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Preoperative CEA and PPD Values as Prognostic Factors for Immunochemotherapy Using PSK and 5-FU*

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Abstract. *Purpose: Immunochemotherapy using PSK, used as postoperative adjuvant chemotherapy for colorectal cancer in Japan, is a treatment that depends on the immunocompetence of the host. Therefore, we analyzed the data of Hokuriku district conducted by the CIP study group to compare the long-term survival for preoperative CEA level and PPD reaction. Patients and Methods: Between February 1991 and March 1993, 87 patients with primary colon cancer and macroscopic lymph node metastasis (macroscopic Dukes' C) underwent macroscopic curative resection. The patients were randomly allocated to receive 5-FU/PSK therapy or 5-FU alone. The 7-year disease-free survival (DFS), 7-year overall survival (OS) and 7-year cancer death survival (CDS) were compared using the preoperative CEA levels and PPD values. Results: In cases with preoperative CEA level ≥3.0 ng/mL, the 7-year DFS, 7-year OS and 7-year CDS were significantly better in the PSK group (85.7, 90.5, 90.5%) than in the control group (52.4, 52.4, 57.1%; p=0.019, 0.007, 0.014,). In cases with preoperative PPD level <19.0 mm, the 7-year DFS, 7-year OS and 7-year CDS were significantly better in the PSK group (85.7, 85.7, 89.3%) than in the control group (56.7, 60.0, 63.3%; p=0.018, 0.036, 0.028). Recurrence was significantly less in the PSK group. The DFS tended to be superior in the PSK group (87.4%) compared to the control group (69.9%) for hematogenous metastasis. Conclusion: The present study demonstrated that preoperative CEA and PPD, that can be measured easily in the clinical setting, may be effective indicators of postoperative adjuvant immunochemotherapy using PSK.*

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In the early nineties, the 5-fluorouracil (5-FU)/levamisole combination was considered to be the standard postoperative adjuvant chemotherapy for colon cancer (1). With the subsequent development of leucovorin (LV), 5-FU/LV became globally accepted as the standard therapy. According to the review of postoperative adjuvant therapy for colon cancer reported by Chau *et al.* at the 38th ASCO Meeting in 2002, the 3- to 5-year disease-free survival (DFS) with 5-FU/LV therapy was 59-74%, and the 3- to 5-year overall survival (OS) was 65-83% (2).

In Japan, immunochemotherapy using PSK in combination with fluoropyrimidines is used widely as postoperative adjuvant chemotherapy for colorectal cancer. PSK is a protein-bound polysaccharide isolated from the mycelium of *Coriolus versicolor* and has a putative mean molecular weight of 100 kD. PSK has been confirmed to be effective for gastric cancer (3, 4), colorectal cancer (5, 6, 7) and small cell lung cancer (8), and is being used widely in Japan as a treatment for these cancer groups.

Recently, Ito *et al.* reported the final results of a randomized clinical trial on alternate PSK and 5-FU therapy for Dukes' C colon cancer (the CIP study, upon which the present study is based), with a 7-year cancer death survival (CDS) of 83.4% (7). In addition, Ohwada *et al.* reported the results of a randomized controlled trial of PSK and

tegafur/uracil (UFT) combination therapy for stages II and III colorectal cancer, with 5-year DFS of 73.0% and 5-year OS of 81.8% (5). These results suggest that 5-FU/PSK or UFT/PSK combination therapy achieve survival rates comparable to that of 5-FU/LV. However, immunochemotherapy is a treatment that depends on the immunocompetence of the host. An indicator that accurately identifies patients who will respond to PSK is needed.

In the randomized controlled trial conducted by the CIP study group in the Chubu and Hokuriku districts in Japan, randomization was conducted independently in the districts. In particular, since the surgical techniques are more uniform in the Hokuriku district, the data from this district were used to compare the 7-year DFS, 7-year OS and 7-year CDS for preoperative carcinoembryonic antigen (CEA) level and purified protein derivative (PPD) reaction, two parameters that can be easily measured in the clinical setting. This study attempted to verify whether preoperative CEA and PPD are effective markers for the indication of postoperative adjuvant therapy using PSK and fluoropyrimidines.

Patients and Methods

Study design. This study was conducted by the CIP study group, and the overall results together with a detailed description of the study design have already been reported by Ito *et al.* (7).

Patients who had received at least three sessions of 5-FU induction therapy were centrally registered and randomly allocated to either the PSK group (n=43) or the control group (n=44).

The postoperative treatment regimen consisted initially of 48-h intravenous infusion of 5-FU (1,000 mg/m^2/24 h x 2) once a week for 3 to 4 weeks. From week 4, PSK (300 mg/day) was given for 4 weeks followed by 5-FU (200 mg/day) for 4 weeks to the PSK group, and this constituted one course of the alternate treatment. Ten courses of the alternate administration were conducted. The control group received 10 courses of intermittent treatment with 5-FU, and received no treatment during the PSK administration period.

Follow-up was conducted monthly up to 18 months after surgery, and then every 3 to 6 months up to 7 years. Recurrence was examined during follow-up.

Setting cut-off values for preoperative CEA level and preoperative PPD value. The preoperative CEA levels ranging from 2.0 to 15.0 ng/mL were assigned cut-off values at 1 mg/mL increments. Using a stratified log-rank test, the 7-year DFS of the control group and the PSK group were compared by stratifying the cases according to each cut-off value (<cut-off and ≥cut-off). The CEA level showing the greatest difference (smallest *p* value) between the control and PSK groups in cases with CEA levels above cut-off was selected as the cut-off value for prognosis analysis.

The preoperative PPD values, ranging from 6.0 to 20.0 mm, were assigned cut-off values at 1.0 mm increments. Using a stratified log-rank test, the 7-year DFS of the control group and the PSK group were compared by stratifying the cases according to each cut-off value (<cut-off and ≥cut-off). The PPD value giving the greatest difference (smallest *p* value) between the control and PSK groups in cases with PPD values below cut-off was selected as the cut-off value for prognosis analysis.

Statistical analysis. The eligibility of patients was determined by the intention-to-treat principle. Patient background was compared with χ^2 test, Mann-Whitney *U*-test and *t*-test. Survival curves (DFS, OS and CDS) were constructed by the Kaplan-Meier method, and intergroup comparisons were conducted by an unadjusted log-rank test. The cut-off values for preoperative CEA level and PPD value were determined by a stratified log-rank test adjusted for Dukes' stage. The prognostic factors affecting DFS were evaluated by multivariate analysis using the Cox proportional hazards model. All statistical analyses were performed using the Statistical Analysis System software (SAS version8.2; Cary, NC, USA). Two-tailed *p* values less than 0.05 were considered to be significantly different.

Results

Patient background. Eighty-seven cases registered at the CIP study group in the Hokuriku district were randomized into 44 cases in the control group and 43 cases in the PSK group. One case in the control group and 2 cases in the PSK group were ineligible. The ineligible case in the control group had multiple cancers, and the 2 cases in the PSK group had non-curative resections. Finally, 43 cases in the control group and 41 cases in the PSK group were eligible. Table I shows the background of the eligible cases. The 2 groups did not differ significantly in the background factors.

Survival rates of eligible cases. Figure 1 shows the 7-year DFS and 7-year OS of the eligible cases. The 7-year DFS was significantly superior ($p=0.046$) in the PSK group (80.5%) compared to the control group (60.5%), and the hazard ratio of the PSK group was 0.450 (Figure 1a). The 7-year OS was also significantly better ($p=0.035$) in the PSK group (85.4%) than in the control group (65.1%), and the hazard ratio of the PSK group was 0.399 (Figure 1b). Similarly, the 7-year CDS was significantly superior ($p=0.029$) in the PSK group (87.7%) compared to the control group (67.4%), and the hazard ratio of the PSK group was 0.367 (data not shown).

Multivariate analysis of the 7-year DFS was performed using the Cox proportional hazards model for the variables of gender, age, performance status, tumor site, curability, preoperative PPD value, preoperative CEA level, histological type, depth of invasion, lymph node metastasis, and treatment arm. The hazard ratios were significantly high for invasion to *s* and *si* (5.249; $p=0.004$), histological lymph node metastasis (10.026; $p=0.001$), and control arm (5.990; $p=0.005$).

Prognosis analysis by preoperative CEA level. Table II shows the results of comparison of the cut-off values of preoperative CEA level for the range of 2.0 to 15.0 ng/mL. At the cut-off value of 3.0 ng/mL, the *p* value was smallest for the cases above cut-off. Therefore, subsequent analysis was performed using the cut-off values of 3.0 ng/mL for preoperative CEA levels.

Table I. *Patients' background of the eligible cases.*

Sex	Male	24 (55.8)	24 (58.5)	0.97 (χ^2)
	Female	19 (44.2)	17 (41.5)	
Age				
(years)	-49	4 (9.3)	7 (17.1)	0.36 (U)
	50-59	12 (27.9)	12 (29.3)	
	60-69	21 (48.8)	17 (41.5)	
	70-75	6 (14.0)	5 (12.2)	
	mean	61.2	59.5	0.36 (t)
Performance status	0	40 (93.0)	38 (92.7)	1.00 (χ^2)
(PS)	1	3 (7.0)	3 (7.3)	
Tumor location	cecum	5 (11.6)	8 (19.5)	0.19 (χ^2)
	ascending colon	8 (18.6)	6 (14.6)	
	transverse colon	11 (25.6)	5 (12.2)	
	descending colon	0 (0.0)	3 (7.3)	
	sigmoid	19 (44.2)	19 (46.3)	
Curability	absolute	38 (88.4)	40 (97.6)	0.23 (χ^2)
	relative	5 (11.6)	1 (2.4)	
PPD	≥10 mm	24 (55.8)	23 (56.1)	1.00 (χ^2)
(balancing factor)	<10 mm	19 (44.2)	18 (43.9)	
CEA	≥5.0 ng/dL	12 (27.9)	10 (24.4)	0.91 (χ^2)
(balancing factor)	<5.0 ng/dL	31 (72.1)	31 (75.6)	
Histological typing	well	25 (58.1)	18 (43.9)	0.41 (χ^2)
	mod	16 (37.2)	21 (51.2)	
	others	2 (4.7)	2 (4.9)	
Depth of invasion	sm	1 (2.3)	2 (4.9)	0.48 (U)
	mp	7 (16.3)	5 (12.2)	
	ss	27 (62.8)	23 (56.1)	
	se	8 (18.6)	10 (24.4)	
	si	0 (0.0)	1 (2.4)	
Lymph node metastases	n0	21 (48.8)	21 (51.2)	0.71 (U)
(balancing factor)	n1	13 (30.2)	13 (31.7)	
	n2	5 (11.6)	5 (12.2)	
	n3	4 (9.3)	2 (4.9)	
Dukes' stage	A	6 (14.0)	3 (7.3)	0.92 (U)
(histological)	B	15 (34.9)	18 (43.9)	
	C	22 (51.2)	20 (48.8)	

well: well-differentiated adenocarcinoma
mod: moderately-differentiated adenocarcinoma

Figure 2 shows the 7-year DFS and 7-year OS for cases having CEA levels below (<3.0 ng/mL) and above (≥3.0 ng/mL) the cut-off value. The 7-year DFS was significantly better ($p=0.019$) in the PSK group (85.7%) than in the control group (52.4%) and the hazard ratio of the PSK group was 0.269 in cases with preoperative CEA level ≥3.0 ng/mL (Figure 2b), whereas no significant differences in 7-year DFS (control group: 68.2%, PSK group: 75.0%; $p=0.644$) were observed between the two groups in cases with preoperative CEA level <3.0 ng/mL (Figure 2a). The 7-year OS was significantly better ($p=0.007$) in the PSK group (90.5%) than in the control group (52.4%) and the hazard ratio of the PSK group was 0.206 in cases with preoperative CEA level ≥3.0 ng/mL (Figure 2d), whereas no significant differences in 7-year OS (control group: 77.3%, PSK group: 80.0%; $p=0.890$) were observed between the two groups in cases with preoperative CEA level <3.0 ng/mL (Figure 2c). The 7-year CDS was also significantly better

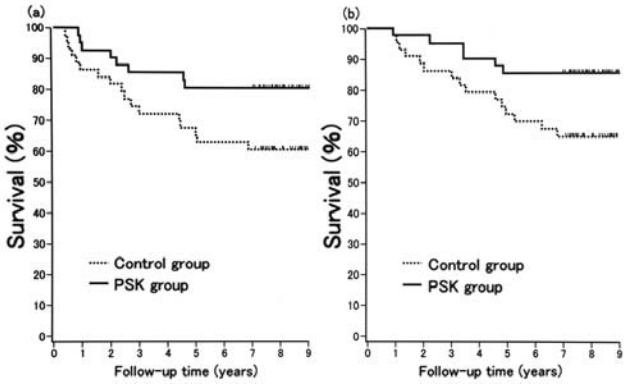

Figure 1. *The 7-year disease-free survival (DFS) (a) and 7-year overall survival (OS) (b) of the eligible cases. The dotted line represents the control group and the solid line represents the PSK group.*

(p=0.014) in the PSK group (90.5%) than in the control group (57.1%) and the hazard ratio of the PSK group was 0.224 in cases with preoperative CEA level ≥3.0 ng/mL, whereas no significant differences in 7-year CDS (control group: 77.3%, PSK group: 85.0%; p=0.596) were observed between the two groups (data not shown). In both groups with preoperative CEA level <3.0 ng/mL and ≥3.0 ng/mL, no significant differences in the background factors were observed between the control group and the PSK group.

Prognosis analysis by preoperative PPD value. Table III shows the results of comparison of the cut-off values of preoperative PPD value for the range of 6.0 to 20.0 mm. At the cut-off value of 19 mm, the p value was smallest for cases below cut-off. Therefore subsequent analysis was performed using the cut-off value of 19 mm.

Figure 3 shows the 7-year DFS and 7-year OS for cases having PPD values below (<19.0 mm) and above (≥19.0 mm) the cut-off value. The 7-year DFS was significantly better (p=0.018) in the PSK group (85.7%) than in the control group (56.7%) and the hazard ratio of the PSK group was 0.317 in cases with preoperative PPD level <19.0 mm (Figure 3a), whereas no significant differences were observed in the 7-year DFS (control group: 69.2%, PSK group: 69.2%; p=0.897) between the two groups in cases with preoperative PPD level ≥19.0 mm (Figure 3b). The 7-year OS was significantly better (p=0.036) in the PSK group (85.7%) than in the control group (60.0%) and the hazard ratio of the PSK group was 0.350 in cases with preoperative PPD level <19.0 mm (Figure 3c), whereas no significant differences were observed in the 7-year OS (control group: 76.9%, PSK group: 84.6%; p=0.573) between the two groups in cases with preoperative PPD level ≥19.0 mm (Figure 3d). The 7-year CDS was also significantly better (p=0.028) in the PSK group (89.3%)

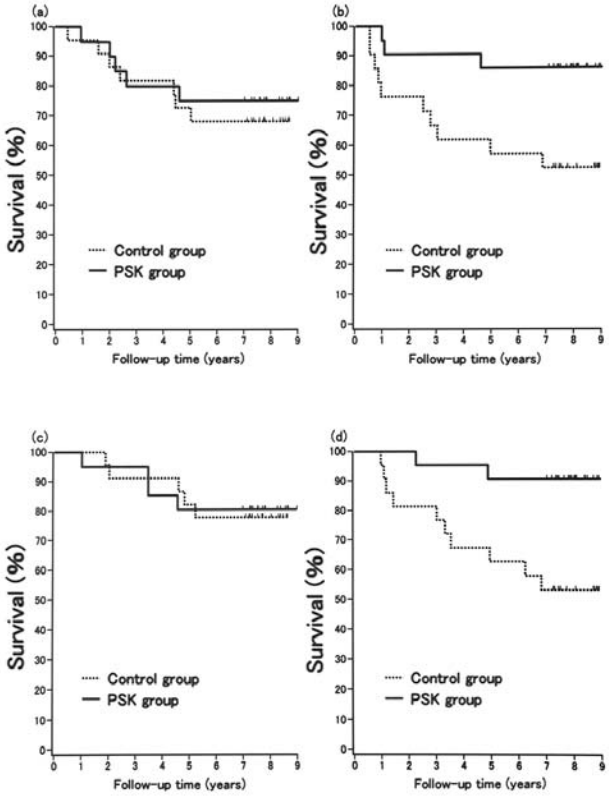

Figure 2 *The 7-year DFS and 7-year OS comparison between cases having CEA levels below (<3.0 ng/mL) and above (≥3.0 ng/mL) the cut-off value. (a) The 7-year DFS for cases having CEA levels <3.0 ng/mL); (b) the 7-year DFS for cases having CEA levels ≥3.0 ng/mL; (c) the 7-year OS for cases having CEA levels <3.0 ng/mL; (d) the 7-year OS for cases having CEA levels ≥3.0 ng/mL. The dotted line represents the control group and the solid line represents the PSK group.*

than in the control group (63.3%) and the hazard ratio of the PSK group was 0.308 in cases with preoperative PPD level <19.0 mm, whereas no significant differences were observed in 7-year CDS (control group: 76.9%, PSK group: 84.6%; p=0.573) between the two groups (data not shown). In both groups, with preoperative PPD values <19.0 mm and ≥19.0 mm, no significant differences in the background factors were observed between the control group and the PSK group.

No significant differences in 7-year DFS, OS and CDS were observed between the control group and the PSK group in cases with both low CEA levels and high PPD reaction (control group: 71.4%, 85.7%, 85.7%, PSK group: 60.0%, 80.0%, 80.0%; p=0.774, 0.854, 0.854, respectively), although both the patient numbers were small (control group: n=7, PSK group: n=5). On the other hand, the 7-year DFS, OS and CDS were significantly better in the PSK group than in the control group in cases with high

Table II. *Comparison of the cut-off values of preoperative CEA level for the range of 2.0 to 15.0 ng/mL.*

Preoperative CEA (ng/mL)	< cut-off value			≥ cut-off value		
	Control group	PSK group	*p*-value	Control group	PSK group	*p*-value
2.0	12	13	0.732	31	28	0.032
3.0	22	20	0.934	21	21	0.007
4.0	25	29	0.208	18	12	0.108
5.0	31	31	0.165	12	10	0.075
6.0	32	31	0.119	11	10	0.135
7.0	32	31	0.119	11	10	0.135
8.0	34	34	0.063	9	7	0.293
9.0	34	36	0.099	9	5	0.249
10.0	34	36	0.099	9	5	0.249
11.0	34	36	0.099	9	5	0.249
12.0	34	37	0.072	9	4	0.372
13.0	34	37	0.072	9	4	0.372
14.0	35	38	0.154	8	3	0.159
15.0	35	39	0.118	8	2	0.281

Stratified log-rank test adjusted by Dukes stage

CEA levels and/or low PPD reaction (control group: 58.3%, 61.1%, 63.9%, PSK group: 83.3%, 86.1%, 88.8%; $p=0.021$, 0.019, 0.015, respectively).

Recurrence. Recurrence was found in 15 cases in the control group and 6 cases in the PSK group, being significantly fewer in the PSK group ($p=0.04$). By site of metastasis, liver metastasis was observed in 6 cases in the control group and 1 case in the PSK group, lung metastasis in 4 cases in the control group and 2 cases in the PSK group, lymph node metastasis in 1 case each in the control and PSK groups, peritoneum metastasis in 3 cases in the control group and 1 cases in the PSK group, and ovary metastasis in 1 case each in the control and PSK groups; with no significant differences between the two groups for all metastatic sites.

Liver metastasis and lung metastasis were grouped as hematogenous metastasis and others as non-hematogenous metastasis, and the 7-year DFS was compared (Figure 4). For non-hematogenous metastasis, the DFS was not significantly different ($p=0.446$) between the control group (80.4%) and the PSK group (87.1%) (Figure 4a). For hematogenous metastasis, the DFS tended to be superior ($p=0.066$) in the PSK group (87.4%) compared to the control group (69.9%), and the recurrence hazard ratio of the PSK group was 0.409 (Figure 4.b).

Discussion

In the early nineties, the 5-fluorouracil (5-FU)/levamisole combination was considered to be the standard postoperative adjuvant chemotherapy for colon cancer (1). With the subsequent development of leucovorin (LV), 5-FU/LV became globally accepted as the standard therapy. According to the review of postoperative adjuvant therapy for colon cancer reported by Chau *et al.* at the 38th ASCO Meeting in 2002, the 3- to 5-year disease-free survival (DFS) with 5-FU/LV therapy was 59-74%, and the 3- to 5-year overall survival (OS) was 65-83% (2).

In Japan, clinical studies have been conducted to examine the usefulness of immunochemotherapy in combination with PSK, a biological response modifier (BRM) developed in Japan, for the treatment of colorectal cancer. In the report of Mitomi *et al.,* who treated stage III colorectal cancer with 5-FU/PSK therapy, the 5-year DFS was 72.3% and 5-year OS was 78.5%, while the 5-year DFS for colon cancer alone was 80.1% (6). Ohwada *et al.* used UFT/PSK to treat stages II and III colorectal cancer and achieved 5-year DFS of 73.0% and 5-year OS of 81.8% (5). In the final report by the CIP study group on macroscopic Dukes' C colon cancer, upon which the present study is based, alternate 5-FU/PSK therapy yielded a 7-year DFS of 74.1%, a 7-year OS of 79.6%, and a 7-year CDS of 83.4% (7). Our results were 80.5%, 84.5% and 87.7%, respectively, and also reproduced the efficacy of 5-FU/PSK therapy for colon cancer.

However, PSK is a BMR that exhibits antitumor effects mainly through the immune functions of the cancer-bearing host. This implies that the beneficial effect of the therapy largely depends on the host factors. In the present study, we examined the factors that may potentially predict the prognosis of 5-FU/PSK therapy. The preoperative CEA level and preoperative PPD value were identified as strong candidates as prognostic factors. When the cut-off point of

Table III. *Comparison of the cut-off values of preoperative PPD value for the range of 6.0 to 20.0 mm.*

Preoperative PPD (mm)	< cut-off value Control group	PSK group	*p*-value	≥ cut-off value Control group	PSK group	*p*-value
6.0	16	14	0.063	27	27	0.262
7.0	16	15	0.050	27	26	0.262
8.0	18	17	0.054	25	24	0.350
9.0	19	18	0.074	24	23	0.259
10.0	20	18	0.087	23	23	0.259
11.0	22	19	0.053	21	22	0.401
12.0	23	20	0.035	20	21	0.517
13.0	23	21	0.064	20	20	0.365
14.0	25	24	0.046	18	17	0.385
15.0	26	25	0.029	17	16	0.550
16.0	27	26	0.019	16	15	0.705
17.0	27	27	0.013	16	14	0.903
18.0	30	28	0.010	13	13	0.991
19.0	30	28	0.010	13	13	0.991
20.0	31	29	0.030	12	12	0.563

Stratified log-rank test adjusted by Dukes stage

preoperative CEA level was set at 3.0 ng/mL, cases in the PSK group with CEA ≥3.0 ng/mL showed significantly more favorable 7-year DFS, OS and CDS than those in the control group. Furthermore, when the cut-off point of preoperative PPD value was set at 19.0 mm, cases in the PSK group with PPD <19.0 mm showed significantly superior 7-year DFS, OS and CDS to those in the control group.

The involvement of preoperative CEA level in immunochemotherapy using PSK has been reported by Munemoto *et al.* (9). In their study, patients who underwent curative resection for colorectal cancer were treated with fluoropyrimidine/PSK as adjuvant therapy, and the patients were stratified into two groups by the preoperative CEA level of 8.0 ng/mL. The 5-year OS in patients with CEA <8.0 ng/mL was 80.0% and was significantly lower than the rate of 37.5% in patients with CEA ≥8.0 ng/mL. However, these results were obtained by analysis within a single arm. In contrast, our present study was a comparison between two arms; PSK treatment and control treatment without PSK. Our results showed that when the preoperative CEA level is above 3.0 ng/mL, the survival advantage of PSK is promoted. Medoff *et al.* reported that an immunosuppressive factor existed in malignant ascites was released from human lymphocyte by CEA stimulation (10, 11). PSK has been shown clinically to suppress immunosuppressive substances that increase in the cancer-bearing state, such as immunosuppressive acidic protein (IAP) (12) and immunosuppressive substance (IS) (13). These observations may suggest that in patients with high CEA level, PSK relieves the CEA-induced immunosuppression and contributes to prolong survival. In patients with low CEA level, the contribution of relieving immunosuppression by PSK is relatively small and, therefore, the difference may not be observable in long-term results.

The PPD reaction is delayed-type hypersensitivity and is caused by various cytokines such as migration inhibition factor (MIF) secreted from T-cells activated by PPD (14). The involvement of the PPD reaction has been reported by Tamada *et al.* in a histological study of curatively resected gastric cancer (15). In their study, a PPD reaction of 10 mm or above was rated as positive and a reaction less than 10 mm as negative. When 4-year OS was compared between chemotherapy (MMC+FT) and immunochemotherapy (MMC+FT+PSK), there was no significant difference between the two groups in PPD-negative cases, but the 4-year OS was significantly higher for immunochemotherapy using PSK (78.6%) compared with chemotherapy alone (70.3%) in PPD-positive cases, showing improvement in long-term results. These results show that the survival advantage of PSK may be expected in patients with preserved immune capacity to an extent that the PPD intradermal reaction can be elicited. Based on these results, we also adopted PPD intradermal reaction as a potential prognostic factor in the present study. Using the evaluation criteria of positivity and negativity based on a 10-mm reaction, we were not able to detect a difference between the control and PSK groups (Table III). No significant differences in 7-year DFS, OS and CDS were observed at the 10 mm PPD reaction within the control group (DFS: 60.0% *vs* 60.9%; $p=0.974$, OS: 65.0% *vs* 65.2%; $p=0.994$,

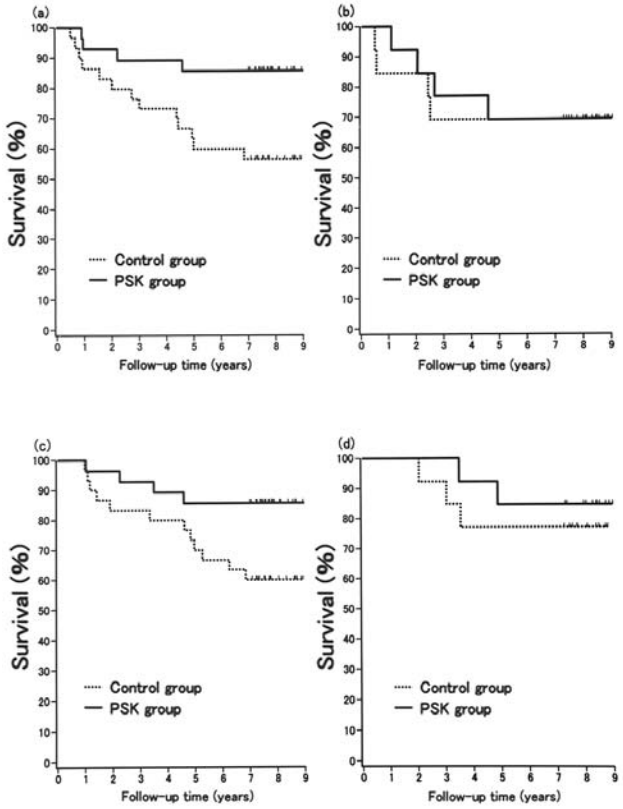

Figure 3. *The 7-year DFS and 7-year OS comparison between cases having PPD values below (<19.0 mm) and above (≥19.0 mm) the cut-off value. (a) The 7-year DFS for cases having PPD values <19.0 mm; (b) the 7-year DFS for cases having PPD values ≥19.0 mm; (c) the 7-year OS for cases having PPD values <19.0 mm; (d) the 7-year OS for cases having PPD values ≥19.0 mm. The dotted line represents the control group and the solid line represents the PSK group.*

CDS: 70.0% *vs* 65.2%; $p=0.776$, below 10 mm *vs* above 10 mm, respectively) or within the PSK group (DFS: 83.3% *vs* 78.3%; $p=0.681$, OS: 83.3% *vs* 87.0%; $p=0.723$, CDS: 88.9% *vs* 87.0%; $p=0.901$, below 10 mm *vs* above 10 mm, respectively). Although the reason why the cut-off value became 19 mm is uncertain, it is possible that the patients with preoperative PPD value of 19 mm or above have well preserved immune competence, such that the promotional effect of PSK may not be apparent.

Concerning metastasis, Mitomi *et al.* reported that PSK inhibited lymphatic metastasis (6), while our group and Ohwada *et al.* (5) observed inhibition of hematogenous metastasis, and Nakazato *et al.* also reported inhibition of hematogenous metastasis in gastric cancer (3). Therefore, PSK mainly inhibits hematogenous metastasis to suppress recurrence, and this mechanism may be relevant to the improvement of long-term results.

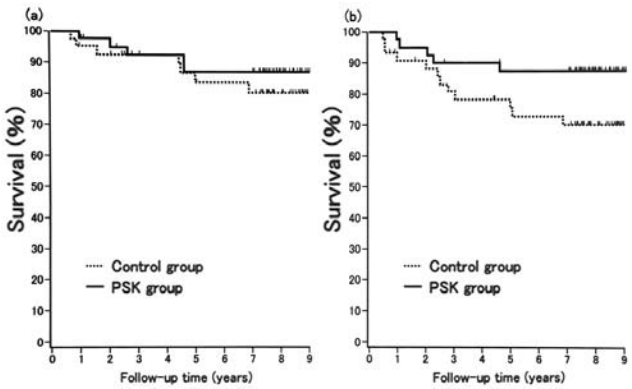

Figure 4. *Comparison of 7-year DFS between non-hematogenous metastasis (a) and hematogenous metastasis (b). The dotted line represents the control group and the solid line represents the PSK group.*

The present study demonstrated that the preoperative CEA level and preoperative PPD value, which can easily be measured in the clinical setting, may be effective indicators of postoperative adjuvant immunochemotherapy using PSK in combination with fluoropyrimidines. However, the present study was a sub-analysis of a randomized controlled study and, therefore, was of a small scale. A large-scale controlled clinical study is necessary to validate the usefulness of these factors

References

1 Moertel CG, Fleming TR, MacDonald JS, Haller DG, Laurie JA, Goodman PJ, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, Veeder MH and Mailliard JA: Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med *322*: 352-358, 1990.

2 Chau I and Cunningham D: Adjuvant chemotherapy in colon cancer: state of art. The 38th ASCO Meeting Educational Book 228-239, 2002.

3 Nakazato H, Koike A, Saji S, Ogawa N and Sakamoto J: Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Lancet *343*: 1122-1126, 1994.

4 Nakajima T, Inokuchi K, Hattori T, Inoue K, Taguchi T, Kondou T, Abe O, Kikuchi K, Tanabe T and Ogawa N: Multi-institutional cooperative study of adjuvant immunochemotherapy for gastric cancer: five-year survival rate. Jpn J Cancer Chemother *16*: 799-806, 1989.

5 Ohwada S, Ikeya T, Yokomori T, Kusaba T, Roppongi T, Takahashi T, Nakamura S, Kakinuma S, Iwazaki S, Ishikawa H, Kawate S, Nakajima T and Morishita Y: Adjuvant immunochemotherapy with oral tegafur/uracil plus PSK in patients with stage II or III colorectal cancer: a randomised controlled study. Br J Cancer *90*: 1003-1010, 2004.

6 Mitomi T, Tsuchiya S, Iilima N, Aso K, Nishiyama K, Amano T, Takahashi T, Oka H, Murayama N, Oya K, Noto T and Ogawa N: Randomized controlled study on adjuvant immunochemotherapy with PSK in curatively resected colorectal cancer: 5 year follow-up after surgery (a final report). J Jpn Soc Cancer Ther *28*: 71-83, 1993.

7 Ito K, Nakazato H, Koike A, Takagi H, Saji S, Baba S, Mai M, Sakamoto J and Ohashi Y: Int J Colorectal Dis *19*: 157-164, 2004.
8 Konno K, Motomiya M, Oizumi K, Sato M, Yamamoto F, Tamiya K, Hasuike T, Yokosaea A, Uchiyama T, Ogawa N and Nakai Y: Effects of protein-bound polysaccharide preparation (PSK) in small cell carcinoma of the lung. Haigan *28*: 19-28, 1988.
9 Munemoto Y, Iida Y, Abe J, Saito H, Fujisawa K, Kasahara Y, Mitsui T, Asada Y and Miura S: Significance of postoperative adjuvant immunochemotherapy after curative resection of colorectal cancers: association between host or tumor factors and survival. Int J Oncol *20*: 403-411, 2002.
10 Medoff JR, Jegasothy BV and Roche JK: Carcinoembryonic antigen-induced release of a suppressor factor from normal human lymphocytes *in vitro*. Cancer Res *44*: 5822-5827, 1984.
11 Medoff JR, Clack VC and Roche JK: Characterization of an immunosuppressive factor from malignant ascites that resembles a factor induced *in vitro* by carcinoembryonic antigen. J Immunol *137*: 2057-2064, 1986.
12 Nio Y, Tamura K, Masai Y, Hayashi H, Araya S, Imai S, Shiraishi T, Tseng CC, Kawabata K, Tsuboi K, Tsubono M, Sato M and Imamura M: Multi-institutional study on the immunomodulatory effect of protein-bound polysaccharide, PSK on the immunity of patients after gastric and colorectal cancer surgery. J Jpn Soc Cancer Ther *30*: 1623-1634, 1995.
13 Hayashibe A, Tanaka H, Kitoh H, Sakamoto K, Taruya E, Nakaue K, Yanagi Z, Tokura K, Asada K and Takebayashi J: Pre- and postoperative investigation on immunoactivity of the patients with gastric cancer: with a special reference to the effect of PSK. J Jpn Soc Cancer Ther *29*: 647-653, 1994.
14 Vyakarnam A and Lachmann PJ: Migration inhibition factor secreting human T-cell lines reactive to PPD: a study of their antigen specificity, MHC restriction and the use of Epstein-Barr virus-transformed B-cell lines as requirement for antigen-presenting cells. Immunol *53*: 601-610, 1984.
15 Tamada R, Inokuchi K, Hattori T, Inoue K, Taguchi T, Kondo T, Abe O, Kikuchi K, Tanabe T, Nakajima T and Ogawa N: A multi-institutional study on postoperative adjuvant immunochemotherapy of gastric cancer (II). Gan To Kagaku Ryoho *14*: 716-722, 1987.

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