early recurrence than those with late recurrence ($p=0.023$) (Figure 2C and D).

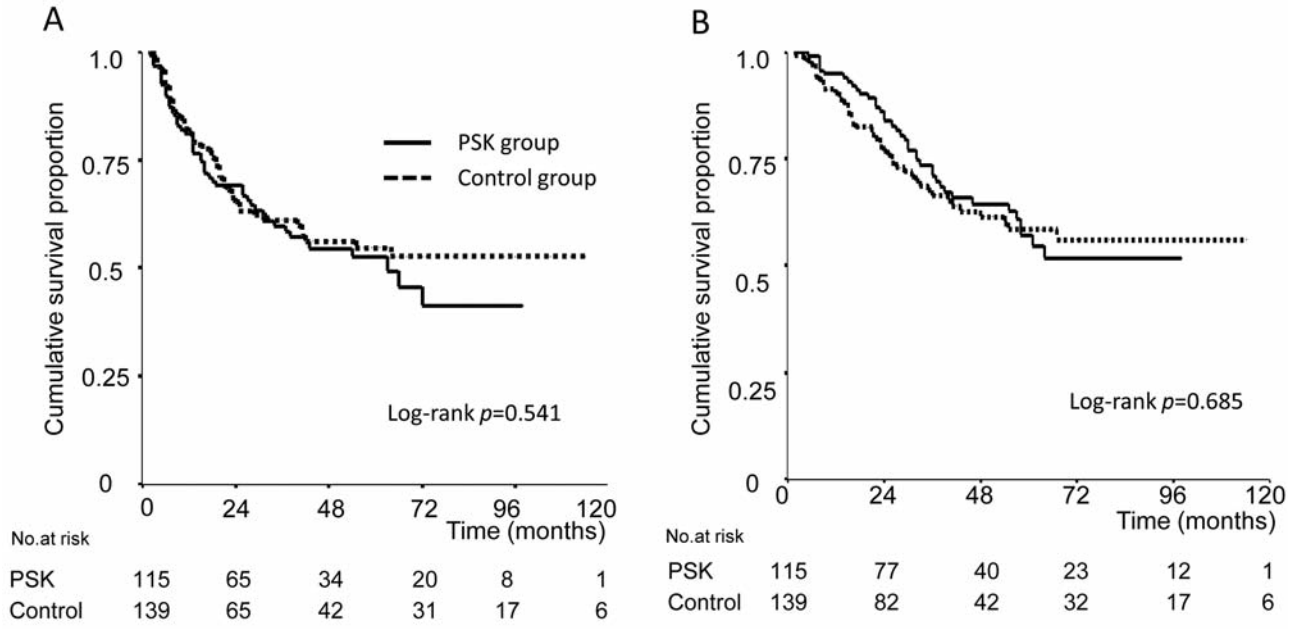

Figure 1. *Data on relapse-free survival (A) and overall survival (B) of 254 pateints with stage II/III gastric cancer are shown according to treatment group. There was no significant difference between treatment with and without PSK in relapse-free and overall survival.*

Table II. *Hazard ratios for overall survival*

	Univariate analysis				Multiivariate analysis			
	p-Value	HR	95% CI		*p*-Value	HR	95% CI	
pT	0.011	1.451	1.089	1.933	0.041	2.057	1.029	4.114
pN	<0.001	1.454	1.208	1.751	0.022	1.955	1.100	3.474
pStage	<0.001	1.476	1.246	1.749	0.171	0.632	0.327	1.220
V	<0.001	1.534	1.215	1.936	0.020	1.343	1.047	1.721
Tumor size	<0.001	1.009	1.005	1.013	0.020	1.007	1.001	1.012
Complication	0.041	1.638	1.020	2.631	0.033	1.692	1.043	2.746

V: Venous infiltration; HR: Hazard ratio; CI: confidence interval.

Analysis of prognostic factors influencing overall survival. Univariate analysis showed that the factors that influenced overall survival were tumor invasion, lymph node status, TNM stage, venous invasion, tumor size and postoperative complications (Table II). In multivariate analysis, the most relevant factors to overall survival were tumor invasion and lymph node status (Table II). Based on this result, we performed a survival analysis in the subpopulation: we did not observe a correlation of tumor invasion with good prognosis following PSK treatment. From the point of view of lymph node metastasis status, however, the prognosis of patients with more than seven instances of lymph node metastasis was improved by PSK treatment (Figure 3): the 5-year overall survival rate of these patients was 47.8% in the PSK group *versus* 22.8% in the control group (p=0.0317).

Discussion

In this study, we have observed that adjuvant immunochemotherapy with PSK appeared to improve the overall survival of patients with pathological stage II and III gastric cancer. Of particular significance is the finding that survival was improved in patients with multiple lymph node metastases following administration of PSK.

As shown in the report by Nakazato *et al*., PSK combined with 5-FU and mitomycin improved both the 5-year disease-free rate (70.7 *vs*. 59.4% in the standard treatment group) and 5-year survival (73.0 *vs*. 60.0%) for stage II/III gastric cancer (7). In our study, the 5-year overall survival rate was 54% in all patients, which is worse than what is seen in the other reports (10, 11). This was likely to be due to

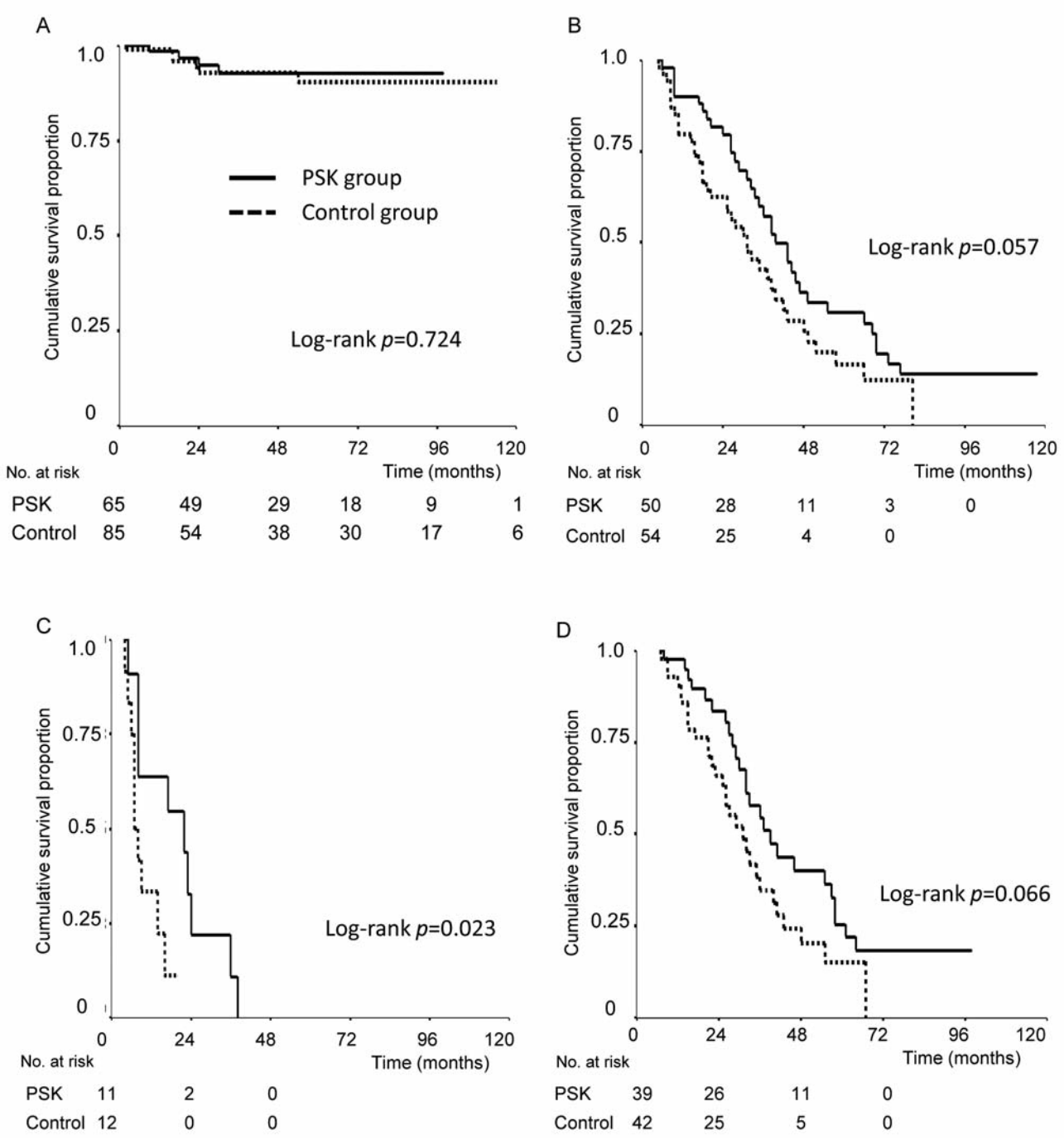

Figure 2. *The overall survival rates stratified by postoperative relapse. The Kaplan–Meier curves are shown for relapse-free patients (A), relapse patients (B). Patients received by PSK had better overall survival than patients without PSK (p<0.057 for both comparisons by the two-sided log-rank test). C: Early recurrence patients treated with PSK had significant better overall survival compared with control group (p<0.023). D: In late relapse patients, median survival time of patients with PSK was longer than those without PSK.*

complexities in our patients' backgrounds, such as extensive pathological stage or tumor invasion. However, this outcome does not compromise our analysis regarding the impact of PSK on overall survival.

Currently, S-1 is accepted as a standard regimen for adjuvant chemotherapy by many oncologists in Asia (12). It is currently unclear whether PSK is superior to S-1-based chemotherapy for patients with pathological stage II or III

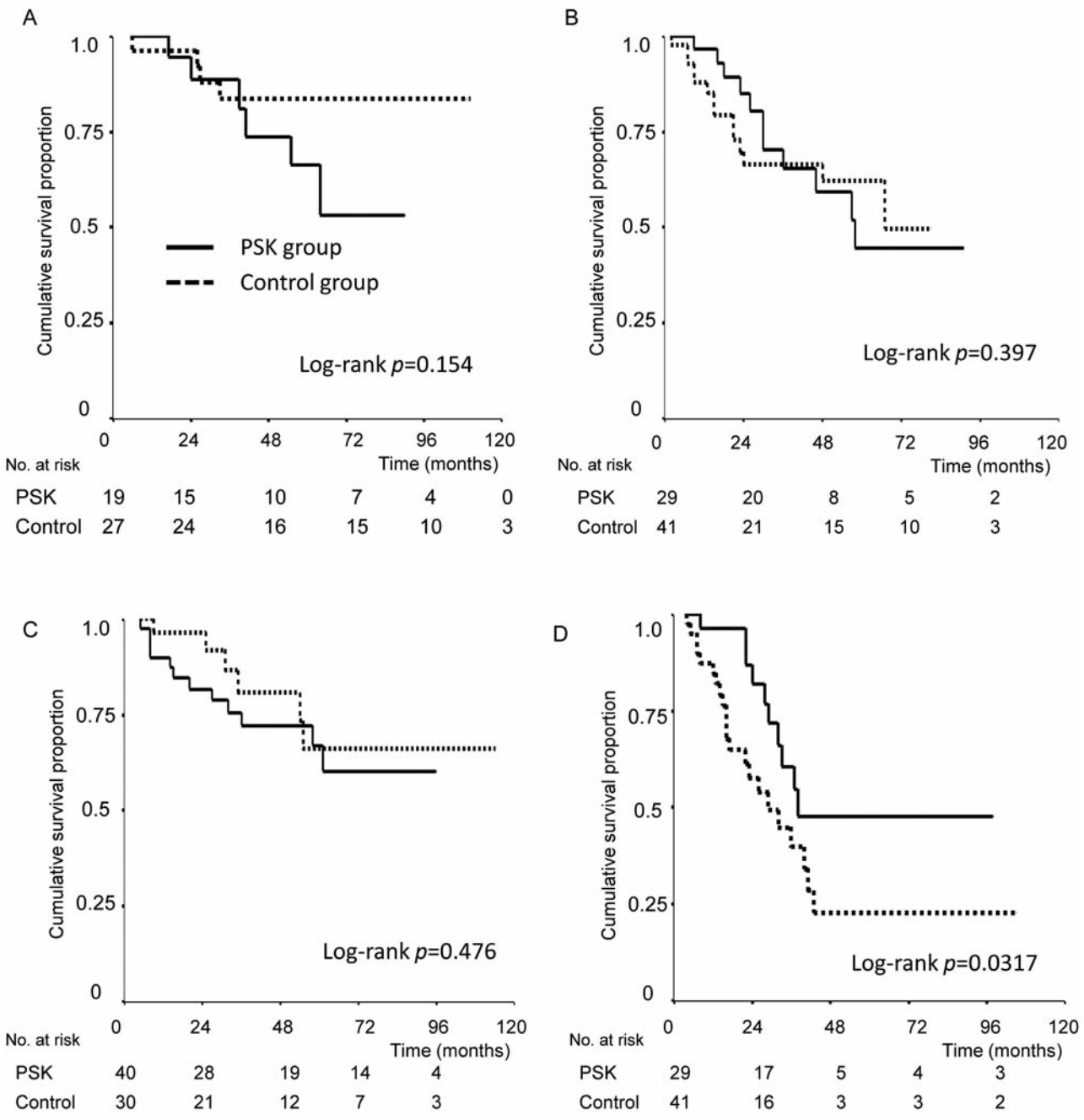

Figure 3. *The overall survival rates stratified by lymph nodes metastasis graded pN0, pN1,pN2 and pN3 (panels A, B,C and D respectively). In patients with pN3 metastasis, PSK group had significantly better overall survival than control group (p<0.033 for both comparisons by the log-rank test).*

gastric cancer. Our cohort included 29 patients who had received S-1 plus PSK, but no significant synergistic benefit was seen; however, this could be a result of study design or scale. The results of a randomized phase III trial designed to compare S-1 alone with S-1 plus PSK will be interesting (13).

PSK is a unique antitumor agent commonly used in Japan for gastric or colorectal cancer (14-16). Its actions include immunological effects such as the induction of cytotoxic T-lymphocyte activity and the suppression of immuno-suppressive cytokines (17, 18). It can also act directly on cancer cells, inducing of apoptosis and augmentation of

major histocompatibility complex class I expression (19, 20). Several studies also revealed the synergistic effect of PSK with chemotherapeutic agents, including suppression of apoptosis of circulating T-cells and augmentation of docetaxel-induced apoptosis of cancer cells through NF-kB inhibition (5, 21).

In recent years, immunological science has evolved, and new immunotherapies for cancer have been developed (22). Unlike chemotherapy, which acts directly on the tumor, cancer immunotherapies act on the immune system. It takes time, however, to trigger the translation from immune response to antitumor response. It has been reported that Kaplan Meier curves for immunotherapeutic agents showed a delayed separation of the survival effect (22, 23). In our study, the overall survival curve for patients with early relapse exhibited a delayed pattern, where the separation of the Kaplan Meier curves occurred approximately 6 months after surgery, indicating that PSK has an immunological antitumor effect.

Our study did not demonstrate significantly better RFS in the PSK group than the control group. The most likely explanation for this is that the patients with stage II disease in the PSK group had several worse prognostic factors compared with the control group. Pathological stage and venous invasion determined the prognosis of patients of our cohort by multivariate analysis (data not shown). In pathological stage II, stage IIB accounted for 82% of patients in the PSK group but only for 62% in the control group (p=0.086). Moreover, positive venous invasion was found in 27% of the PSK group as opposed to 11% of the control group (p=0.021).

It is of particular interest that the overall survival of patients in the PSK group who had unpromising prognoses due to multiple lymph node metastases was significantly better compared with the control group. Lymph node metastasis is considered to be the most important prognostic factor in patients with gastric cancer (24). It has been reported that the infiltration of forkhead box P3 ($Foxp3^+$) T-regulatory cells in regional lymph nodes was associated with lymph node metastasis, suggesting that weakened-antitumor immunity of cancer patients could trigger lymph node metastasis culminating in poor prognosis (25). In addition, early relapse could be associated with an immune response to cancer. It has been reported that the absence of early signs of metastatic invasion correlated with a significant increase of the density of memory T-cells *in situ* (26). Here, amelioration of the overall survival of the patients with early recurrence in the PSK group may be induced by the immunological effect of PSK.

In conclusion, immunochemotherapy with PSK should augment antitumor treatment of and immunological response to gastric cancer. PSK might have a key role in reactivating antitumor immunity and improving prognosis in gastric cancer patients with multiple lymph node metastases.

References

1 Endoh H, Matsunaga K, Yoshikumi C, Kawai Y, Suzuki T and Nomoto K: Production of antiserum against antitumor protein-bound polysaccharide preparation, PSK (Krestin) and its pharmacological application. Int J Immunopharmacol *10*: 103-109, 1988.

2 Hirahara N, Fujioka M, Edamatsu T, Fujieda A, Sekine F, Wada T and Tanaka T: Protein-bound polysaccharide-K (PSK) induces apoptosis and inhibits proliferation of promyelomonocytic leukemia HL-60 cells. Anticancer Res *31*: 2733-2738, 2011.

3 Kato M, Hirose K, Hakozaki M, Ohno M, Saito Y, Izutani R, Noguchi J, Hori Y, Okumoto S, Kuroda D *et al*: Induction of gene expression for immunomodulating cytokines in peripheral blood mononuclear cells in response to orally administered PSK, an immunomodulating protein-bound polysaccharide. Cancer Immunol Immunother *40*: 152-156, 1995.

4 Zhang H, Morisaki T, Matsunaga H, Sato N, Uchiyama A, Hashizume K, Nagumo F, Tadano J and Katano M: Protein-bound polysaccharide PSK inhibits tumor invasiveness by down-regulation of TGF-beta1 and MMPs. Clin Exp Metastasis *18*: 343-352, 2000.

5 Zhang H, Morisaki T, Nakahara C, Matsunaga H, Sato N, Nagumo F, Tadano J and Katano M: PSK-mediated NF-kappaB inhibition augments docetaxel-induced apoptosis in human pancreatic cancer cells NOR-P1. Oncogene *22*: 2088-2096, 2003.

6 Ohwada S, Kawate S, Ikeya T, Yokomori T, Kusaba T, Roppongi T, Takahashi T, Nakamura S, Kawashima Y, Nakajima T and Morishita Y: Adjuvant therapy with protein-bound polysaccharide K and tegafur uracil in patients with stage II or III colorectal cancer: randomized, controlled trial. Dis Colon Rectum *46*: 1060-1068, 2003.

7 Nakazato H, Koike A, Saji S, Ogawa N and Sakamoto J: Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Study Group of Immunochemotherapy with PSK for Gastric Cancer. Lancet *343*: 1122-1126, 1994.

8 Oba K, Teramukai S, Kobayashi M, Matsui T, Kodera Y and Sakamoto J: Efficacy of adjuvant immunochemotherapy with polysaccharide K for patients with curative resections of gastric cancer. Cancer Immunol Immunother *56*: 905-911, 2007.

9 Kwon SJ: Evaluation of the 7th UICC TNM Staging System of Gastric Cancer. J Gastric Cancer *11*: 78-85, 2011.

10 Jang YJ, Park MS, Park SS, Kim JH, An H, Park SH, Kim SJ, Kim CS and Mok YJ: Surgeon subspecialty as a factor in improving long-term outcomes for gastric cancer: Twenty years of experience in Korea. Arch Surg *145*: 1091-1096, 2010.

11 Niimoto M, Hattori T, Tamada R, Sugimachi K, Inokuchi K and Ogawa N: Postoperative adjuvant immunochemotherapy with mitomycin C, futraful and PSK for gastric cancer. An analysis of data on 579 patients followed for five years. Jpn J Surg *18*: 681-686, 1988.

12 Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, Furukawa H, Nakajima T, Ohashi Y, Imamura H, Higashino M, Yamamura Y, Kurita A and Arai K: Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med *357*: 1810-1820, 2007.

13 Ueda Y, Fujimura T, Kinami S, Hirono Y, Yamaguchi A, Naitoh H, Tani T, Kaji M, Yamagishi H and Miwa K: A randomized

phase III trial of postoperative adjuvant therapy with S-1 alone *versus* S-1 plus PSK for stage II/IIIA gastric cancer: Hokuriku-Kinki Immunochemo-Therapy Study Group-Gastric Cancer (HKIT-GC). Jpn J Clin Oncol *36*: 519-522, 2006.

14 Nio Y, Tsubono M, Tseng CC, Morimoto H, Kawabata K, Masai Y, Shiraishi T, Imai S, Ohgaki K and Tobe T: Immunomodulation by orally administered protein-bound polysaccharide PSK in patients with gastrointestinal cancer. Biotherapy *4*: 117-128, 1992.

15 Sakamoto J, Morita S, Oba K, Matsui T, Kobayashi M, Nakazato H and Ohashi Y: Efficacy of adjuvant immunochemotherapy with polysaccharide K for patients with curatively resected colorectal cancer: a meta-analysis of centrally randomized controlled clinical trials. Cancer Immunol Immunother *55*: 404-411, 2006.

16 Sakai T, Yamashita Y, Maekawa T, Mikami K, Hoshino S and Shirakusa T: Immunochemotherapy with PSK and fluoropyrimidines improves long-term prognosis for curatively resected colorectal cancer. Cancer Biother Radiopharm *23*: 461-467, 2008.

17 Asai H, Iijima H, Matsunaga K, Oguchi Y, Katsuno H and Maeda K: Protein-bound polysaccharide K augments IL-2 production from murine mesenteric lymph node CD4+ T cells by modulating T cell receptor signaling. Cancer Immunol Immunother *57*: 1647-1655, 2008.

18 Yamaguchi Y, Minami K, Ohshita A, Kawabuchi Y, Noma K and Toge T: Enhancing effect of PS-K on IL-2-induced lymphocyte activation: possible involvement of antagonistic action against TGF-beta. Anticancer Res *24*: 639-647, 2004.

19 Iguchi C, Nio Y, Takeda H, Yamasawa K, Hirahara N, Toga T, Itakura M and Tamura K: Plant polysaccharide PSK: cytostatic effects on growth and invasion; modulating effect on the expression of HLA and adhesion molecules on human gastric and colonic tumor cell surface. Anticancer Res *21*: 1007-1013, 2001.

20 Hattori TS, Komatsu N, Shichijo S and Itoh K: Protein-bound polysaccharide K induced apoptosis of the human Burkitt lymphoma cell line, Namalwa. Biomed Pharmacother *58*: 226-230, 2004.

21 Kono K, Kawaguchi Y, Mizukami Y, Mimura K, Sugai H, Akaike H and Fujii H: Protein-bound polysaccharide K partially prevents apoptosis of circulating T cells induced by anti-cancer drug S-1 in patients with gastric cancer. Oncology *74*: 143-149, 2008.

22 Hoos A, Eggermont AM, Janetzki S, Hodi FS, Ibrahim R, Anderson A, Humphrey R, Blumenstein B, Old L and Wolchok J: Improved endpoints for cancer immunotherapy trials. J Natl Cancer Inst *102*: 1388-1397, 2010.

23 Bilusic M and Gulley JL: Endpoints, patient selection, and biomarkers in the design of clinical trials for cancer vaccines. Cancer Immunol Immunother *61*: 109-117, 2012.

24 Coburn NG: Lymph nodes and gastric cancer. J Surg Oncol *99*: 199-206, 2009.

25 Mansfield AS, Heikkila PS, Vaara AT, von Smitten KA, Vakkila JM and Leidenius MH: Simultaneous Foxp3 and IDO expression is associated with sentinel lymph node metastases in breast cancer. BMC Cancer *9*: 231, 2009.

26 Camus M, Tosolini M, Mlecnik B, Pages F, Kirilovsky A, Berger A, Costes A, Bindea G, Charoentong P, Bruneval P, Trajanoski Z, Fridman WH and Galon J: Coordination of intratumoral immune reaction and human colorectal cancer recurrence. Cancer Res *69*: 2685-2693, 2009.

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