Comparison between a new assay system, Elecsys® Anti-p53, and conventional MESACUP™ for the detection of serum anti-p53 antibodies: A multi-institutional study

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Abstract. The sensitivity and specificity of a new automated electrochemiluminescence immunoassay system, Elecsys® Anti-p53 (Elecsys), were compared with that of the conventional serum anti-p53 antibody (s-p53-Ab) enzyme-linked immunosorbent assay kit [MESACUP anti-p53 test (MESACUP)]. Elecsys and MESACUP were used to analyze the levels of s-p53-Abs in patients with esophageal, colorectal and breast cancer. A total of 532 controls and 288, 235 and 329 patients with esophageal, colorectal and breast cancer, respectively, were enrolled. Additionally, the sera of patients with benign diseases of the esophagus, colorectal system and breast, patients with autoimmune diseases and healthy volunteers were analyzed as controls. Sensitivity and specificity were compared between the two assay systems. Positive agreement rates were 58.7% in all samples, 71.2% in esophageal samples, 73.6% in colorectal samples and 35.1% in breast samples. Negative agreement rates for the different cancer types were ≥97.1% and the overall agreement rates were ≥92.3%. When the specificities of the two assays were aligned for all samples, Elecsys demonstrated higher sensitivities for all types of analyzed cancer together, as well as for esophageal, colorectal and breast cancer, respectively. Although positive concordance between the two assay systems was low in terms of specificity, Elecsys had a higher sensitivity than the MESACUP.

Introduction

It has previously been reported that the prevalence of serum anti-p53 antibodies (s-p53-Abs) is correlated with the prevalence of p53 mutations in different types of cancers, including esophageal, colon, lung and uterine cancer (1). The accumulation of p53 in tumors and the subsequent immune response are attributable to a self-immunization process linked to the strong immunogenicity of the p53 protein (2-5). Although the clinical value of s-p53-Abs remains debatable, several studies have reported consistent results in colon, esophageal, breast and gastric cancer types, in which s-p53-Abs have been associated with high-grade tumors and a poor prognosis (2-5). Furthermore, the addition of s-p53-Abs may enhance the diagnostic sensitivity of conventional tumor markers without a decrease in specificity, suggesting a promising role for s-p53-Abs as part of a panel of tumor markers (2-5). For these reasons, the quantitative p53-Abs enzyme-linked immunosorbent assay (ELISA) Kit II [MESACUP™ anti-p53 test (MESACUP); Medical & Biological Laboratories Co., Ltd.] for measuring s-p53-Abs was developed, approved by the Japanese government and covered by national healthcare insurance in 2007 ahead of other countries (6-10). In patients with various types of cancer, s-p53-Abs can be used for the diagnosis and monitoring of treatment response and tumor recurrence (2-5). In a previous multi-institutional study, a cutoff value of 1.3 U/ml, with >95.5% specificity, was determined and applied in clinical practice (3). Although the ELISA method

is clinically significant for s-p53-Abs, it is time-consuming and shows only semiquantitative values.

Compared with manual ELISAs, electrochemiluminescence immunoassays (ECLIAs) are highly sensitive, quantitative and quick (11). The s-p53-Abs ECLIA Kit Elecsys® Anti-p53 (Elecsys) (Roche Diagnostics K.K.) was developed recently (12) and approved by the Pharmaceuticals and Medical Devices Agency of Japan. Results of a clinical study showed that the new s-p53-Abs assay, Elecsys, was useful in the detection of esophageal and colorectal cancer, with a specificity of >98.0%. Also, the addition of s-p53-Abs to conventional tumor markers increased the positivity rates in these cancer types (12). However, no direct comparison has been conducted between the clinical performance of Elecsys and MESACUP.

In the present multi-institutional study, the clinical performance of the novel Elecsys system was compared with that of conventional MESACUP for the measurement of s-p53-Abs in patients with esophageal, colorectal and breast cancer. To the best of our knowledge, this is the first study to directly compare the diagnostic utility of these two assay systems, which rely on distinct technologies.

Patients and methods

Patients and controls. This was a multicenter, prospective study designed to compare the analytical performance of two diagnostics kits. Patients with pathologically defined primary esophageal, colorectal or breast cancer and disease controls were enrolled from seven hospitals (Chiba Foundation for Health Promotion and Disease Prevention, Chiba University Hospital, Chiba; Keio University Hospital, Tokyo; Showa University Hospital, Tokyo; Toho University Sakura Medical Center, Chiba; Toho University Ohashi Medical Center, Chiba; Toho University Omori Medical Center, Tokyo; Tokyo Center Clinic, Tokyo Medical and Dental University, Tokyo, Japan) (12). All participants were aged ≥20 years, and provided written, informed consent prior to enrolment. Serum samples from healthy volunteers and patients (subjects) who met all the following inclusion criteria and did not meet any of the following exclusion criteria were measured, and followed by statistical analysis. The subjects who violated the ethical guidelines were excluded from the study and the remaining subjects were handled as the full analysis set. The subjects who met any of the following criteria ii) to v) were excluded from the full analysis set and the remaining subjects were handled as the per protocol set. i) Subjects with violation of the ethical guidelines: Subjects whose serum samples may have been collected not in compliance with the ethical guidelines, including those for whom no consent was obtained, those for whom the consent was obtained in a questionable manner or those whose serum samples were tested before the consent was obtained. ii) Subjects with deviations: Subjects in whom designated examinations were not performed with the procedure or at intervals specified by the protocol, or those excluded from analysis by the investigator due to illness or other reasons. iii) Ineligible subjects: Subjects who should not have been included in the study, as it was found after registration that they did not meet any of the inclusion criteria or meet any of the exclusion criteria. iv) Discontinued subjects: Subjects discontinued from the study by the inves-

tigator due to meeting any of the discontinuation criteria, etc. v) Subjects for whom no measurement was obtained for either of the test or control drug. This study was conducted between October 2016 and September 2018. The mean age of healthy subjects, patients with autoimmune diseases and cancer patients was 40.9 (range, 20-73), 59.6 (range, 22-92) and 64.9 (range, 30-97) years, respectively. A total of 288 patients with esophageal cancer (stage I, n=59; stage II, n=45; stage III, n=138; stage IV, n=40; recurrent or unknown, n=6), 235 patients with colorectal cancer (stage 0, n=1; stage I, n=50; stage II, n=70; stage III, n=82; stage IV, n=30; recurrent or unknown, n=2) (12) and 329 patients with breast cancer (stage 0, n=65; stage I, n=150; stage II, n=95; stage III, n=14; stage IV, n=3; recurrent or unknown, n=2) were enrolled in this multi-institutional study. Control samples were obtained from 137 healthy volunteers, 105 patients with autoimmune diseases (rheumatoid arthritis, n=36; polymyalgia rheumatica, n=12; systemic lupus erythematosus, n=7; adult Still's disease, n=7; eosinophilic granulomatosis with polyangitis, n=6; Sjögren's syndrome, n=5; scleroderma, n=5; microscopic polyangiitis, n=5; other diseases, n=22) and 290 patients with benign diseases, including 100 with a benign disease of the esophagus (reflux esophagitis, n=91; other diseases, n=9), 100 with a benign disease/s of the colorectal system (hemorrhoid, n=45; diverticulosis, n=23; polyp, n=19; adenoma, n=6; hemorrhoid and diverticulosis, n=2; hemorrhoid, diverticulosis and polyp, n=1; other diseases, n=4) and 90 with a benign disease/s of the breast (mastopathy, n=40; fibroadenoma, n=19; mastitis, n=8; lactocele, n=8; mastopathy and fibroadenoma, n=1; mastopathy and lactocele, n=1; other diseases, n=13) (12).

From the subjects, 5 ml of blood was drawn for the study. The blood was held at room temparature until coagulation was complete and then the serum was separated. Serum samples were obtained before treatment, divided into two tubes and stored at -20°C. Patient recruitment and sample collections were performed within the guidelines of protocols approved by the Ethics Committee of Toho University (Tokyo, Japan; approval no. A16049) and the Institutional Review Boards of each participating hospital. In addition, written informed consent was obtained from all participants.

Enzyme immunoassay for s-p53-Abs. s-p53-Abs were assessed via immunoassay for the *in vitro* quantitative determination of anti-p53 autoantibodies in human serum using the anti-p53 ECLIA Kit (catalog no. 07751605174; Elecsys; Roche Diagnostics K.K.) according to the manufacturer's instructions (12). In brief, to allow for the formation of complexes of capture antigen-anti-p53 antibody-detection antigen, the biotinylated capture antigen, 20 μl of the sample and the ruthenylated detection antigen were incubated at 37°C. If anti-p53 antibodies were present in the sample, they formed a bridge between the capture and detection antigens, resulting in the formation of a stable complex. The complexes were immobilized on streptavidin-coated beads that interacted with the biotin on the capture antigen, and the chemiluminescence signal detection was performed using the Cobas 6000 analyzer (Roche Diagnostics K.K.). Electrogenerated chemiluminescence generates species at the electrode surfaces, which undergo electron-transfer reactions and form excited states to emit light. The signal output is expressed in arbitrary
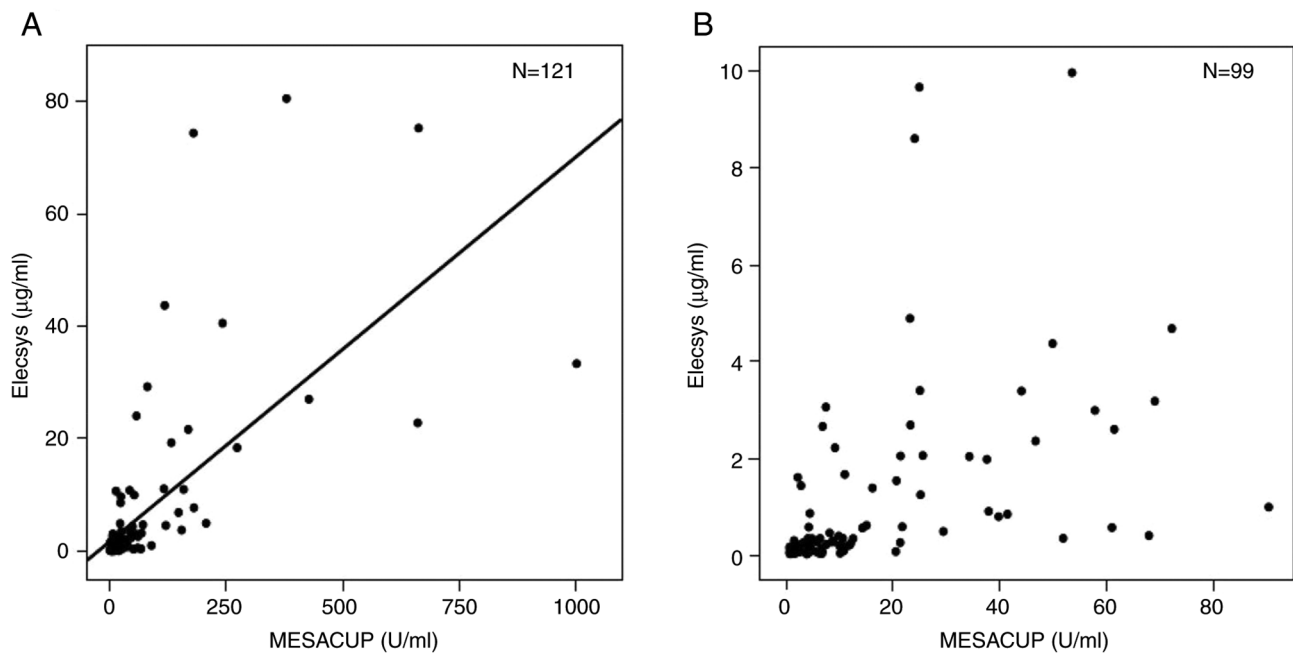
A

B

Figure 1. Correlation of s-p53-Abs titers between the two assay systems. (A) Correlation in the whole group. (B) Correlation in the low-titer group.

light units, which is equivalent to the concentration of the analyte, providing a fully quantitative result (12). This is a fully automated immunoassay system with a high throughput of 300 samples/h, and the reaction time is as short as 18 min. In addition, three different peptides that can strongly capture the wild-type sequences of the p53 antibodies were designed and included in the assay, for use as antigens to maximize the sensitivity and specificity of the assay.

Simultaneously, samples from the same subjects were sent to LSI Medience Corporation for assessment by the p53-Abs ELISA Kit II (MESACUP; RG-7640E; Medical & Biological Laboratories Co., Ltd.) based on the manufacturer's instructions (3). The rationale for setting the cutoff value (Elecsys, 0.05 μg/ml; MESACUP, 1.3 U/ml) and their package inserts are as previously described (3,12).

Statistical analysis. The correlation between the two assay systems was evaluated using Pearson's correlation analysis. To compare the sensitivity and specificity of the two different systems, as well as the values from patients with cancer and those with benign disease according to each assay system, an exact McNemar test was applied. $P<0.05$ was considered to indicate a statistically significant difference. R version 3.6.3 (R Foundation for Statistical Computing; https://www.r-project.org/foundation/) was used for all the statistical analyses. The P-values were calculated using Fisher's exact test with JMP version 15.2 (J.M.P, Co., Ltd.).

Results

Correlation between the two assay systems with regard to s-p53-Ab titer. Fig. 1 shows the correlation between the two assay systems with regard to the s-p53-Ab titer. The overall correlation was calculated as y=0.068x + 1.633, with r=0.674 (Fig. 1A). To examine the correlation around cut off values,

when focusing on the low-titer group (0.02-10 μg/ml for Elecsys and 0.7-100 U/ml for MESACUP), the values were widely spread (n=99; Fig. 1B). Fig. 2 shows the distribution of measurement values by disease for the two assay systems. The positive p53 detection rates in the patients with cancer were higher than those of healthy and benign subjects for both assay systems. In addition, higher s-p53-Ab titers were observed in the patients with cancer compared with those in the healthy and benign subjects for the two assay systems.

Agreement rates and judgments of the two assay systems for each cancer type. Table I shows the rates of agreement between the two assay systems for each cancer type when compared with the cutoff values in the package inserts (Elecsys: 0.05 μg/ml; MESACUP: 1.3 U/ml). Positive agreement rates were 58.7% in all samples, 71.2% in esophageal samples, 73.6% in colorectal samples and 35.1% in breast samples (Table I). Negative agreement rates for each cancer type were ≥97.1%, and overall agreement rates were ≥92.3% (Table I). Checking the agreement between the two assay systems, 6 control and 16 cancer samples were positive by Elecsys only, and 35 control and 39 cancer samples were positive by MESACUP only (Table II).

Table III shows the determinations for s-p53-Abs of the two assay systems for each cancer type when compared with the cutoff values of 0.05 μg/ml for Elecsys and 1.3 U/ml for MESACUP. Those specimens that were above the cutoff were defined as positive, and those below the cutoff were defined as negative. Of the 852 patients with cancer, 117 were positive by Elecsys and 140 were positive by MESACUP. Of the 532 control subjects, 10 were positive by Elecsys and 39 were positive by MESACUP. In general, compared with MESACUP, Elecsys exhibited lower sensitivities and higher specificities. This tendency was observed in esophageal, colorectal and breast cancer. The two assay systems significantly discriminated

Table I. Agreement rates between Elecsys (cutoff, 0.05 μg/ml) and MESACUP (cutoff, 1.3 U/ml).

A, All samples[a]

Method	MESACUP, n			Positive agreement rate, % (95% CI)	Negative agreement rate, (95% CI)	Overall agreement rate, % (95% CI)
	+	-	Total			
Elecsys, n				58.7 (51.1-66.0)	98.2 (97.2-98.9)	93.1 (91.6-94.3)
+	105	22	127			
-	74	1,183	1,257			
Total	179	1,205	1,384			

B, Esophageal samples[b]

Method	MESACUP, n			Positive agreement rate, % (95% CI)	Negative agreement rate, (95% CI)	Overall agreement rate, % (95% CI)
	+	-	Total			
Elecsys, n				71.2 (59.4-81.2)	97.1 (94.6-98.7)	92.3 (89.1-94.7)
+	52	9	61			
-	21	306	327			
Total	73	315	388			

C, Colorectal samples[c]

Method	MESACUP, n			Positive agreement rate, % (95% CI)	Negative agreement rate, (95% CI)	Overall agreement rate, % (95% CI)
	+	-	Total			
Elecsys, n				73.6 (59.7-84.7)	97.5 (95.0-99.0)	93.7 (90.6-96.1)
+	39	7	46			
-	14	275	289			
Total	53	282	335			

D, Breast samples[d]

Method	MESACUP, n			Positive agreement rate, % (95% CI)	Negative agreement rate, (95% CI)	Overall agreement rate, % (95% CI)
	+	-	Total			
Elecsys, n				35.1 (20.2-52.5)	99.0 (97.3-99.7)	93.3 (90.5-95.5)
+	13	4	17			
-	24	378	402			
Total	37	382	419			

[a]Cancer, healthy volunteers, autoimmune diseases and benign diseases. [b]Esophageal cancer and esophageal benign diseases. [c]Colorectal cancer and colorectal benign diseases. [d]Breast cancer and breast benign diseases. CI, confidence interval.

patients with cancer from control subjects overall (P<0.001), but MESACUP did not find a significant difference (P=0.3742) between the breast cancer and control cases.

Comparison of the sensitivity and specificity between the two assay systems. As shown in Tables II and III, when comparing the assays using the cutoff values of the package

Table II. Breakdown of agreement between Elecsys (cutoff, 0.05 μg/ml) and MESACUP (cutoff, 1.3 U/ml).

A, Controls

Volunteers and patients	Elecsys +/ MESACUP +, n	Elecsys +/ MESACUP -, n	Elecsys-/ MESACUP +, n	Elecsys-/ MESACUP -, n	Total, n
Healthy volunteers	1	0	10	126	137
Autoimmune diseases	0	2	5	98	105
Esophageal benign diseases	1	2	6	91	100
Colorectal benign diseases	2	2	5	91	100
Breast benign diseases	0	0	9	81	90
Subtotal	4	6	35	487	532

B, Cancer

Volunteers and patients	Elecsys +/ MESACUP +, n	Elecsys +/ MESACUP -, n	Elecsys-/ MESACUP +, n	Elecsys-/ MESACUP -, n	Total, n
Esophageal cancer	51	7	15	215	288
Colorectal cancer	37	5	9	184	235
Breast cancer	13	4	15	297	329
Subtotal	101	16	39	696	852
Total	105	22	74	1,183	1,384

inserts, Elecsys tended to demonstrate a higher specificity, whereas MESACUP tended to demonstrate a higher sensitivity. To facilitate a head-to-head comparison, the performance of the two assay systems was compared using the cutoff values when the specificities were aligned for all samples (specificity, 98.1%; Elecsys: 0.05 μg/ml; MESACUP: 5.3 U/ml; Tables IV and V), as aligning with the specificity of MESACUP would have resulted in an Elecsys cutoff value that was below the lower end of the measuring range. Also, Table IV shows the determinations for s-p53-Abs of the two assay systems with the aligned cutoff as in Table III. As shown in Tables III and IV, by setting the cutoff value of MESACUP higher than the cutoff value in the package insert, the results of 78 subjects (healthy volunteers, n=9; autoimmune diseases, n=1; esophageal benign diseases, n=5; colorectal benign diseases, n=6; breast benign diseases, n=8; esophageal cancer, n=21; colorectal cancer, n=10; breast cancer, n=18) changed from positive to negative.

The sensitivities of Elecsys for all samples (13.7 vs. 10.7%; P<0.001), esophageal samples (20.1 vs. 15.6%; P=0.002) and breast samples (5.17 vs. 3.04%; P=0.039) were significantly higher than those of MESACUP (Table V). Although the sensitivity of Elecsys was higher than that of MESACUP for the colorectal samples (17.9 vs. 15.3; P=0.210), the difference was not considered significant. The sensitivities for each stage of cancer tended to be higher for Elecsys, except for colorectal cancer (stage I); however, none of the differences were significant. The specificity adjusted for all samples was not significantly different between the two assay systems for each sample (Table V).

Discussion

In this study, the sensitivities and specificities of a new ECLIA-based assay (Elecsys) and an existing ELISA-based assay (MESACUP) were compared using a large number of cancer (n=852) and control (n=532) samples, in a multi-institutional study.

The two assay systems could clearly distinguish between patients with cancer and those with benign disease, and a correlation (r=0.674) was found between the two assay systems. The remaining differences can partially be explained by the characteristics of the systems, such as the differences in detection antigens, units of measurement and quantitative factors (3,12). For example, whereas MESACUP uses the full-length p53 protein, Elecsys uses three different peptides containing epitopes of the p53 protein that are recognized through anti-p53 antibodies (3,12). This can lead to differences in reactivity to the antibodies in each method (3,12). Therefore, no conversion factor between the two products can be provided.

Conversely, Table I shows a relatively low positive agreement rate for all samples [58.7%; 95% confidence interval (CI), 51.1-66.0] due to the lower positive agreement rate observed in the breast cancer group (35.1%; 95% CI, 20.2-52.5). Indeed, Table II shows that MESACUP detected 9 of the 90 (10%) patients with benign breast disease as false-positives, whereas Elecsys detected no patients with benign breast disease. Table I shows that there were relatively good positive rate agreements between the two methods for the esophageal (71.2%; 95% CI, 59.4-81.2) and colorectal (73.6%; 95% CI, 59.7-84.7) cancer types. However, when MESACUP was compared with Elecsys, higher false-positive rates were observed overall, including

Table III. Determination of the serum anti-p53 antibodies of the two assay systems with the cutoff in the package insert.

A, All samples[a]

Method	Cancer, n	Control, n	Total, n	P-value
Elecsys				
+	117	10	127	
-	735	522	1,257	
Total	852	532	1,384	<0.0001
MESACUP				
+	140	39	179	
-	712	493	1,205	
Total	852	532	1,384	<0.0001

B, Esophageal samples[b]

Method	Cancer, n	Control, n	Total, n	
Elecsys				
+	58	6	64	
-	230	336	566	
Total	288	342	630	<0.0001
MESACUP				
+	66	23	89	
-	222	319	541	
Total	288	342	630	<0.0001

C, Colorectal samples[c]

Method	Cancer, n	Control, n	Total, n	
Elecsys				
+	42	7	49	
-	193	335	528	
Total	235	342	577	<0.0001
MESACUP				
+	46	23	69	
-	189	319	508	
Total	235	342	577	<0.0001

D, Breast samples[d]

Method	Cancer, n	Control, n	Total, n	
Elecsys				
+	17	3	20	
-	312	329	641	
Total	329	332	661	0.001
MESACUP				
+	28	25	53	
-	301	307	608	
Total	329	332	661	0.3742

[a]Cancer, healthy volunteers, autoimmune diseases, and benign diseases. [b]Esophageal cancer and esophageal benign diseases. [c]Colorectal cancer and colorectal benign diseases. [d]Breast cancer and breast benign diseases.

Table IV. Determination of the serum anti-p53 antibodies of the two assay systems with the aligned cutoff for MESACUP of 5.3 U/ml.

A, All samples[a]

MESACUP	Cancer, n	Control, n	Total, n
+	91	10	101
-	761	522	1,283
Total	852	532	1,384

B, Esophageal samples[b]

MESACUP	Cancer, n	Control, n	Total, n
+	45	2	47
-	243	98	341
Total	288	100	388

C, Colorectal samples[c]

MESACUP	Cancer, n	Control, n	Total, n
+	36	1	37
-	199	99	298
Total	235	100	335

D, Breast samples[d]

MESACUP	Cancer, n	Control, n	Total, n
+	10	1	11
-	319	89	408
Total	329	90	419

[a]Cancer, healthy volunteers, autoimmune diseases, and benign diseases. [b]Esophageal cancer and esophageal benign diseases. [c]Colorectal cancer and colorectal benign diseases. [d]Breast cancer and breast benign diseases.

those for healthy volunteers and autoimmune diseases (Tables II and III). False-positive results may lead to unnecessary invasive procedures, such as biopsies, to confirm the diagnosis, and a higher specificity for an assay is desirable for daily clinical use, especially when tumor markers are used in combination. Different positivity rates of patients with cancer could also influence the inconsistency in results between the two methods. Table II shows that MESACUP missed a total of 16 patients with cancer, counting them as false-negatives, whereas the results were positive with Elecsys. In addition, Elecsys missed 39 patients with cancer, whereas positive results were obtained using MESACUP. A comparison of the cutoff values listed in the package inserts may suggest that Elecsys was developed with a focus on specificity, whereas MESACUP may focus on sensitivity.

Table V. Sensitivity and specificity between the two assay systems [Elecsys (cutoff, 0.05 μg/ml) and MESACUP (cutoff, 1.3 U/ml)] with the aligned cutoff.

A, All samples[a]

Diagnostic accuracy	Elecsys, % (95% CI)	MESACUP, % (95% CI)	P-value
Sensitivity	13.7 (11.5-16.2)	10.7 (8.7-13.0)	<0.001
Specificity	98.1 (96.6-99.1)	98.1 (96.6-99.1)	1.000

B, Esophageal samples[b]

Diagnostic accuracy	Elecsys, % (95% CI)	MESACUP, % (95% CI)	P-value
Sensitivity (All)	20.1 (15.7-25.2)	15.6 (11.6-20.3)	0.002
Sensitivity at stage I	11.9 (4.9-22.9)	10.2 (3.8-20.8)	>0.999
Sensitivity at stage II	24.4 (12.9-39.5)	15.6 (6.5-29.5)	0.219
Sensitivity at stage III	21.0 (14.5-28.8)	18.8 (12.7-26.4)	0.375
Sensitivity at stage IV	27.5 (14.6-43.9)	15.0 (5.7-29.8)	0.063
Specificity	97.0 (91.5-99.4)	98.0 (93.0-99.8)	>0.999

C, Colorectal samples[c]

Diagnostic accuracy	Elecsys, % (95% CI)	MESACUP, % (95% CI)	P-value
Sensitivity (All)	17.9 (13.2-23.4)	15.3 (11.0-20.6)	0.210
Sensitivity at stage I	10.0 (3.3-21.8)	12.0 (4.5-24.3)	>0.999
Sensitivity at stage II	18.6 (10.3-29.7)	14.3 (7.1-24.7)	0.250
Sensitivity at stage III	17.1 (9.7-27.0)	15.9 (8.7-25.6)	>0.999
Sensitivity at stage IV	33.3 (17.3-52.8)	23.3 (9.9-42.3)	0.250
Specificity	96.0 (90.1-98.9)	99.0 (94.6-100.0)	0.375

D, Breast samples[d]

Diagnostic accuracy	Elecsys, % (95% CI)	MESACUP, % (95% CI)	P-value
Sensitivity (All)	5.2 (3.0-8.1)	3.0 (1.5-5.5)	0.039
Sensitivity at stage I	3.1 (0.4-10.7)	1.5 (0.00-8.3)	>0.999
Sensitivity at stage II	5.3 (2.3-10.2)	3.3 (1.1-7.6)	0.375
Sensitivity at stage III	7.4 (3.0-14.6)	4.2 (1.2-10.4)	0.250
Sensitivity at stage IV	0.0 (0.0-23.2)	0.0 (0.0-23.2)	1.00
Specificity	0.0 (0.0-70.8)	0.0 (0.0-70.8)	1.00

[a]Cancer, healthy volunteers, autoimmune diseases, and benign diseases. [b]Esophageal cancer and esophageal benign diseases. [c]Colorectal cancer and colorectal benign diseases. [d]Breast cancer and breast benign diseases. CI, confidence interval.

To assess the diagnostic accuracy between the two methods, the clinical performance (sensitivity and specificity) after aligning the specificity of the two assay systems was compared (Fig. 2; Tables III and IV). A total of 49 samples from patients with cancer and 29 samples from controls changed status from positive to negative when applying the convetntional MESACUP assay. The new Elecsys assay was shown to demonstrate significantly higher sensitivity than MESACUP for esophageal and breast cancer. In addition, Elecsys was shown to be more sensitive than MESACUP in colorectal cancer, although the difference was not considered significant. These results indicate that in daily clinical practice, Elecsys performs as well as the MESACUP for the detection of esophageal and colorectal cancer.

Elecsys exhibits low sensitivity as a single-marker test, but its high specificity ($\geq$96.0%) allows its effective use in combination with other tumor markers, such as carcinoembryonic antigen (CEA), cytokeratin 19 fragment and squamous cell carcinoma antigen. Moreover, when combined with other tumor markers, Elecsys showed increased sensitivity
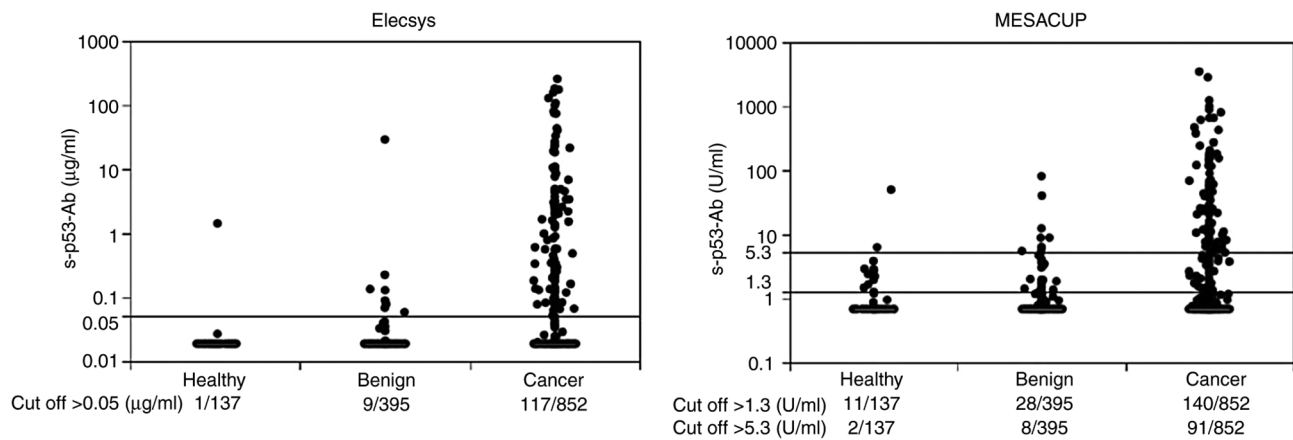
Figure 2. Distribution of s-p53-Abs titers by control and cancer groups for the two assay systems. Cutoff values of 0.05 for the Elecsys Anti-p53 and 1.3 and 5.3 for the MESACUP anti-p53 test, when specificity was aligned. Owing to the fact that many samples were measured below the limit of detection, statistical analysis was not performed.

in esophageal and colorectal cancer (12). The routine clinical use of anti-p53 in combination with other tumor markers is facilitated by its availability on an automated platform that allows s-p53-Abs to be measured simultaneously with multiple tumor markers vs. the manual MESACUP. As a successor to MESACUP, the STACIA MEBLux™ test anti-p53 (catalog no. 2385; Medical & Biological Laboratories Co., Ltd.) has become commercially available since starting the present study, and the reagent can be run on an automated platform with the same cutoff value and the same clinical performance (e.g., sensitivity and specificity) as the manual MESACUP. However, only limited parameters are available for analysis on the same platform. This means that the parallel measurement of other tumor markers, such as CEA or CA19-9, must rely on another platform, resulting in additional costs and reduced testing efficiency.

The present study exhibited several limitations. First, data on the immunoreactivity of p53 expression in the tumor tissues were not evaluated, and no data were collected after treatment. To evaluate tumor recurrence, it may be useful to monitor the antibody titer changes over time using these two assay systems. A prospective study should be conducted to confirm the clinical significance and the practical usefulness of anti-p53 monitoring using Elecsys. Second, as aforementioned, the accumulation of p53 in tumors and the subsequent immune response that is associated with the strong immunogenicity of the p53 protein has already been reported (2-5). Therefore, immunohistochemical staining to confirm the protein expression status was not conducted in this study.

In conclusion, Elecsys was found to be as useful as MESACUP and could be used to stratify patients with esophageal, colorectal and breast cancer. Understanding the diagnostic accuracy of tumor markers may facilitate the appropriate evaluation and treatment of patients.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TS, YO, SY, HO, HSh and HSu were responsible for the study design, and Hsu was responsible for performing the Elecsys. SY, FS, MS, HO, TH, SN, TNak, TNan and MU were responsible for sample data collection and data analysis. ME

performed the statistical data analysis. TS, YO, HSh and ME confirm the authenticity of all the raw data. TS, YO, and HSh drafted the initial version of the manuscript. All authors critically reviewed the manuscript, edited and approved the final version.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of Toho University Graduate School of Medicine (Tokyo, Japan; approval no. A16049) and the Institutional Review Boards of each participating hospital. Serum was collected from patients who had provided written informed consent.

Patient consent for publication

Written informed consent for publication was collected.

Competing interests

HSh received research funding from Ono Pharmaceutical, Taiho Pharmaceutical and Roche Diagnostics K.K. HSu was an employee of Roche Diagnostics K.K. ME is an employee of Roche Diagnostics GmbH. The Elecsys assay system is manufactured by Roche Diagnostics K. K.

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