Original Article

Diagnostic and Prognostic Impact of Serum p53 Antibody Titration in Colorectal Cancer

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ABSTRACT

Background: Although s-p53-Abs titers of cancer patients are distributed in wide range, the diagnostic and prognostic significance of preoperative and perioperative s-p53-Abs titers have not been evaluated in detail.

Methods: Preoperative and postoperative s-p53-Abs titers were assayed in 527 consecutive colorectal cancer patients surgically treated at Toho University Hospital between January 2010 and December 2014, and their associations with prognostic and clinicopathological variables were evaluated. To evaluate the clinical impact of s-p53-Abs titers, seropositive patients were divided into four groups by antibody titer as follows: 1.3 − 10 U/mL (very low); 10.1 − 50 U/mL (low); 50.1 − 200 U/mL (medium-high); and ＞200 U/mL (extremely high). The impact of perioperative change in the titer on survival was also evaluated.

Results: Among 527 patients, 155 (29.4%) were positive for s-p53-Abs. The positive rate of combination with CEA, CA19-9, and s-p53-Abs was significantly higher than the combination with CEA and CA19-9. Tumor depth, lymphatic invasion, and CA19-9 were significantly associated with s-p53-Abs status. Although the overall prognostic value of s-p53-Abs was not significant, the subgroup analysis found that extremely high titers were not associated with recurrence or poor survival. Tumor recurrences were more likely to occur in patients with medium-high titers. Although the medium-high-titer group showed poor survival, the extremely high-titer group showed better survival than the other groups.

Conclusions: Combination assay with CEA, CA19-9, and s-p53-Abs was useful to increase positive rates to detect colorectal cancers. Although s-p53-Abs seropositivity itself was not independently associated with survival, high titers of s-p53-Abs had a paradoxical impact on tumor recurrence and patient survival.

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KEYWORDS: serum p53 antibody, antibody titer, colon cancer, surgery, monitoring

Introduction

p53 gene mutations are common in colorectal cancer and can be detected even in stage I patients. Most p53 mutations result in the synthesis of a stable protein with a much longer half-life than that of the wild-type p53 protein. Overexpression of mutant p53 protein is associated with serum p53 IgG antibody (s-p53-Abs) production in 18.2% − 40% of patients with colorectal cancer.[1−5)] We previously reported the case of a patient with rectal cancer in whom

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the s-p53-Abs titer was useful to predict tumor recurrence, possibly because the antibodies were a response to residual cancer cells.[6] Although the s-p53-Abs seropositivity before treatment has been shown to correlate with lymph-node metastasis in colorectal cancer,[2,3] the prognostic impact of s-p53-Abs is controversial.[3,4] Moreover, the significance of specific titer ranges and perioperative titer changes have not been well evaluated.[5,7]

The study objective was to determine the diagnostic and prognostic impact of s-p53-Abs titer ranges and perioperative titer changes in patients with colorectal cancer.

Patients and methods

Patients

A total of 527 consecutive patients with primary colorectal cancer were enrolled and included 319 men (61%) and 208 women (39%) with a median age of 69 (range, 34 − 92) years. They were surgically treated at Toho University Hospital between January 2010 and December 2014, followed by the end of 2015 for recurrence. Patients with neoadjuvant chemotherapy or chemoradiotherapy were excluded. TNM classification[8] was established by pathological evaluation of resected specimens. Fourteen of the 527 patients had stage 0, 118 patients had stage I, 161 had stage II, 154 had stage III, and 80 had stage IV. All patients with stage 0 − III disease were considered curable by resection of the primary tumor with D2 or more extended lymphadenectomy. All patients with stage IV disease had liver or lung metastases. Serum samples were obtained preoperatively and one month postoperatively, and they were stored at −80℃ until assayed. Patient recruitment was performed following the protocol guidelines of the institutional review board (#26-256). Informed consent was obtained from all patients. After surgery, all patients had routine follow-ups for postoperative clinical examination and imaging studies until the end of December 2015 or their death. Postoperative recurrence was defined as positive findings on successive bimonthly clinical examinations or annual computed tomography scans. Recurrent disease was treated with previously described standard protocols.[9]

Assay of serum p53 antibodies and conventional tumor markers

s-p53-Abs was assayed with a highly specific, quantitative enzyme-linked immunosorbent assay (ELISA) kit (MESACUP anti-p53 test; Medical & Biological Laboratories, Nagoya, Japan).[10] Briefly, samples were added to the wells of microtiter plates coated with either wild-type human p53 or control protein and incubated for 1 h. Then, peroxidase-conjugated goat anti-human immunoglobulin G binding anti-p53 antibody was added; substrate solution was added after 1 h of incubation, and stop solution was added after a further 30 min. Absorption at 450 nm was read immediately using a spectrophotometer. A calibration curve was constructed from standards containing specific amounts of antibody, and s-p53-Abs was determined from the calibration curve; the cutoff value was set at 1.3 U/mL. Using this cutoff value, the false positive rate of healthy donor was less than 5%.[11] We divided positive serum titers into four ranges, which were 1.3 − 10 U/mL (very low), 10.1 − 50 U/mL (low), 50.1 − 200 U/mL (medium high), and >200 U/mL (extremely high).

Carcinoembryonic antigen (CEA) levels were measured with a CEA-2 enzyme immune assay (EIA) kit (Elecsys CEAII; Roche Diagnostics K.K., Tokyo, Japan) following the manufacturer's instructions with a cutoff value of 5.0 ng/mL. Cancer antigen 19-9 (CA19-9) levels were measured with a CA19-9 EIA kit (Elecsys CA19-9; Roche Diagnostics K.K., Tokyo, Japan) with a cutoff value of 37 U/mL.

Statistical analysis

Data were expressed as means ± standard deviation. Comparisons of paired groups were made with the Wilcoxon signed rank test. Survival probabilities beginning at the time of surgery were calculated by the Kaplan − Meier product limit method, and between-group differences were tested with the log-rank test. Clinicopathological variables associated with overall survival were evaluated by univariate analysis and followed by multivariate analysis using Cox proportional hazards models. Statistical analyses were performed using EZR statistical software.[12] Two-sided *P*-values of <0.05 were considered statistically significant.

Results

Clinicopathological significance of s-p53-Abs

Before surgery, 155 of 527 patients (29.4%) were positive for s-p53-Abs; 126 (23.9%) were positive after surgery. The 10 clinicopathological variables, including CEA and CA19-9, that were evaluated for their impact on s-p53-Abs positivity are shown in Table 1. Depth of tumor, lymphatic invasion, and CA19-9 were significantly associated with of s-p53-Abs seropositivity. Tumor histology and CEA were not significantly associated with s-p53-Abs seropositivity.

Table 1 Clinicopathological characteristics of patients with colorectal cancer

Variables		s-p53-Ab				p value *
		Number of patients	Number of seropositive patients	Number of seronegative patients	%	
		527	155	372	29.4	
Stage	0+I	132	29	103	22.0	
	II	161	49	112	30.4	
	III	154	55	99	35.7	
	IV	80	22	58	27.5	
Gender	Male	319	99	152	31	0.331
	Female	208	56	152	26.9	
Age	<65	177	59	118	33.3	0.160
	≥65	350	96	254	27.4	
Tumor location	Colon	347	95	252	27.4	0.155
	Rectum	180	60	120	33.3	
Differentiation	Well or mod	500	150	350	30.0	0.091
	por, sig, or muc	27	4	23	14.8	
Depth of tumor	Tis, T1, T2	152	34	118	22.4	0.024
	T3, T4	375	121	254	32.3	
Lymph-node metastasis	Positive	207	69	138	33.3	0.112
	Negative	320	86	234	26.9	
Lymphatic invasion	Positive	379	124	255	32.7	0.008
	Negative	148	31	117	20.9	
Vascular invasion	Positive	335	106	229	31.6	0.138
	Negative	192	49	143	25.5	
CEA	Positive	232	78	154	33.6	0.060
	Negative	295	77	218	26.1	
CA19-9	Positive	71	15	56	21.1	0.010
	Negative	456	140	316	30.7	

*chi-squared test

The s-p53-Abs seropositive rates at each TNM stage were as follows: 29 of 132 patients with stage 0/I (22.0%), 49 of 161 patients with stage II (30.4%), 55 of 154 patients with stage III (35.7%), and 22 of 80 patients with stage IV (27.5%) (Fig. 1). CEA was positive in 44.0% patients and CA19-9 was positive in 13.5%. The s-p53-Abs seropositivity rate was lower than the CEA-positivity rate, and was higher than that of CA19-9 at each TNM stage (Fig. 1). For stage I, stage II, and stage III disease, the proportions of patients positive for any of these three serum markers were significantly higher than the proportions positive for CEA and/or CA19-9 (Fig. 2).

Comparison of preoperative and postoperative s-p53-Ab titers

We found that s-p53-Abs titers had decreased after surgery in 133 of the 155 seropositive patients (86%) and increased in 22 (14%). Seropositive rates after surgery were 16.7% (stage 0/I), 23.6% (stage II), 29.2% (stage III), and 26.3% (stage IV). Overall, the postoperative s-p53-Abs level was lower than the preoperative level (57.17±506 U/mL vs 19.27±131 U/mL, $P = 0.019$). Titers at each TNM stage also decreased after surgery (Fig. 3), but the differences in before and after titers were not significant.

Prognostic significance of each range of s-p53-Abs titers for survival

Median follow-up of survivors was >30 months. A total of 457 (86.7%) patients were alive, and 70 (13.3%) patients had died by the end of December 2015. In the multivariate analysis, tumor depth and presence of distant metastasis were significantly associated with survival, but s-p53-Abs was not found to be a significant prognostic factor (Table 1). There was a trend toward worse survival in preoperative s-p53-Abs seropositive patients compared with seronegative patients, but the difference did not reach significance (Fig. 4). Antibody titers in preoperative serum samples varied very widely from 1.31 U/mL to 1,020 U/mL.

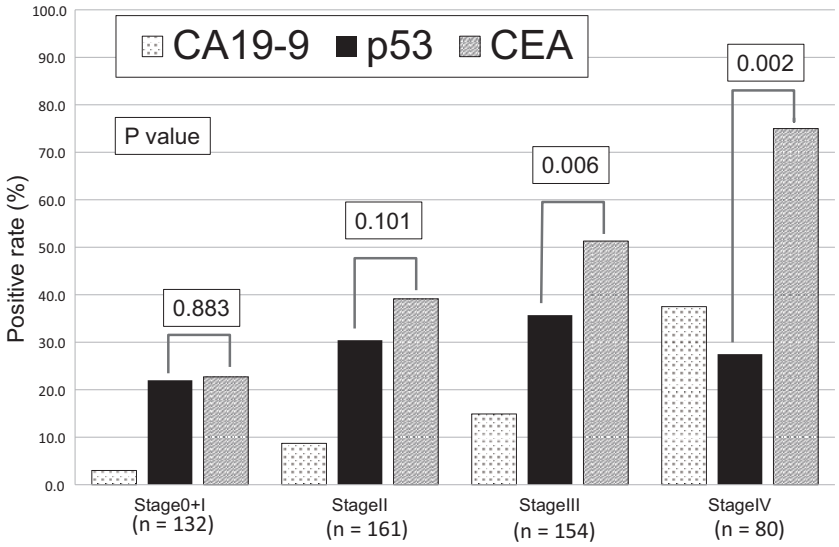

Fig. 1 Positive rates of CEA, CA19-9, and s-p53-Ab at each tumor stage.

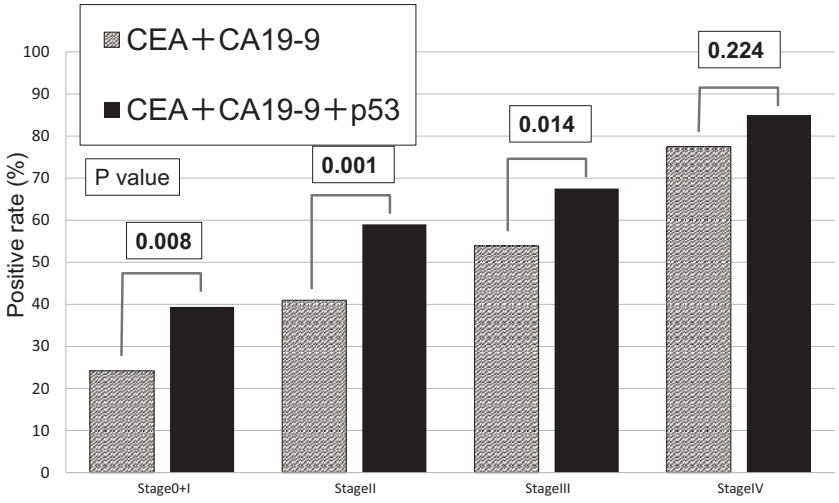

Fig. 2 Combined positive rates of CEA＋CA19-9 and CEA＋CA19-9＋s-p53-Ab at each tumor stage.

To analyze the prognostic significance of s-p53-Abs in stage II and stage III seropositive patients, we divided the serum titers into four ranges, which were 1.3 − 10 U/mL, 10.1 − 50 U/mL, 50.1 − 200 U/mL (medium high), and ＞200 U/mL (extremely high) (Fig. 5). Interestingly, patients with extremely high titers, ＞200 U/mL, had relatively better survival than patients in the other groups. Patients with titers between 50 and 200 U/mL had the worst survival among seropositive patients. Although the difference in survival of these two groups was not significant, the extremely high group showed better prognosis than medium high group. The perioperative changes of serum s-p53-Abs levels in patients with medium-high and extremely high serum titers are shown in Fig. 6. The titers of all patients in both groups were significantly lower after surgery than before surgery. Six of the 16 patients with medium-high titers developed recurrent disease; only two of the 13 patients with extremely high titers developed recurrent disease ($P = 0.07$).

In a representative patient with tumor recurrence, (Fig. 7), data from four years of follow-up showed that s-p53-Abs levels precisely reflected tumor recurrence even though neither CEA nor CA19-9 had been negative. After surgery, s-p53-Abs level initially decreased from 141 U/mL to 33.2 U/mL, but the serum titer remained positive throughout the postoperative course. From the moment that lung

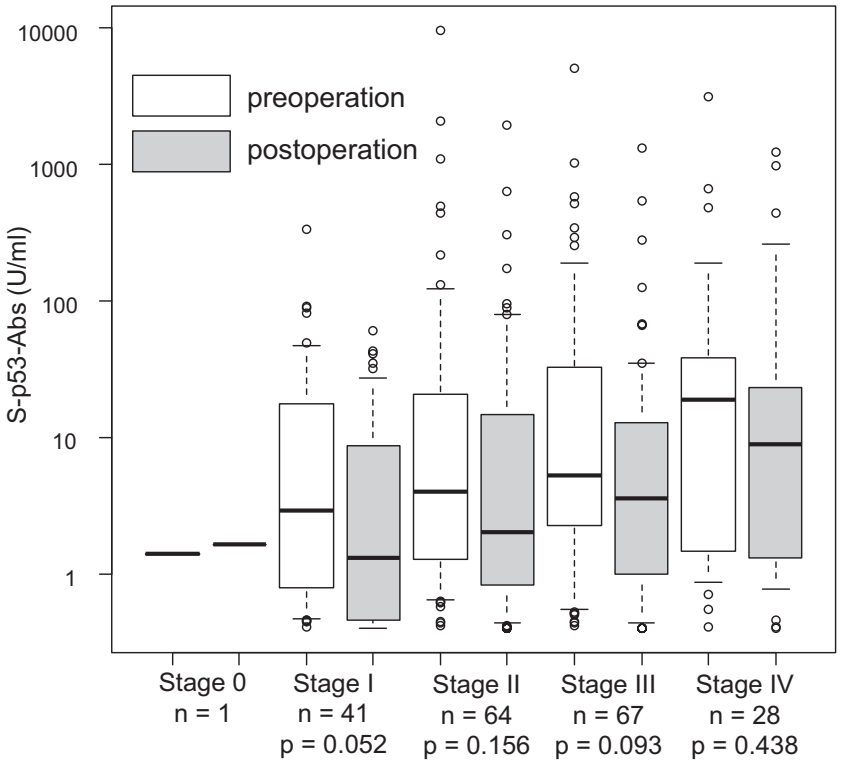

Fig. 3 Comparison of preoperative and postoperative s-p53-Ab titers.

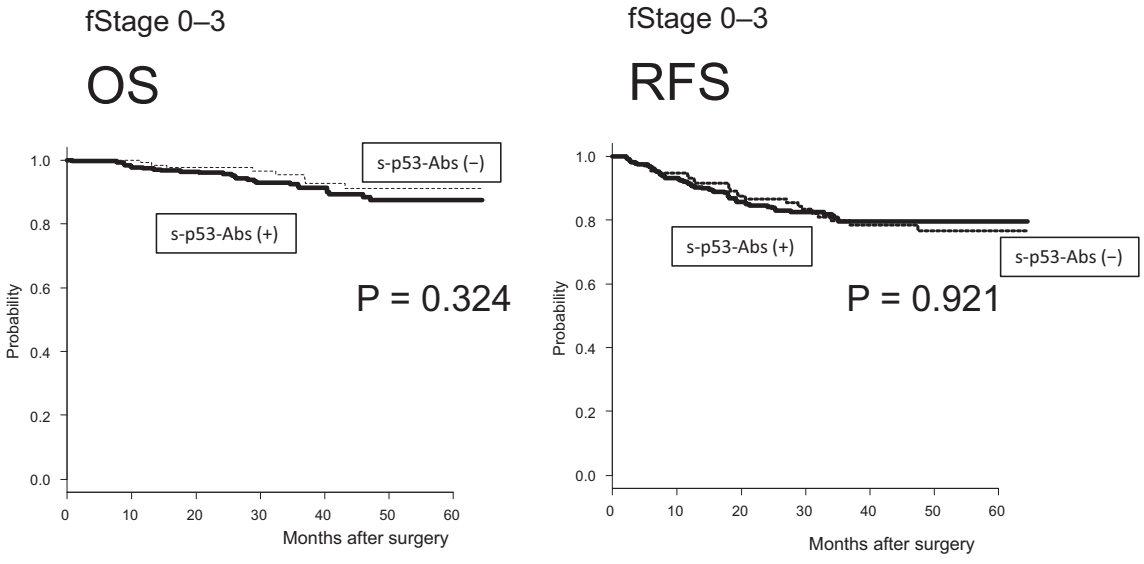

Fig. 4 Overall and relapse free survival after curative resection for colorectal cancer according to preoperative status of s-p53-Ab.

metastases were detected, the titer was seen to increase to 147 U/mL. During second-line chemotherapy with CapeOX+bavacizmab, the titer gradually decreased over a period of 12 months. At that time, lung and liver metastases began to develop rapidly, and the titer increased to extremely high levels of >400 U/mL. Both CEA and CA 19-9 increased only during the last phase of the patient's life.

Discussion

Tumor markers have diagnostic and prognostic value, and are used to monitor treatment and detect recurrence.

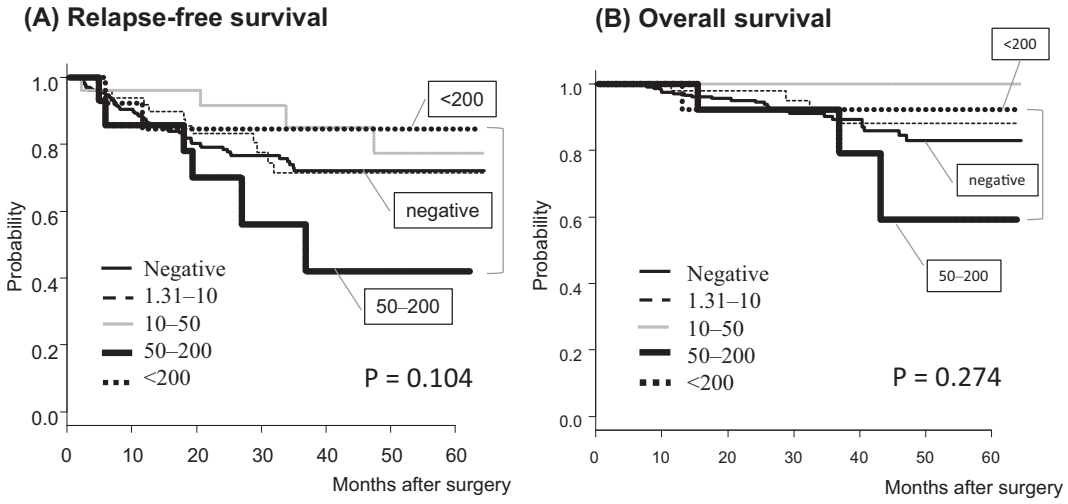

Fig. 5 Survival curves of stage II and stage III patients by s-p53-Ab titers range.

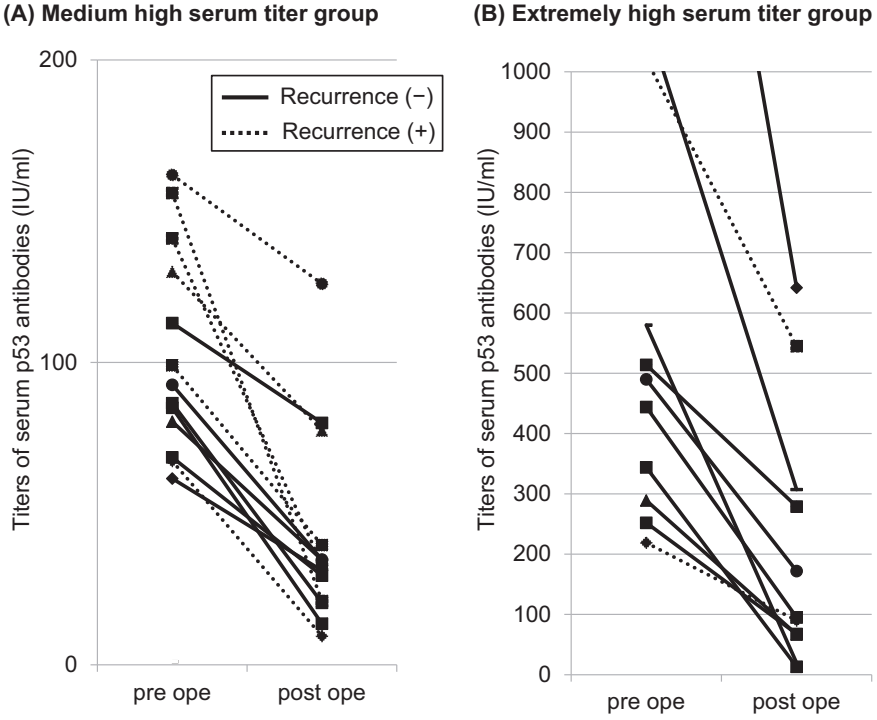

Fig. 6 Change in s-p53-Ab titers in patients with high and extremely high titers. Solid lines indicate patients without tumor recurrence. Dotted lines indicated the patients with tumor recurrence.

In this study, the s-p53-Abs seropositivity rate in colorectal cancer patients was 29.4% overall and 22% for stage 0 or I. The clinical significance of s-p53-Ab for detecting early cancers has been confirmed in previous reports,[1–5] and because the main purpose of this study was to clarify the impact of s-p53-Abs titer on prognosis, we focused on stage II or stage III patients. The overall prognostic value of s-p53-Abs was not significant in this patient series, and a subgroup analysis found that "extremely high titers" >200 U/mL were not associated with recurrence or poor survival. Tumor recurrences were more likely to occur in patients with medium-high titers of 50.1 − 200 U/mL. Tang et al. also reported similar subgroup analyses,[13] but they used a p53-autoantibody ELISA kit (Calbiochem-

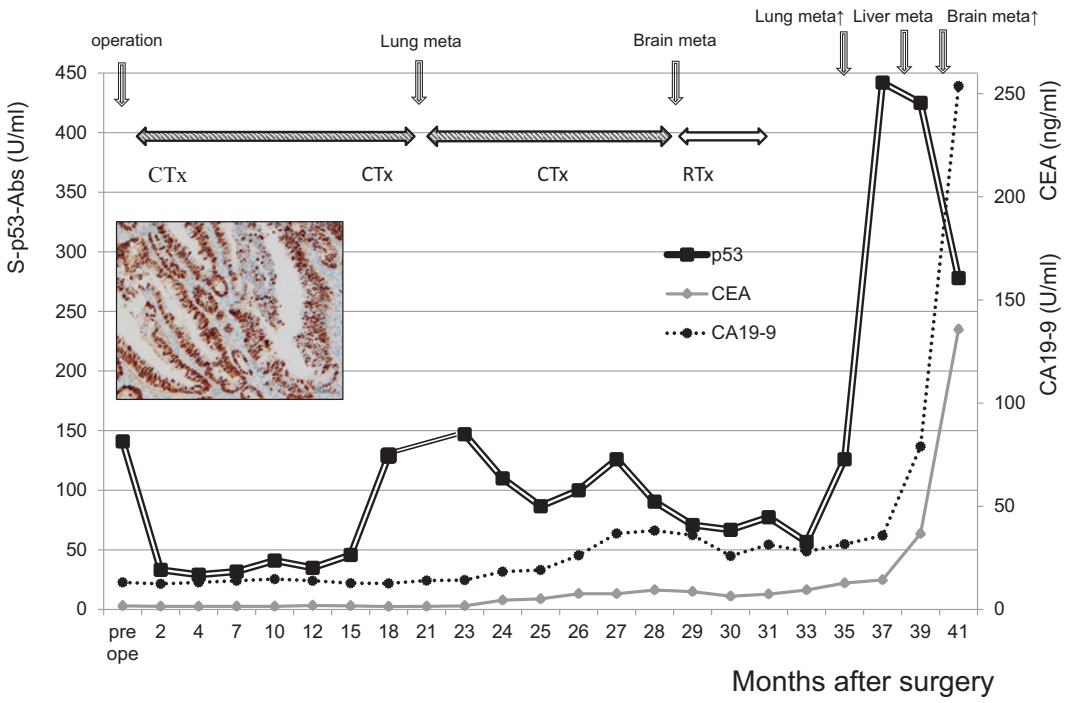

Fig. 7 Clinical course and change in s-p53-Ab titers of a representative patient who was positive only for s-p53-Ab.

Novabiochem, Darmstadt, Germany) of a different type from ELISA kit used in this study, making the results difficult to compare with ours.

Changes in serum titer above the cutoff of 1.3 U/mL seemed to have significant clinical impact. The patients with decreased titers had better survival than the patients without decreased titers, even though seronegative was not achieved. The changes in serum titer can be explained by changes in tumor load. Hammel et al.[14] also observed that s-p53-Abs titer decreased within one month after tumor resection in five of eight patients with colon cancer. Patients with advanced-stage esophageal cancer frequently have very high antibody levels.[15, 16] The study data show decreases in antibody levels, but patients with initially high levels will tend to have detectable levels of antibody for an increased period. Because the half-life of IgG antibodies is approximately 30 days, one month may not have been a long enough time to observe the loss of seropositivity of seropositive patients. Because increasing or decreasing serum titers may prompt cancer detection on standard imaging, at least two years of follow-up would be more helpful than one month to evaluate the clinical impact of s-p53-Abs.

In this study, we found that s-p53-Ab seropositivity was not independently associated with survival, but that a specific range of serum titers did predict long-term survival. A possible explanation for preoperative s-p53-Abs status not being significantly associated with survival is that seronegative patients included loss of both p53 alleles or presence of p53 nonsense mutations. One month after surgery, 31 of the 155 seropositive patients had become seronegative, and the other 124 patients remained seropositive. Of the 372 initially seronegative patients, two (0.5%) became seropositive after surgery. We found that a one month perioperative change in s-p53-Abs was predictive of long-term surgical outcome. Postoperative seropositive patients may have residual cancer cells and are therefore more likely to experience a recurrence.[17]

A limitation of this study was that the mechanism of paradoxical prognostic impact of extremely high group was not clear. There was no difference in clinicopathological factors between extremely high group and medium-high group except venous invasion. The extremely high group showed significantly higher frequency to have venous invasion than the medium-high group. Therefore, such unbalance of poor prognostic factors could not explain the paradoxical prognostic impact of the extremely high group.

Since ELISA is quick and easy to perform, perioperative monitoring of s-p53-Abs titer may prove to be a useful bio-

Table 2

s-p53-Abs		200≦	50≦, 200>	p value
positive		21	17	
Gender	Male	12	8	0.536
	Female	9	9	
Age	<65	13	10	0.847
	≥65	8	7	
Tumor location	Colon	12	11	0.635
	Rectum	9	6	
Differentiation	Well or mod	21	15	0.106
	por, sig, or muc	0	2	
Depth of tumor	Tis, T1, T2	3	1	0.401
	T3, T4	18	16	
Lymph-node metastasis	Positive	10	9	0.744
	Negative	11	8	
Lymphatic invasion	Positive	19	15	0.823
	Negative	2	2	
Venous invasion	Positive	16	5	0.004
	Negative	5	12	
CEA	Positive	11	9	0.650
	Negative	10	6	
CA19-9	Positive	2	2	0.823
	Negative	19	15	

logical marker to identify patients at high risk of tumor recurrence and to predict long-term surgical outcomes. Seropositive patients with no change in s-p53-Abs titer may be good candidates for adjuvant chemotherapy or candidates for more frequent postoperative imaging than other patients. Additional studies with long-term serum follow-up are needed to gain a more precise understanding of the prognostic value of moderate high s-p53-Abs titers. For patients with double-negative CEA/CA19-9 tumors, s-p53-Abs was the only useful monitoring marker. Our representative patient had s-p53-Abs immune response that was precisely associated with recurrence and treatment response. Although CEA and CA9-19 increased during the last phase of the patient's life, the paradoxical reduction of serum titers during progression of recurrent tumors has frequently been reported in the patients with liver metastases.[6, 18]

In conclusion, perioperative changes of s-p53-Abs titer in colorectal cancer patients predicted increased risk of tumor recurrence and poor prognosis. Patients with titers >200 U/mL had relatively better survival than those with titers from 50.1 to 200 U/mL. This paradoxical impact on prognosis warrants further assessment of the significance of s-p53-Abs profiles.

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