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Original Article

Urinary N1, N12-Diacetylspermine Level in the Patients with Various Cancer; A Pilot Study in Seven Types of Cancer

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ABSTRACT

Introduction: N1, N8-Diacetylspermidine and N1, N12-diacetylspermine (DAS) are useful tumor markers. Herein, we investigated the urinary DAS-positive rates for various cancers.

Methods: Urinary DAS levels were measured in 40 urine samples from patients with esophageal (n = 9), gastric (n = 10), breast (n = 10), thyroid (n = 1), biliary tract (n = 3), and pancreatic (n = 5) cancers and hepatocellular carcinoma (HCC) (n = 2). The DAS cut-off value was set so that 95% of healthy subjects were negative.

Results: Overall positive rate of urinary DAS was 30%. The positive rates of DAS and tumor markers in each cancer type were as follows. In esophageal cancer, DAS: 33%, CEA: 13%; SCC-Ag: 63%, serum p53 antibody (s-p53-Abs): 22%. In gastric cancer, DAS: 40%, CEA: 10%, CA19-9: 10%, CA72-4: 10%, s-p53-Abs: 10%, AFP: 0%, CA125: 0%. In breast cancer, DAS: 20%, CEA: 0%, p53: 11%, CA15-3: 11%, BCA225: 11%, NCC-ST 439: 22%. In biliary pancreatic cancer: DAS: 25%, CEA: 13%, CA19-9: 63%, s-p53-Abs: 14%, DUPAN-2: 13%, Span1: 50%, Elastase1: 40%. In hepatocellular carcinoma, the number of DAS-positive cases was 1/2, and CEA was 0/2, CA19-9 was 0/2, p53-Abs was 1/1, AFP was 1/2, AFP-L3 was 0/2, PIVKA-2 was 1/2. In thyroid cancer, the number of DAS-positive cases was 0/1, and thyroglobulin was 1/1. The DAS-positive rates were 20%-50%. Of the 12 DAS-positive patients, 5 were positive only for DAS.

Conclusions: Urinary DAS-positive rates in various cancers ranged from 20% to 50%. Combining DAS with other markers may improve marker sensitivities.

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KEYWORDS: diacetylspermine, urine tumor marker, esophageal cancer, gastric cancer, breast cancer

Introduction

Polyamines, with polyamine concentrations increasing during malignant transformation due to cell proliferation,[1)] are essential for normal cell proliferation, gene expression, membrane stabilization, apoptosis, and organogenesis. Uri-

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Table 1 Characteristics of the patients

Types of cancer	Number of cases	Age	Gender ratio	Number of cases by stage
Esophageal cancer	9	47-75 (average 64.4)	6:3	I:3 II:1 III:4 IV:1
Gastric cancer	10	50-77 (average 65.7)	7:3	I:7 II:1 III:2 IV:0
Breast cancer	10	40-82 (average 60.6)	0:10	I:5 II:2 III:2 IV:1
Thyroid cancer	1	26	0:1	I:0 II:1 III:0 IV:0
Hepatobiliary pancreatic cancer *	10	55-83 (average 71.5)	6:4	I:1 II:7 III:2 IV:0

*biliary tract cancer: 3 cases Pancreatic cancer: 5 cases Hepatocellular carcinoma: 2 cases

nary polyamine excretion in cancer patients was initially reported in 1971.[2] Urinary N1, N8-Diacetylspermidine and N1, N12-diacetylspermine (DAS), which are trace components of human urinary polyamines, account for less than 0.5% of human urinary polyamines.

It has been reported that DAS may be a useful tumor marker[3–5] for urogenital malignancies,[4] breast cancer,[6] colorectal cancer,[1] lung cancer,[7] pancreatic biliary tract cancer,[8] ovarian cancer,[9] and hepatocellular carcinoma.[10] In addition, DAS increases from early stages of colorectal or breast cancer.[11, 12] Recently, urinary DAS levels have been investigated mainly in colorectal cancer. While DAS is a noninvasive, highly sensitive, and specific biomarker,[13] we find that although fecal occult blood tests and blood tumor markers are inexpensive as a tool of mass screening, they showed relatively low sensitivity and specificity. Although only a few reports have evaluated urinary DAS levels in various solid tumors other than colorectal cancer, we see that urinary DAS is also a useful marker for early stage cancer types other than colorectal cancer.[6, 12]

Therefore, while conventional serum tumor markers, CEA, CA19-9, and serum p53 antibody, were also assessed for comparisons, we examined urinary DAS levels in various solid tumors rather than colorectal cancer as an exploratory study.

Methods

Patients

Urinary DAS levels were measured in 40 urine samples from cancer patients with esophageal (n = 9), gastric (n = 10), breast (n = 10), thyroid (n = 1), biliary tract (n = 3), and pancreatic (n = 5) cancers and hepatocellular carcinoma (HCC) (n = 2) (Table 1). The DAS cut-off value was set so that 95% of the healthy subjects (3890 men, 1869 women, total 5759) were negative (Fig. 1, Fig. 2). Patient urine samples were collected at the first visit, and urinary DAS levels were measured and compared with existing tumor markers.

Assay for urinary DAS

Urinary DAS was measured using the colloidal gold agglutination method previously reported by Kawakita.[14] This method is based on the specific binding of bovine serum albumin (BSA)-acetylspermine conjugate (DAS mimic) to the colloidal gold-antibody complex to produce a stable red-purple solution. The BSA-acetylspermine conjugate induces agglomeration of colloidal gold particles, causing a color change from red-purple to gray. With the urine sample with DAS being added, DAS stops the color change by competitively binding to the gold colloid-antibody complex, and the concentration of DAS can be measured by the color change of the solution, and we see that the monovalent antigen, DAS, competes with the BSA-acetylspermine conjugate for binding to the colloidal gold-antibody complex.

Statistical analysis

McNemar's test were employed to compare the groups. All statistical analyses were conducted using EZR,[15] a graphical user interface for R (The R Foundation for Statistics Computing, version 2.13.0). A P-value of <0.05 was considered statistically significant.

Results

Positive rates of DAS and tumor markers in each cancer

The overall positive rates for DAS and CEA were 30% and 8%, respectively (P = 0.03). The positive rates are presented in Fig. 3. The positive rates for the esophageal cancer were as follows: DAS: 33%, CEA: 13%, SCC-Ag: 63%,

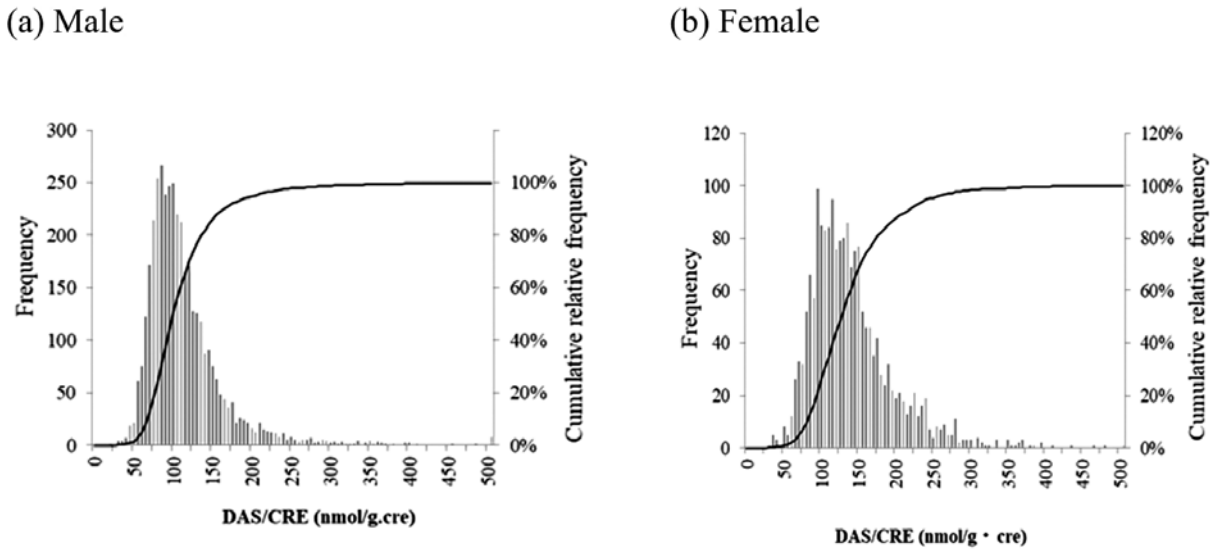

Fig. 1
Histogram of urinary DAS levels in healthy subjects. (a) Male, (b) Female

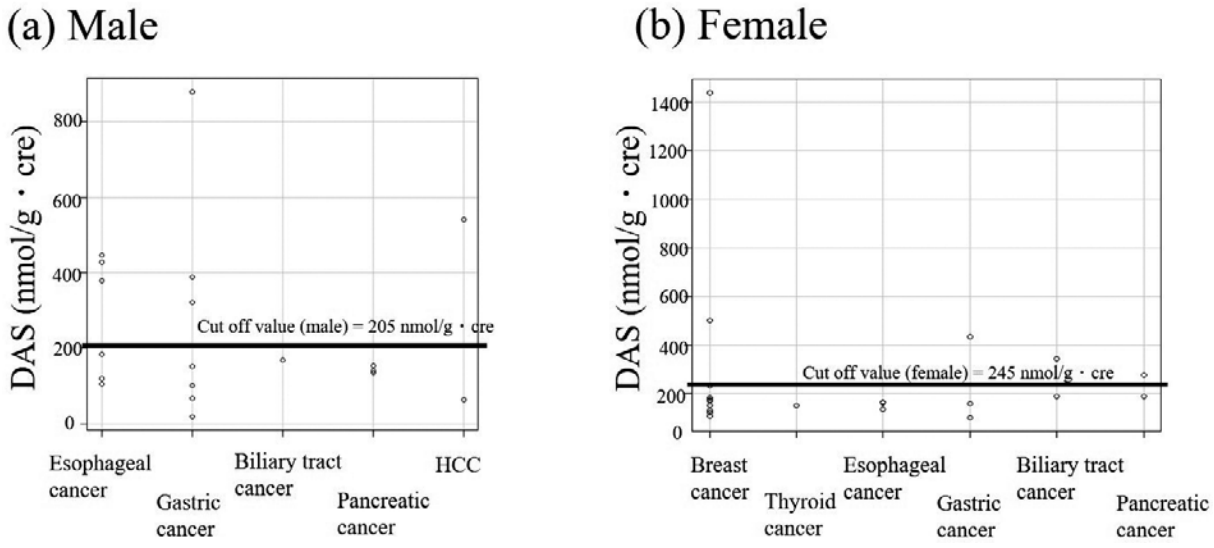

Fig. 2
The values of urine DAS of each cancer type. (a) Male, (b) Female

and s-p53-Ab: 22% (Fig. 3). The positive rates for the gastric cancer were DAS: 40%, CEA: 10%, CA19-9: 10%, CA72-4: 10%, p53: 10%, AFP: 0%, and CA125: 0% (Fig. 3). The positive rates for the biliary pancreatic cancer were DAS: 25%, CEA: 13%, CA19-9: 63%, p53: 14%, DUPAN2 13%, Span1 50%, and Elastase1 40%. The positive rates for the breast cancer were DAS: 20%, CEA: 0%, s-p53-Ab: 11%, CA15-3: 11%, BCA225: 11%, and NCC-ST439: 22% (Fig. 3). In hepatocellular carcinoma, the number of DAS-positive cases was 1/2, and CEA positive cases was 0/2, CA19-9 positive cases were 0/2, p53-Abs positive cases were 1/1, AFP positive cases were 1/2, AFP-L3 positive cases was 0/2, PIVKA-2 positive cases was 1/2. In thyroid cancer, the number of DAS-positive cases was 0/1, and thyroglobulin positive cases was 1/1. The overall urinary DAS-positive rates ranged from approximately 20% to 50% (Fig. 3).

Positive rates of DAS, CEA, and s-p53-Ab at each stage

In stage I cancers (n = 16), while When DAS, CEA, and s-p53-Ab were combined, the positive rates were 26% for stage I, 36% for stage II, and 100% for stage III/IV, the

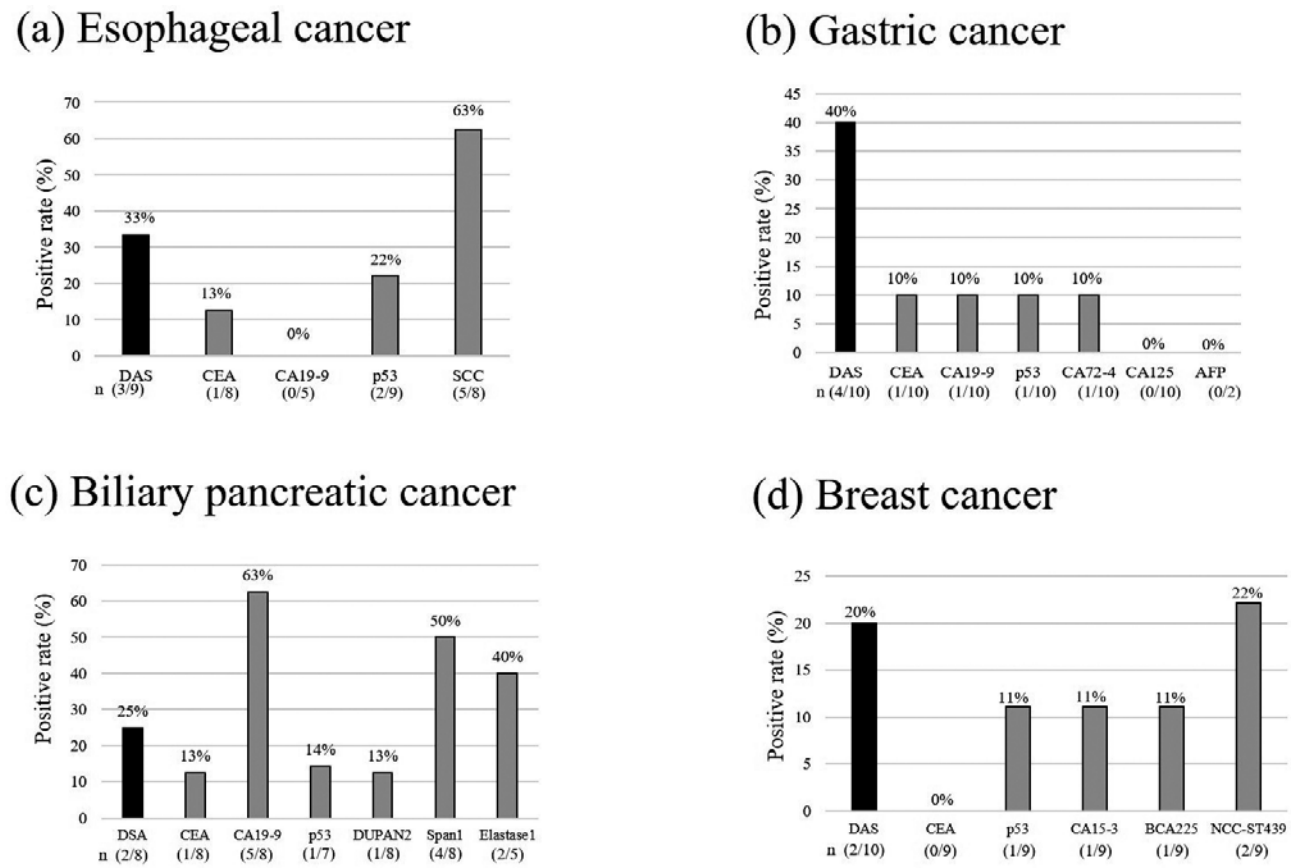

Fig. 3
The positive rates of DAS and conventional tumor markers. (a) Esophageal cancer, (b) Gastric cancer, (c) Biliary pancreatic cancer, (d) Breast cancer

positive rates for DAS, CEA, and s-p53-Ab were 19%, 7%, and 0% respectively (Fig. 4). In stage II cancers (n = 12), the positive rates for DAS, CEA, and s-p53-Ab were 17%, 9%, and 10%, respectively. In stage III cancers (n = 10), the positive rates for DAS, CEA, and s-p53-Ab were 60%, 10%, and 40% respectively.

Clinicopathological characteristics of DAS-positive cases

Of the cases in which conventional tumor markers were negative, DAS was positive in one esophageal cancer case, three gastric cancer cases, and one biliary tract cancer case (Fig. 5). Of the 12 cases in which DAS was positive, 5 were classified as stage I or II. In addition, in 5 of the 12 cases, only DAS was positive, but all conventional markers were negative (Table 2).

Discussion

In this exploratory study, we examined the urinary DAS-positive rates in various solid cancers other than colorectal cancer. The overall DAS-positive rate was 30%. Thus, urinary DAS may also be positive in various cancers other than colorectal cancer. Positive rates for each cancer type with at least 5 cases were as follows: esophagus, 33%; stomach, 40%; breast, 20%; biliary pancreas, 25%. The number of positive cases for each cancer type with fewer than 5 cases was as follows: thyroid, 0/1; liver, 1/2. The positive rates were significantly increased by combining DAS with CEA and serum p53 antibody.

Urinary DAS-positive rates were higher in advanced tumors than the positive rates in relatively early tumors. However, while combining urinary DAS with conventional tumor markers, such as CEA, may improve the sensitivity of the markers, furthermore, urinary DAS was useful even in stage I/II cases, as it was positive even at relatively early stages.

Like CEA, DAS tended to be less specific by cancer type. The characteristic of being positive in various cancers may be useful for comprehensive early cancer screening. The combination of DAS and the serum p53 antibody may be useful for comprehensive early cancer screening,

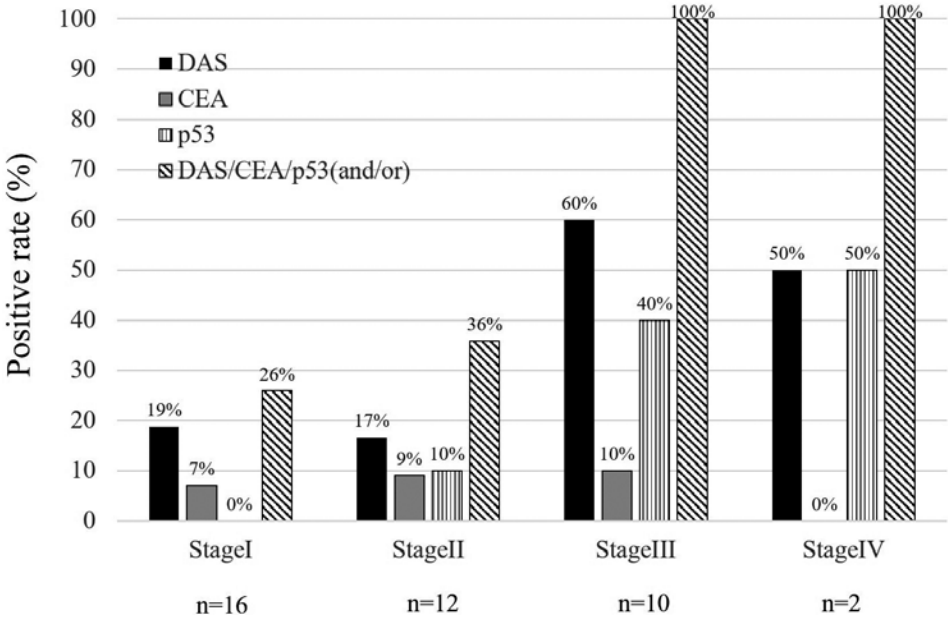

Fig. 5

Comparison of positive rates of DAS, carcinoembryonic antigen, serum p53 antibody, and DAS + carcinoembryonic antigen + serum p53 antibody combination in 7 types of cancer patients according to the tumor stage

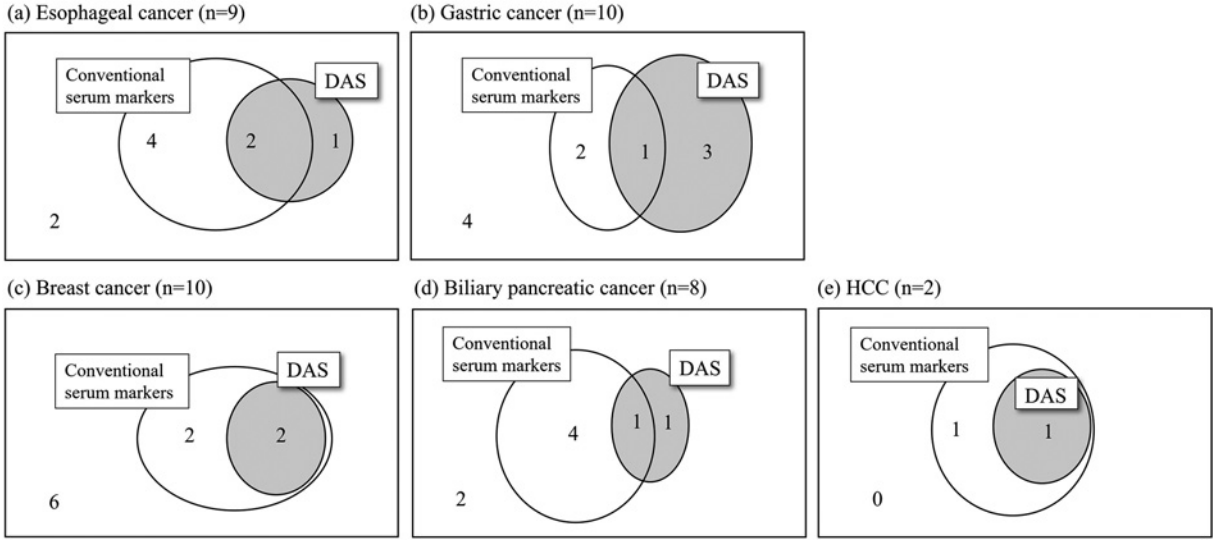

Fig. 5

Relationship between conventional tumor marker positive patients and DAS-positive patients. (a) Esophageal cancer, (b) Gastric cancer, (c) Breast cancer, (d) Biliary pancreatic cancer, (e) HCC

owing to the serum p53 antibody also being positive from a relatively early stage in various cancers.[16] DAS can be measured by an automatic analyzer for biochemistry, which is widely used in clinical laboratories, and results can be obtained in about 10 minutes after the specimen is loaded into the analyzer. It would be minimally invasive and of high public health benefit if early cancer screening using urine samples were possible.

On the other hand, in advanced cancers, it has been reported that cancer recurrence of urogenital malignancies was accompanied by an increase in DAS.[5] There is a possibility that tumor progression can be monitored by a sim-

Table 2 Characteristics of patients positive for urinary DAS

Types of cancer	Age	Gender	Stage	DAS (nmol/g・cre)	CEA (ng/ml)	P53 (U/ml)	Other tumor markers		
Esophageal cancer	62	Male	IVA	*380*	2.7	*55.8*	CA19-9 6.8 U/ml	SCC *2.9* ng/ml	
Esophageal cancer	75	Male	I	*447*	1.8	0.40	CA19-9 7.4 U/ml	SCC 1.03 ng/ml	
Esophageal cancer	68	Male	III	*428*	1.5	0.59	CA19-9 13.1 U/ml	SCC *1.63* ng/ml	
Gastric cancer	62	Male	IA	*880*	0.8	0.40	CA19-9 5.3 U/ml	CA72-4 2.8 U/ml	CA125 14.5 U/ml
Gastric cancer	64	Male	IIA	*388*	2.6	0.40	CA19-9 5.8 U/ml	CA72-4 1.4 U/ml	CA125 14.8 U/ml
Gastric cancer	74	Female	IIIC	*434*	1.0	*13.5*	CA19-9 11.7 U/ml	CA72-4 *10.4* U/ml	CA125 17.4 U/ml
Gastric cancer	77	Male	IIIA	*321*	1.2	0.40	CA19-9 21.1 U/ml	CA72-4 1.5 U/ml	CA125 8.5 U/ml
Breast cancer	56	Female	IIIB	*501*	1.8	*4.90*	CA15-3 18.2 U/ml	BCA225 90.5 U/ml	NCCST439 4.3 U/ml
Breast cancer	40	Female	IIIB	*1439*	1.1	0.40	CA15-3 *61.3* U/ml	BCA225 *523* U/ml	NCCST439 2.4 U/ml
Biliary tract cancer	76	Female	IA	*344*	1.4	0.40	CA19-9 8.3 U/ml	DUPAN2 25 U/ml	Span-1 7.8 U/ml
Pancreatic cancer	70	Female	IIB	*276*	2.4	*22.1*	CA19-9 *54.3* U/ml	DUPAN2 27 U/ml	Span-1 24 U/ml Elastase-1 204 ng/dl
HCC	73	Male	III	*540*	3.0	*229*	CA19-9 27.0 U/ml	AFP 0.9 nfg/ml	AFPL3 0.5% PIVKAII 22 mAU/ml

ple test, which is expected to reduce patient burden.

Limitations of the study

Since this study was an exploratory study, there were several limitations. As we are currently conducting a multi-institutional study, the number of cases in this study was small, and the study was conducted at a single institution. In addition, an evaluation of perioperative changes in urinary DAS levels (comparing DAS levels before and after surgery) is needed. Furthermore, an investigation into the usefulness of DAS in predicting recurrence after surgery should be conducted.

Conclusion

With only approximately half of the urinary DAS-positive cases being positive only for urinary DAS, these urinary DAS levels were positive in solid tumors other than colorectal cancer. Despite the small sample size of the study, the urinary DAS-positive rates for various cancers were in the range of 20%-50%. Therefore, positive detection rates of conventional tumor markers may improve when combined with urinary DAS.

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Authors' contribution: T.N. and H.S. conceived and designed the study. F.S., T.S., Y.O., S.Y., Y.O., H.O., and K.F. analyzed the data. T.N. and H.S. analyzed patient data and drafted the manuscript.

Ethics statement: This study was approved by the Institutional Ethics Committee of Toho University Omori Medical Center Tokyo Japan (approval no. M20199 18143). Additionally, written informed consent was obtained from all patients.

Conflicts of interest: Hideaki Shimada received a research funds from Alfresa Pharma Corporation.

References

1) Venäläinen MK, Roine AN, Häkkinen MR, Vepsäläinen JJ, Kumpulainen PS, Kiviniemi MS, et al. Altered polyamine profiles in colorectal cancer. Anticancer Res. 2018; 38: 3601-7.
2) Russell DH. Increased polyamine concentration in the urine of human cancer patients. Nat New Biol. 1971; 233: 144-5.
3) Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, et al. Determination of amounts of polyamines excreted in urine; demonstration of N1, N8-diacetylspermidine and N1, N12-diacetylspermine as components commonly occurring in normal human urine. J Biochem. 1995; 117: 107-12.
4) Sugimoto M, Hiramatsu K, Kamei S, Kinoshita K, Hoshino M,

Iwasaki K, et al. Significance of urinary N 1, N 8-diacetylspermidine and N1, N12-diacetylspermine as indicators of neoplastic diseases. J Cancer Res Clin Oncol. 1995; 121: 317-9.
5) Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, et al. Diagnostic and prognostic usefulness of N1, N8-diacetylspermidine and N1, N12-diacetylspermine in urine as novel markers of malignancy. J Cancer Res Clin Oncol. 1997; 123: 539-45.
6) Umemori Y, Ohe Y, Kuribayashi K, Tsuji N, Nishidate T, Kameshima H, et al. Evaluating the utility of N1, N12-diacetylspermine and N1, N8-diacetylspermidine in urine as tumor markers for breast and colorectal cancers. Clin Chim Acta. 2010; 411: 1894-9.
7) Takahashi Y, Sakaguchi K, Horio H, Hiramatsu K, Moriya S, Takahashi K, et al. Urinary N1, N12-diacetylspermine is a non-invasive marker for the diagnosis and prognosis of non-small-cell lung cancer. Br J Cancer. 2015; 113: 1493-501.
8) Yamaguchi K, Nakamura M, Shirahane K, Konomi H, Torata N, Hamasaki N, et al. Urine diacetylspermine as a novel tumour maker for pancreatobiliary carcinomas. Dig Liver Dis. 2005; 37: 190-4.
9) Niemi RJ, Roine AN, Häkkinen MR, Kumpulainen PS, Keinänen TA, Vepsäläinen J, et al. Urinary polyamines as biomarkers for ovarian cancer. Int J Gynecol Cancer. 2017; 27: 1360-6.
10) Enjoji M, Nakamuta M, Arimura E, Morizono S, Kuniyoshi M, Fukushima M, et al. Clinical significance of urinary N1, N12-diacetylspermine levels in patients with hepatocellular carcinoma. Int J Biol Markers. 2004; 19: 322-7.
11) Moriya SS, Hiramatsu K, Kimura E, Matsumoto K, Kawakita M. Construction of an immunochromatographic determination system for N^{1}, N^{12}-diacetylspermine. J Clin Lab Anal. 2014; 28: 452-60.
12) Hiramatsu K, Takahashi K, Yamaguchi T, Matsumoto H, Miyamoto H, Tanaka S, et al. N(1), N(12)-diacetylspermine as a sensitive and specific novel marker for early- and late-stage colorectal and breast cancers. Clin Cancer Res. 2005; 11: 2986-90.
13) Deng Y, Yao H, Chen W, Wei H, Li X, Zhang F, et al. Profiling of polar urine metabolite extracts from Chinese colorectal cancer patients to screen for potential diagnostic and adverse-effect biomarkers. J Cancer. 2020; 11: 6925-38.
14) Kawakita M, Hiramatsu K, Yanagiya M, Doi Y, Kosaka M. Determination of N1, N12-diacetylspermine in urine: a novel tumor marker. Methods Mol Biol. 2011; 720: 367-78.
15) Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013; 48: 452-8.
16) Nanami T, Hoshino I, Shiratori F, Yajima S, Oshima Y, Suzuki T, et al. Presence of serum RalA and serum p53 autoantibodies in 1833 patients with various types of cancers. Int J Clin Oncol. 2022; 27: 72-6.