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ORIGINAL ARTICLE – COLORECTAL CANCER

Serum p53 Antibody as Tumor Marker for Follow-Up of Colorectal Cancer After Curative Resection

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ABSTRACT

Background. No large-scale studies have examined the use of serial measurements of serum p53 antibodies (s-p53Abs) combined with carcinoembryonic antigen (CEA) measurements during the follow-up of colorectal cancer (CRC) patients after curative resection.

Methods. A highly specific enzyme-linked immunosorbent assay was used to analyze s-p53Abs levels in 305 CRC patients before and after curative resection at a single institution. Agreement between recurrence and serial s-p53Ab and CEA measurements was evaluated by diagnostic accuracy odds ratio (DOR), kappa, and area under receiver operating characteristic curve (AUC).

Results. Among 305 patients, 76 (25%) patients had disease recurrence during follow-up. None of the 168 s-p53Ab seronegative patients (s-p53Ab < 10 U/μL) without recurrence had an abnormal s-p53Ab test during follow-up. Among the remaining low-level (10 U/μL $\leq$ s-p53Ab ≤ 76 U/μL, $n = 103$) and high-level (s-p53Ab titer > 76 U/μL, $n = 34$) seropositive patients, recurrence defined by s-p53Ab tests resulted in a DOR of 4.3 and ∞, a kappa of 0.35 and 1.00, and an AUC of 0.633 [95% confidence interval (CI), 0.495 to 0.772; $P = 0.047$], and 1.0 (95% CI, 1.000 to 1.000; $P < 0.0001$), respectively. Recurrence defined by CEA tests had an AUC of 0.781 (95% CI, 0.654 to 0.909) for low-level and 0.796 (95% CI, 0.611 to 0.982) for high-level seropositive patients.

Conclusions. Agreement between clinical recurrence and serial s-p53Ab test was dependent upon preoperative s-p53Ab level. Serial s-p53Ab testing outperformed CEA testing when predicting clinical recurrence in colorectal cancer patients with an abnormal preoperative s-p53Ab level.

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Carcinoembryonic antigen (CEA) monitoring has been widely used for more than three decades for gastrointestinal cancers, especially colorectal cancer (CRC).[1–4] However, the clinical usefulness of this marker has remained contentious owing to some studies arguing that it has limited sensitivity when predicting relapse.[5,6] In recent years, although the use of various other serum markers for the monitoring of CRC has been described, few have proved to be superior or even comparable to CEA during the follow-up of CRC patients.[2,3,7]

Circulating anti-p53 antibodies can be found in sera of patients with various human malignancies.[8–12] Most studies of these antibodies note a strong relationship between p53 protein accumulation and gene mutation. The p53 humoral response is believed to result from self-immunization against p53 protein that has accumulated in tumor cells.[13] Most studies have focused on measuring serum p53 antibodies (s-p53Abs) as a prognostic marker for a variety of cancers. Some studies have noted a correlation between preoperative s-p53Ab and a poor outcome in cancer patients.[14–20] However, others have reported a different result.[21–24] A previous study by this research group, as well as several studies from other groups, found that the adverse effects of a high s-p53Ab level on survival in CRC patients is due to an increased frequency of s-p53Ab positivity in patients with lymph node metastasis and advanced disease.[25,26]

The value of serial measurements of s-p53Ab as a marker for early recurrence of disease has not been thoroughly elucidated. Some studies have reported that serial measurement of s-p53Ab provides useful clinical information during follow-up among cancer patients.[27,28]

However, other studies have been inconclusive.[29] Other than one study, which reported a promising role for s-p53Ab when monitoring CRC follow-up in a small group of patients, there been no other large-scale surveillance studies of s-p53Abs in CRC patients.[30] This study analyzes serial s-p53Ab levels in CRC patients during a long-term follow-up in order to test the usefulness of the serial measurement of s-p53Ab alone or in conjunction with the simultaneous measurement of CEA, when used for the early detection of recurrence in curatively resected CRC patients.

MATERIALS AND METHODS

Patients

This prospective study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (CGMH) and undertaken according to the ethical guidelines of human investigation. Informed consent was obtained from all the patients. Patients fulfilling the following criteria were enrolled in the study: (1) prior curative resection for histology-proven primary adenocarcinoma of the colorectum at CGMH between 1995 and 2002, (2) availability of serial serum samples from before the operation and from after the surgery, and (3) follow-up with a definitive clinical outcome. The exclusion criteria were as follows: (1) synchronous or metachronous extracolonic cancers, (2) having neoadjuvant therapy for rectal cancer, and (3) fewer than three follow-up samples available for s-p53Ab analysis. Serum samples were collected before surgery and at the time of regular outpatient follow-up after surgery and stored at −80°C until use. To increase serial serum sample numbers for the present study, some serum samples were retrieved from the hospital serum bank. All cases were followed up at the outpatient department every 3–6 months until death or until December 2007. All the patients were followed according to the hospital guidelines of care. Briefly, all patients underwent a follow-up protocol of an outpatient visits every 3–6 months. The follow-up included physical examination and carcinoembryonic antigen tests as well as chest X-ray, abdominal sonography or abdominal computer-assisted tomography scan, and colonoscopy every 1–3 years after operation. The primary end-point was clinical recurrence, which was defined as relapse confirmed by histology or by an imaging study. All the clinical data relevant to the patients were obtained from the Colorectal Cancer Registry Database.

Assay for S-p53 Antibodies and CEA

All the samples were analyzed within 1 year after collection without previous thawing. Serum anti-p53 antibodies (s-p53Abs) were detected by enzyme-linked immunosorbent assay (ELISA) kit (p53-Autoantibody ELISA^{PLUS}; Calbiochem-Novabiochem, Darmstadt, Germany) in accordance with methods described previously.[26] Briefly, all samples were diluted 1:100 in reconstituted sample dilution buffer and assayed in duplicate. The control samples contained a negative control, control I (calibrator with defined p53 autoantibody titer), control II (calibrator: sample dilution buffer = 1:2.5), control III (calibrator: sample dilution buffer = 1:5), and control IV (calibrator: sample dilution buffer = 1:10), which were all included on each microtiter plate. If the absorptions of the samples were outside the calibration range, they were tested again at a higher dilution. The s-p53Ab titer was determined based on the calibration curve generated by serial dilution of the calibrator. Samples were designated as s-p53Ab seronegative if the titer was less than 10, which is equivalent to the cutoff value of the assay. According to a previous study, in which the sera from 611 normal control subjects were analyzed, the highest titer value detected for s-p53Abs was 76.[26] Thus, for the seropositive samples, a titer value of between 10 and 76 units per microliter (U/μL) was designated as low and a value above 76 U/μL was designated as high.[26] All samples were analyzed successively with blinding to clinical information by the same well-trained technician. The clinical dataset was decoded to analyze any association with the s-p53Abs only after completion of the assays. Serum CEA levels were determined by Abbott Architect 2000 (Abbott Laboratories, Abbott Park, IL; reference threshold <5 ng/mL) at Clinical Pathology, CGMH.[31]

Statistical Analysis

The differences in clinicopathological parameters between seronegative and seropositive groups were tested using Student's *t* test for continuous variables (age, CEA, and follow-up period) and Fisher's exact or chi-square test for categorical variables. A two-tailed *P* value of ≤ 0.05 was considered significant.

To assess seroconversion in seropositive patients after surgery, the lowest values of the serial follow-up s-p53Abs 1 year after surgery were identified for each patient. Four cutoffs of these values for positive classification were used: 10, 20, 30, and 40 U/μL. Receiver operating characteristics (ROCs) were drawn for patients stratified by s-p53Ab serology. The power of postoperative seroconversion was evaluated using the area under the curve. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy odds ratio (DOR), and kappa were calculated for positive s-p53Ab testing in patients stratified by level of s-p53Ab positivity.

The CEA test was defined as positive if two consecutive postoperative CEA values were greater than 5 ng/mL or the elevated preoperative CEA values had not returned to the normal level (<5 ng/mL) after surgery. Kappa values were calculated using the following equation: $2 \times (ad - bc)/[(a + b) \times (b + d) + (a + c) \times (c + d)]$, where the values of a, b, c, and d represents the number of true-negative, false-negative, false-positive, and true-positive patients, respectively. The SPSS software package (version 13.0; SPSS, Chicago, IL) was used for the statistically analysis.

RESULTS

A total of 305 patients were eligible for this study. There were 45 (14.8%), 130 (42.6%), and 130 (42.6%) cases in tumor–node–metastasis (TNM) stages I, II, and III, respectively. The preoperative titers of s-p53Ab varied widely from 0 to 60,000 U/μL. The titers were <10 U/μL (seronegative) in 168 patients, 10–76 U/μL (defined as low-level seropositivity) in 103 patients, and >76 U/μL (defined as high-level seropositivity) in 34 patients. The value of preoperative CEA widely ranged from 0.5 to 544 ng/mL [mean, 15.0; standard deviation (SD), 50.0 ng/mL]. One hundred and one patients had a preoperative value of 5.0 ng/mL or more. The distribution of CEA levels was similar among patients stratified as positive for s-p53Ab (<10 versus ≥10 U/μL) or by levels of positivity for s-p53Ab (<10, 10–76, >76 U/μL). Table 1 shows characteristics of these patients stratified by presence of s-p53Ab (e.g. <10 versus ≥10 U/μL). The two groups did not show any significant differences in terms of gender, tumor stage, histological grade, preoperative CEA levels or frequency of clinical recurrence. However, mucinous adenocarcinomas were significantly associated with seronegativity, and seropositivity was associated with rectal cancers. In addition, seropositive patients were older, had shorter duration of follow-up (47 versus 55 months; $P = 0.020$) and more frequently received postoperative adjuvant therapy (50% versus 31%; $P = 0.001$).

Disease Status at End of Follow-Up and Pattern of Recurrence

The mean follow-up was 61.7 months with a range of 2–134 months. At end of follow-up, 76 (25%) patients had recurrent disease. The initial sites of recurrence included: 7 locoregional recurrences, 18 intra-abdominal or retroperitoneal recurrences, 29 hepatic recurrences, 17 pulmonary recurrences, and 9 recurrences in brain or bone. A total of 80 metastases were noted among 76 patients since 4 patients had simultaneous double metastases (2 with hepatic and pulmonary metastases, 1 with hepatic and

TABLE 1 Characteristics of patients with TNM stage I–III colorectal cancer versus status of preoperative s-p53Ab serology[a]

Characteristics	Seronegative patients ($n = 168$)	Seropositive patients ($n = 137$)	P value
Gender (%)			0.198
Female	42	50	
Male	58	50	
Age at diagnosis, years			0.008
Mean	59	63	
Range	20–90	33–89	
Tumor location (%)			0.001
Colon	58	37	
Rectum	40	61	
Colon and rectum	2	2	
Tumor TNM stage (%)			0.528
I	16	13	
II	44	41	
III	40	46	
Tumor morphology (%)			0.469
Polypoid	17	23	
Ulcerative	77	71	
Other type	6	6	
Histological grade (%)			0.231
Good differentiation	20	15	
Moderate differentiation	74	82	
Poor differentiation	6	4	
Histological type (%)			0.001
Adenocarcinoma	91	99	
Mucinous adenocarcinoma	9	1	
Preoperative CEA, ng/mL			0.064
Mean	19.9	9.2	
Range	0.5–544.0	0.5–138.0	
Preoperative CEA (%)			0.690
≤5 ng/mL	68	66	
>5 ng/mL	32	34	
Adjuvant therapy[b] (%)			0.001
None	69	50	
Yes	31	50	
Duration of follow-up, months			0.020
Mean	55	47	
Range	3–113	2–116	
Relapse during follow-up (%)			0.762
Absence	74	76	
Presence	26	24	

[a] Seronegative when the value of preoperative s-p53Ab was less than 10 U/μL; seropositive when s-p53Ab ≥ 10 U/μL

[b] 5-Fluorouracil-based chemotherapy for colon cancer and postoperative combined chemoradiation therapy for rectal cancer

retroperitoneal metastases, and 1 with bone and intra-abdominal metastases). The pattern of recurrence was not different between seronegative and seropositive group (data not shown). Briefly, liver was the most common site of disease failure among both groups (seronegative 34.9%, seropositive 33.3%). Intra-abdominal or retroperitoneal metastases were more frequent in the seronegative group (seronegative 27.9%, seropositive 12.1%) whereas lung (seronegative 16.3%, seropositive 24.2%) and bone/brain metastases (seronegative 7.0%, seropositive 15.2%) were seen more often in the seropositive group.

Determination of Cutoff Value of Postoperative Serial s-p53Ab

For s-p53Ab seronegative patients, the relationship between recurrence and cutoff value of postoperative serial s-p53Ab was not significant at any level; the areas under the ROC curve (AUC) were 0.508, 0.512, 0.500, and 0.500 for cutoff value of 10, 20, 30, and 40 U/μL, respectively (Fig. 1a). Among these 168 seronegative patients, 160 cases (95.2 %) remained seronegative and another 8 cases had at least a follow-up titer ranging from 10–23 U/μL, with a mean of 15.6 U/μL. For the low-level seropositive patients, the AUC values were 0.620 (95% CI, 0.488 to 0.752; $P = 0.73$), 0.633 (95% CI, 0.496 to 0.771, $P = 0.047$), 0.633 (95% CI, 0.495 to 0.772; $P = 0.047$), and 0.593 (95% CI, 0.455 to 0.732; $P = 0.164$) for cutoff value of 10, 20, 30, and 40 U/μL, respectively (Fig. 1b). For the high-level seropositive patients, the AUC values were 0.891 (95% CI, 0.779 to 1.004; $P < 0.001$), 0.978 (95% CI, 0.928 to 1.029; $P < 0.0001$), 1.0 (95% CI, 1.000 to 1.000; $P < 0.0001$), and 1.0 (95% CI, 1.000 to 1.000; $P < 0.0001$) for cutoff value of 10, 20, 30, and 40 U/μL, respectively (Fig. 1c). Taken together, the cutoff score of 30 U/μL performed best with regard to AUC among low-level and high-level seropositive patients combined. In addition, Table 2 shows that the cutoff value at 30 U/μL outperforms the other three cutoff values in terms of DOR, kappa, and AUC. Thus, an s-p53Ab of 30 U/μL was chosen as the cutoff value for the later analyses.

Seroconversion and Reconversion Among s-p53Abs-Positive Patients

Seroconversion was defined as postoperative serial s-p53Ab titers that were decreasing and with a lowest level less than 30 U/μL. Among 137 seropositive patients, 126 (92%) had complete seroconversion and 11 (8%) did not undergo seroconversion. The recurrence rates for those with seroconversion and for those without were 17% (22/126) and 100% (11/11), respectively ($P < 0.0001$). Among 126 patients with seroconversion, 14 (11%) reconverted to seropositive (with a titer >30 U/μL). Compared with 12 out of 112 (11%) patients without reconversion, 10 out of these 14 (71%) patients with reconversion had a recurrence

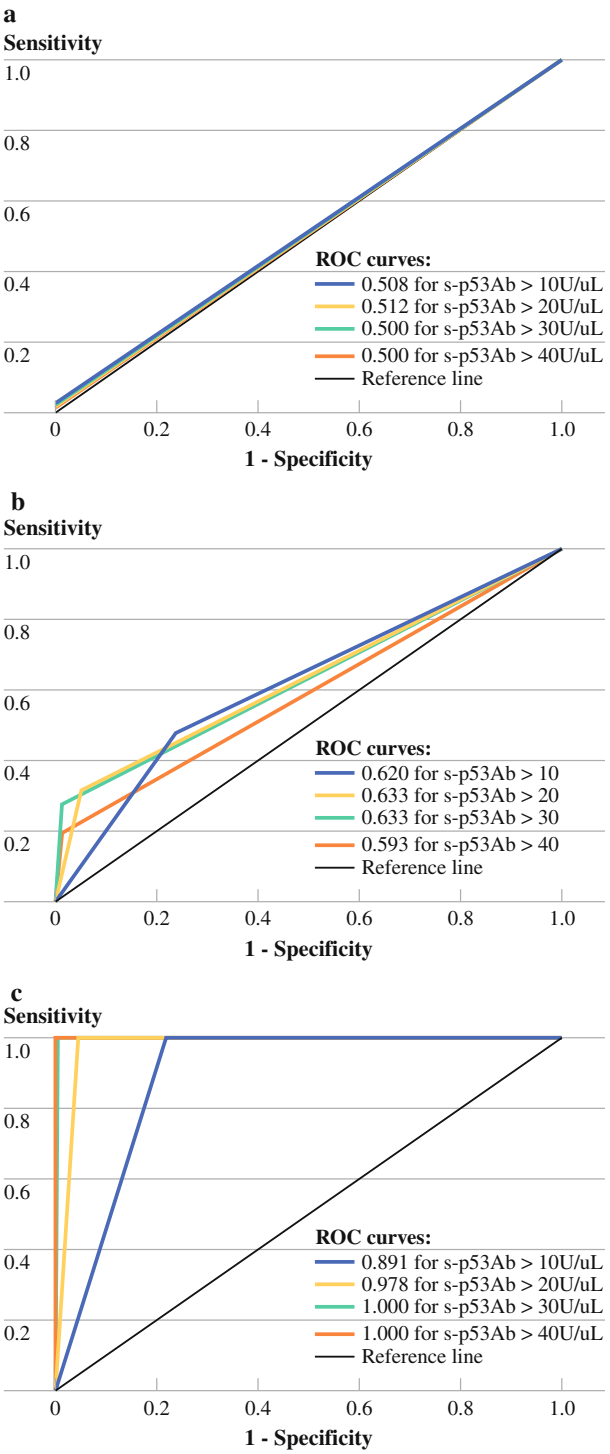


FIG. 1 Receiver operating characteristic (ROC) curves for patients with **a** negative, **b** low-level, and **c** high-level preoperative s-p53Ab drawn with four cutoff values for the serial s-p53Ab levels (10 U/μL, *blue line*; 20 U/μL, *green line*; 30 U/μL, *red line*, and 40 U/μL, *purple line*)

TABLE 2 Results for serial s-p53Ab cutoff values for recurrence in patients with preoperative high-level and low-level s-p53Ab

Preoperative level and postoperative cutoff value (U/μL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DOR	Kappa	Area under ROC	
							Area	95% CI
High-level s-p53Ab (>76 U/μL)								
≥10	100.0	78.3	64.3	100.0	5.4	0.67	0.891	0.779–1.004
≥20	100.0	95.7	90.0	100.0	31.0	0.93	0.978	0.928–1.029
≥30	100.0	100	100.0	100.0	∞	1.00	1.000	1.000–1.000
≥40	100.0	100	100.0	100.0	∞	1.00	1.000	1.000–1.000
Low-level s-p53Ab (10–76 U/μL)								
≥10	48.0	76.0	40.0	81.4	2.2	0.23	0.620	0.488–0.752
≥20	32.0	94.7	66.7	80.7	3.8	0.32	0.633	0.496–0.771
≥30	28.0	98.7	87.5	80.4	4.3	0.35	0.633	0.495–0.772
≥40	20.0	98.7	83.3	78.7	3.8	0.25	0.593	0.455–0.732

ROC receiver operating characteristics, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic accuracy odds ratio

($P < 0.0001$). Accordingly, patients were defined as having positive postoperative s-p53Ab tests if (1) they did not have seroconversion after surgery or (2) they had a seroconversion but with a later reconversion back to the initial state.

Agreement Between Clinical Recurrence and Serial s-p53Ab and CEA Testing

The overall agreement between clinical recurrence and serial CEA measurements during follow-up was fair, with a kappa of 0.70 (sensitivity, 74%; specificity, 94%; positive predictive value, 80%; negative predictive value, 92%). When stratified by preoperative CEA level, the sensitivity of the serial CEA test was 67% in patients with normal preoperative CEA levels and 82% in those with abnormal preoperative CEA levels.

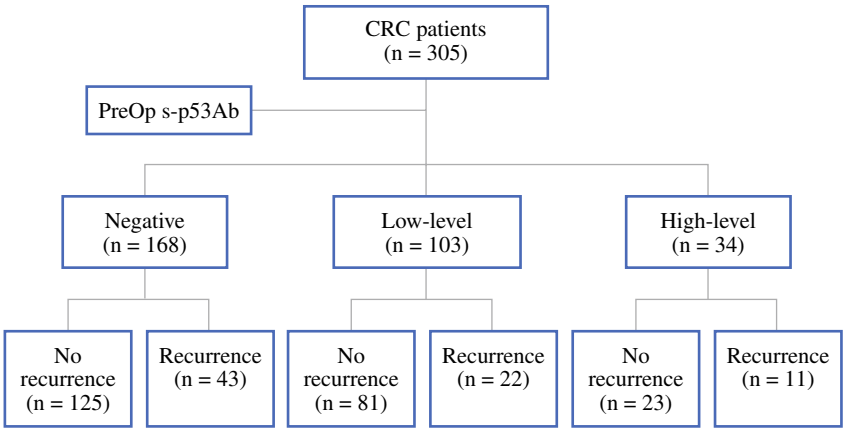
Figure 2 shows the flow of patients from preoperative s-p53Ab level to clinical recurrence during follow-up and the testing results (serial s-p53Ab and CEA measurements) for each corresponding subgroup. A total of 76 patients had clinical recurrence. Time to recurrence ranged from 2 to 58 months (mean, 20.4 months). Positive s-p53Ab tests were observed in 21 patients prior to development of clinical recurrence (lag time ranging from 0.1 to 23.7 months with a mean of 6.8 months and an SD of 6.0 months). The remaining two patients had an abnormal test at the time of clinical recurrence. The pattern of recurrence was not related to their status in terms of serial s-p53Ab testing (data not shown).

Table 3 summarizes the diagnostic efficacy of the serial measurements of s-p53Ab, CEA, and the combination of both for monitoring CRC stratified by preoperative s-p53Ab levels. Firstly, these values showed a strong association between the diagnostic efficacy of serial s-p53Ab testing with the preoperative level of s-p53Ab. The CEA test performed better than the s-p53Ab test (AUC, 0.879 versus 0.500; κ, 0.76 versus 0) in the seronegative group. Furthermore, it did as well as the s-p53Ab test in the low-level group, but worse than the s-p53Ab test (AUC, 0.796 versus 0.978; κ, 0.64 versus 0.93) in the high-level seropositive group. Secondly, the performance of serial CEA measurements was not related to the preoperative level of s-p53Ab. Thirdly, the results also show that adding s-p53Ab to CEA testing was not better than CEA alone when detecting recurrent disease among the s-p53Ab seronegative patients. Likewise, adding CEA to s-p53Ab testing did not improve the sensitivity, kappa or AUC of serial sp53Ab testing alone among the s-p53Ab seropositive patients.

DISCUSSION

The analytical results indicated that serum anti-p53 antibody (s-p53Ab) levels may be a reliable tumor marker for recurrence during follow-up for s-p53Ab seropositive patients with curatively resected CRC. This may be particularly true for the group of patients with a high preoperative serum anti-p53 antibody level (>76 U/μL), where s-p53Ab monitoring outperforms CEA in terms of sensitivity, specificity, and diagnostic accuracy for detecting disease recurrence among CRC patients.

Of the numerous tumor markers suggested for CRC, CEA is the surveillance marker of choice and has been recommended for monitoring purposes on many occasions.[1–3,31,32] However, as noted in several studies, its clinical use is limited since the sensitivity and specificity for serial CEA measurements when detecting recurrent disease are limited.[5,6] In the current study, the sensitivity of CEA monitoring was low at 64% in the seropositive patients, compared with 81% in the seronegative patients. These analytical results also confirmed that increased CEA



Serial s-p53Ab testings						
–	125	43	77	10	22	0
+	0	0	4	12	1	11
Serial CEA testings						
–	118	8	75	8	22	4
+	7	35	6	14	1	7

FIG. 2 Consolidated chart of 305 patients with serial s-p53Ab and CEA testing results stratified by preoperative s-p53Ab level and clinical recurrence during follow-up. Patients were defined as having positive postoperative s-p53Ab tests if (1) they did not have seroconversion after surgery or (2) they had a seroconversion but with a later reconversion of s-p53Ab serology. The CEA test was defined as positive if two consecutive postoperative CEA values were greater than 5 ng/mL or the elevated preoperative CEA values did not returned to the normal level (<5 ng/mL) after surgery

in association with disease recurrence is not uncommon (67% in the current series) among patients with a normal preoperative CEA level.[33] Thus, CEA tests are needed for postoperative follow-up in all patients, both with and without an elevated CEA value.

The role that s-p53Abs could play in the follow-up of CRC patients after curative resection has not been adequately elucidated. Hammel et al., in a study of eight patients over 12–24 months of follow-up, reported that s-p53Ab monitoring might help physicians to follow up CRC patients.[30] Another small series that included 27 patients with early CRC treated by endoscopic resection suggested that p53 antibody seroconversion might indicate local curability after endoscopic treatment.[34] In the current study we observed that the sensitivity of s-p53Ab monitoring was highly correlated with s-p53Ab level before resection, being 0% for seronegative patients, 55% for low-level seropositive patients, and 100% for high-level seropositive patients. Likewise, the diagnostic efficacies were highly correlated with the preoperative s-p53Ab level with regard to DOR, κ, and AUC (Table 3). Abnormal s-p53Ab titers were noted only in the seropositive patients but not in seronegative patients. Therefore, postoperative serial s-p53Ab tests are needed only in patients having an elevated s-p53Ab titer before surgery.

This leaves open the question as to why the sensitivity of the s-p53Ab test is highly correlated with preoperative s-p53Ab level. It is possible that the anti-p53 antibodies are of the immunoglobulin G (IgG) isotype, which is dependent on helper T cells and therefore the majority of such patients display long-lasting anti-p53 T helper (Th) immunity even in the absence of measurable s-p53Ab. This might easily last for several years after resection of the primary colorectal cancer.[35] Thus, the capacity of a patient to produce circulating anti-p53 antibodies in response to p53 protein from recurrent tumor cells will depend on whether the patient has been pre-immunized against the p53 protein. However, whether CRCs harboring p53 alterations (p53 mutations or accumulated proteins) are more immunogenic than wild-type p53 also requires further investigation.[9,36,37] Other possible explanations include individual differences in major histocompatibility complex-based presentation of the p53 protein, the position of the p53 mutations, the presence of multiple steps in the carcinogenesis process, and a tissue-specific humoral response against the p53 antibody.[9,26,36,38]

Alteration of the *P53* gene, the most frequent genetic alteration in human cancer, causes an accumulation of mutant p53 in the nucleus of tumor cells. It is noteworthy in the context of this paper that p53 antibodies have been found in the sera of patients with a variety of different forms of cancer as well as in those with inflammatory bowel disease.[38,39] Although the frequency of abnormal s-p53Ab levels varies among different cancers, Lubin et al. demonstrated that the immune response of patients with p53 antibodies is restricted to a small subset of peptides regardless of the form of cancer.[13] Thus, examination of s-p53Ab in addition to other specific tumor markers might also serve as a useful indicator of tumor recurrence in other malignancies that express p53 alterations.

In conclusion, serial s-p53Ab testing outperformed CEA testing in predicting clinical recurrence in colorectal cancer

TABLE 3 Statistics of serial measurements of s-p53Ab alone, CEA alone, and the combination of both among patients with three levels of preoperative s-p53Ab

Level of preoperative s-p53Ab and serum markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DOR	κ	Area under ROC	
							Area	(95%CI)
Negative (<10 U/μL)								
CEA	81.4	94.4	83.3	93.7	10.2	0.76	0.879	0.807–0.951
s-p53Ab	0.0	100.0	–	74.4	2.9	0.00	0.500	0.400–0.600
CEA + s-p53Ab	81.4	94.4	83.3	93.7	10.2	0.76	0.879	0.807–0.951
Low-level (10–76 U/μL)								
CEA	63.6	92.6	70.0	90.4	6.4	0.58	0.781	0.654–0.909
s-p53Ab	54.5	95.1	75.0	88.5	6.4	0.55	0.748	0.613–0.883
CEA + s-p53Ab	81.8	87.7	64.3	94.7	6.4	0.63	0.847	0.745–0.950
High-level (>76 U/μL)								
CEA	63.6	95.7	87.5	84.6	5.8	0.64	0.796	0.611–0.982
s-p53Ab	100.0	95.7	91.7	100	33.0	0.93	0.978	0.928–1.028
CEA + s-p53Ab	100.0	91.3	84.6	100	16.0	0.87	0.957	0.886–1.027

CEA carcinoembryonic antigen, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic accuracy odds ratio, κ kappa. Dash inidates denominator = 0

patients with an abnormal preoperative s-p53Ab level. Such monitoring would seem to be a valuable and cost-effective component of the postoperative follow-up program for those with elevated preoperative s-p53Ab levels. This should take the form of postoperative CEA monitoring for s-p53Ab seronegative patients, with combined CEA and s-p53Ab tests for low-level seropositive patients and s-p53Ab monitoring for high-level seropositive patients. These findings require confirmation by further independent studies before the tumor marker (s-p53Ab) can be recommended for routine clinical use.

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