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The performance of a novel amino acid multivariate index for detecting lung cancer: A case control study in Korea

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ABSTRACT

Introduction: Previous studies have shown that plasma free amino acid (PFAA) profiles are altered in cancer patients compared with healthy controls. A multivariate index based on PFAAs was generated from a Japanese dataset and has been previously demonstrated to be clinically valuable for discriminating patients in the early stages of lung cancer. However, it remains unclear whether similar PFAA profile changes occur in cancer patients from other populations. Therefore, this study aimed to validate the performance of this index in discriminating lung cancer patients from controls in the Korean population.
Methods: Samples were collected from a total of 142 Korean subjects (72 lung cancer/70 controls) for this study. PFAAs were quantified by high-performance liquid chromatography–electrospray ionization-mass spectrometry, and the clinical performance characteristics of the amino acid multivariate index were evaluated across cancer stages and histological types.
Results: The concentrations of several PFAAs were significantly decreased in the Korean lung cancer patients compared with the controls. Significant decreases in threonine, citrulline, histidine and tryptophan and increases in proline, isoleucine, phenylalanine and ornithine were observed, which are similar to the PFAA changes reported by a previous Japanese study. The area under the receiver-operator characteristic curve (AUC of the ROC) for the index was 0.80, and similar performances were demonstrated for the different histological types.
Conclusions: These results suggest that the amino acid multivariate index previously developed from a Japanese dataset has the potential to aid in the early detection of lung cancers of different histological types in Korean patients.

1. Introduction

Amino acids play important roles as both basic metabolites and physiological regulators, and their metabolism is closely related to other metabolic networks, such as glucose and lipid metabolism. Accordingly, their metabolism in organs is strictly regulated, and the concentrations of plasma free amino acids (PFAAs), which are abundant in the circulation and exchanged among all organ systems, remain constant in a healthy state. However, numerous studies have shown that various diseases, such as hepatic failure, kidney failure, cancer, diabetes and Alzheimer's disease, are able to influence the concentrations of PFAAs [1–5], suggesting that they may be suitable biomarkers to discriminate these patients from healthy subjects. For example, a previous study has reported that

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among 61 potential metabolites, five branched-chain and aromatic amino acids are biomarkers for predicting the future onset of type 2 diabetes [6].

Many groups have reported that PFAA profiles differ significantly in cancer patients, even among those with different types of cancer [7–12]. The recently reported "AminoIndex Technology" facilitates the evaluation of certain health conditions and the possibility of disease by analyzing the balance of PFAAs [13–15]. These publications have established amino acid multivariate indices using the above technology and have clinically validated them for the discrimination of various cancer types, including lung, gastric, colorectal, breast, prostate and gynecological cancers [13]. These indices compress the multidimensional PFAA profile information into a single dimension and maximize differences between patients and controls. Using this technology, the "AminoIndex® Cancer Screening" service is now commercially available in Japan. The robustness of the amino acid multivariate indices has been verified using large case-control datasets collected at multiple medical institutes in Japan [16,17], and they have been shown to be able to detect cancer regardless of the stage or histological type. However, there are currently no data regarding the PFAA profiles in cancer patients other than Japanese patients or of the differences in test performance characteristics among populations.

In Korea, approximately 218,000 people are diagnosed with cancer annually. Approximately 72,000 persons died from cancer in 2011, and this number is increasing [18]. Although national cancer screening programs were initiated in 1999 in Korea, there is no screening program for lung cancer, which is the leading cause of cancer deaths in Korea [19]. Chest X-rays and sputum cytology are two screening tests that have been used to detect markers of lung cancer, but neither is sufficiently sensitive to detect this cancer at the early stages across different histological types. For example, chest X-rays are not always suitable for the early detection of cancer [20] and require highly skilled technicians to achieve sufficient accuracy. In addition, it has been reported that sputum cytology is useful only for the detection of squamous cell carcinoma but not adenocarcinoma [20]. Although low-dose helical computed tomography (LDCT) has been recently reported to be a new screening method, it may produce a high rate of false positives [21] and exposes subjects to radiation. Therefore, there is an urgent need for new, non-invasive methods to detect lung cancer at an early stage for all histological subtypes.

In the present study, we focus on a previously developed amino acid multivariate index for lung cancer, and we aimed to investigate whether the PFAA profile changes in Korean lung cancer patients are similar to those in Japanese patients and to evaluate the performance of the index for the detection of lung cancer across different stages and histological types in Korean patients.

2. Research design and methods

2.1. Methods

2.1.1. Ethics

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committees of Konkuk University Medical Center, Inha National University Hospital, Chonnam National University Hwasun Hospital, Chungnam National University Hospital, Hallym University Medical Center, Kyungpook National University Medical Center, Pusan National University Hospital, and Kosin University Gospel Hospital. All subjects gave their written informed consent for inclusion before participating in the study. All data were analyzed anonymously throughout the study.

2.1.2. Subjects

The participants comprised Korean patients who had been histologically diagnosed with lung cancer at any of the 8 included Korean medical hospitals ($n = 75$), as well as sex- and age-matched control subjects without cancer ($n = 80$).

2.1.3. Quantification of plasma amino acid concentration

Blood samples (5 ml) were collected from the forearm veins in the morning after overnight fasting into tubes containing ethylenediaminetetraacetic acid (EDTA) and immediately placed on ice. Plasma was prepared by centrifugation at 3000 rpm at 4 °C for 15 min and was then stored at −80 °C until analysis. The plasma samples were deproteinized using acetonitrile to a final concentration of 80% and subjected to precolumn derivatization followed by high-performance liquid chromatography (HPLC)–electrospray ionization (ESI)-mass spectrometry (MS) for amino acid quantification, as described previously [22–24]. The 19 amino acids that were quantified included the following: alanine (Ala), arginine (Arg), asparagine (Asn), citrulline (Cit), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val). The concentrations of the amino acids in the plasma are expressed in μM, and the percentages of the amino acid concentrations compared with the sum of all of the amino acids (the

Table 1
Clinical characteristics of the subjects.

		Lung cancer	Controls	*P*
Number	Total	72	70	
	(Male, female)	(51, 21)	(44, 26)	
Age, year	Mean ± SD	65.6 ± 9.2	63.2 ± 8.9	NS
	Range	45–82	39–78	
BMI, kg/m^2	Mean ± SD	22.2 ± 3.4	23.3 ± 2.3	0.042*
	Range	13.9–29.5	17.8–28.2	
Smoking status	Never	19	34	
	Ex	13	25	
	Current	40	8	
	Unknown	0	3	
Clinical stage	I	14		
	II	5		
	III	21		
	IV	32		
Histology	Adenocarcinoma	37		
	Squamous cell carcinoma	30		
	Other NSCLC	4		
	Unknown	1		

Significant differences were evaluated by Student's *t*-test (*$P < 0.05$).

relative amino acid value) are expressed by the following previously described equation:

$$X2_{i,j} = \frac{X_{i,j}}{\sum_k X_{i,k}}$$

where $X2_{i,j}$ is the relative amino acid value of the j-th amino acid of the i-th subject, and $X_{i,j}$ is the plasma concentration (μM) of the j-th amino acid of the i-th subject.

2.1.4. Calculation of amino acid multivariate index scores

The PFAA profiles of the subjects were evaluated with a previously established amino acid multivariate index. These indices are logistic regression functions that discriminate lung cancer patients from healthy controls. Index-1, which is used in the "AminoIndex® Cancer Screening" service in Japan, consists of Ser, Gln, Ala, His, Orn and Lys [17,25], and index-2, which has been previously reported, comprises Ala, Val, Ile, His, Trp and Orn [16].

2.2. Statistical analysis

2.2.1. Mean and standard deviation (SD)

The mean amino acid concentrations ± SD were calculated to determine the overall PFAA profiles for both the controls and patients.

2.2.2. Mann–Whitney U-test

The Mann–Whitney *U*-test was used to evaluate the differences in the PFAA profiles between the controls and patients.

2.2.3. ROC curve analysis

Receiver-operator characteristic (ROC) curve analyses were performed to evaluate the performances of both the PFAA concentrations and the multivariate PFFA index in discriminating between patients and controls. The patient labels were fixed as positive class labels. Thus, a value <0.5 for the area under the ROC curve (AUC of the ROC) indicated that the amino acid level was lower in the patients than in the controls, whereas an AUC of the ROC of >0.5 indicated a higher level in the controls. A 95% confidence interval (95% CI) for the AUC of the ROC for the discrimination of patients based on amino acid concentrations and relative values was also estimated, as described by Hanley and McNeil [26].

2.2.4. Software

All of the analyses were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Characteristics of Korean subjects in this study

Between February 2013 and November 2013, 80 lung cancer patients and 75 controls were enrolled in this study. Subjects whose blood was collected during the afternoon were excluded from the analysis based on previous study data indicating the circadian fluctuations of PFAAs [27]. Therefore, 72 lung cancer patients and 70 controls were analyzed in total. Table 1 summarizes the characteristics of the subjects in this study. No significant differences in sex distribution or age were observed between the lung cancer patients and controls. The body mass index (BMI) of the patients was significantly lower than that of the controls.

Disease stages were determined according to the Sixth Edition of the International Union Against Cancer (UIGC) Tumor-Node-Metastasis (TNM) Classification of Malignant Tumors. The fractions of patients at each stage were as follows: ~20% stage I, ~5% stage II, ~30% stage III, and ~45% stage IV. The patients were also subdivided according to histological tumor type. Approximately 50% of the patients had adenocarcinoma, and approximately 40% had squamous cell carcinoma.

Table 2
Comparison of plasma amino acid concentrations between cancer patients and healthy controls.

	Lung Cancer		Controls				
Amino acids (μM)	Mean	SD	Mean	SD	AUC	*P*	
Thr	109.8	30.6	127.2	28.8	0.340	**<0.001**	***
Ser	110.4	27.3	112.6	21.2	0.464	0.459	
Asn	44.4	11.0	49.2	9.1	0.352	**0.002**	**
Gln	556.8	115.2	603.9	69.9	0.337	**<0.001**	***
Pro	165.0	48.9	152.3	39.1	0.572	0.140	
Gly	204.8	55.2	225.2	44.7	0.383	**0.016**	*
Ala	357.0	124.2	383.0	86.7	0.386	**0.019**	*
Cit	28.9	9.0	37.1	10.3	0.261	**<0.0001**	****
Val	222.0	43.9	241.1	43.7	0.370	**0.008**	**
Met	25.8	6.7	28.3	5.1	0.361	**0.004**	**
Ile	69.6	16.4	68.6	14.4	0.503	0.958	
Leu	123.9	29.1	133.4	26.6	0.385	**0.018**	*
Tyr	68.4	15.7	70.3	12.7	0.469	0.523	
Phe	68.5	16.3	63.4	9.6	0.571	0.145	
His	66.8	13.8	82.6	10.3	0.180	**<0.0001**	****
Trp	48.9	10.7	59.3	10.9	0.222	**<0.0001**	****
Orn	70.3	24.3	68.1	18.9	0.517	0.736	
Lys	183.5	42.5	198.5	38.3	0.384	**0.017**	*
Arg	90.4	25.8	100.2	20.2	0.374	**0.009**	**

Significant differences were determined by the Mann–Whitney *U*-test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
**** $P < 0.0001$.

3.2. PFAA profiles of lung cancer patients

To compare the PFAA profiles between the lung cancer patients and controls, the amino acids concentrations were quantified, and the AUCs of the ROC for each amino acid were determined (Table 2). Significant decreases in the concentrations of Thr, Asn, Gln, Gly, Ala, Cit, Val, Met, Leu, His, Trp, Lys and Arg were observed in the lung cancer patients. Because a global decrease in the PFAA concentrations was observed, ROC analysis using the relative values of each amino acid was conducted (Fig. 1A). Comparisons of the relative values revealed significant decreases in the Thr, Cit, His and Trp levels and significant increases in the Pro, Ile, Phe and Orn levels.

The PFAA profiles across the clinical stages were also compared (Fig. 1B). In the early stages of lung cancer (stages I and II), significant decreases in the relative levels of Thr, Asn, Cit, Met, His and Trp and significant increases in the relative levels of Pro and Ile were observed. Significant decreases in the relative levels of Thr, Cit, His and Trp and significant increases in the Pro, Ile, Phe and Orn levels were observed during the late stages (stages III and IV).

3.3. Clinical performance evaluation

The amino acid multivariate index for the discrimination of lung cancer patients from controls includes the following six amino acids: Ser, Gln, Ala, His, Orn and Lys. This index (index-1) is used by the "AminoIndex® Cancer Screening" service in Japan and was generated from logistic regression models using PFAA data collected from a Japanese multisite study consisting of 996 controls and 200 lung cancer patients [25]. To evaluate the performance of this index in Korean subjects, the ROC curve of the index for discriminating lung cancer patients from controls in the current study is illustrated in Fig. 2. The AUC of the ROC reached 0.80 (95% CI: 0.72–0.87). The AUC of ROC of another function (index-2), which

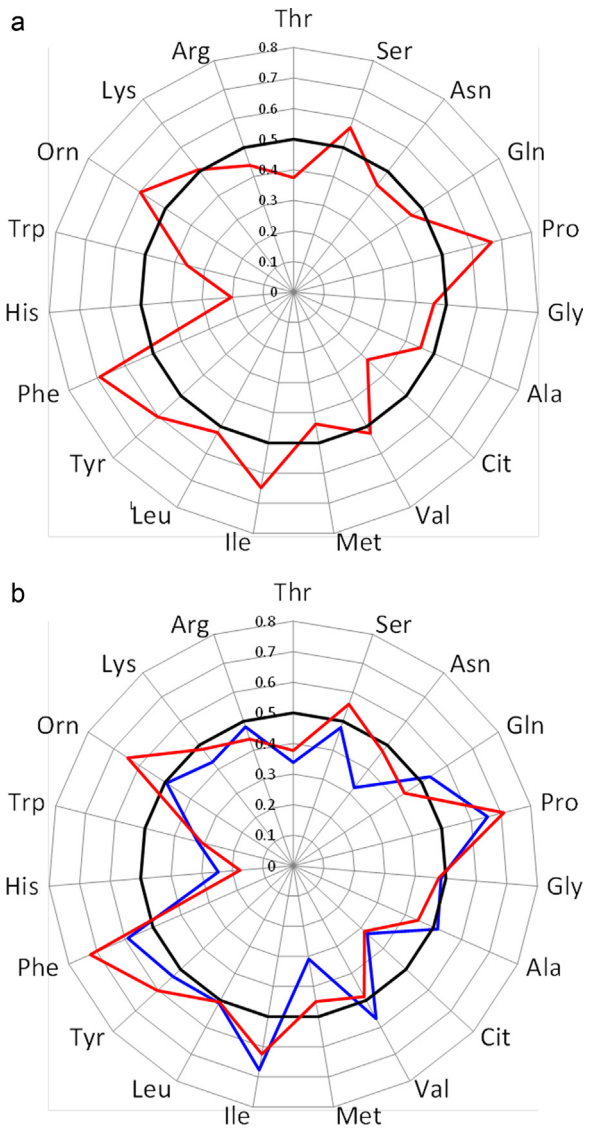

Fig. 1. PFAA profiles of Korean lung cancer patients.
The axes show the AUC of the ROC for the relative value of each amino acid to discriminate lung cancer patients from controls. The bold black line indicates the point at which the AUC of the ROC = 0.5. (A) PFAA profiles of lung cancer patients. Significant increases in Pro, Ile, Phe, and Orn and significant decreases in Thr, Cit, His, and Trp were observed. (B) Comparison of the PFAA profiles of lung cancer patients across clinical stages. The blue line corresponds with the early stages of cancer (stages I and II), during which significant increases in Pro and Ile and significant decreases in Thr, Asn, Cit, Met, His and Trp were observed. The red line indicates the later stages of cancer (stages III and IV), during which significant increases in Pro, Ile, Phe and Orn and significant decreases in Thr, Cit, His and Trp were observed.

has also been reported to discriminate lung cancer patients in a previous Japanese study, was 0.72 (95% CI: 0.64–0.81).

3.4. Performance of index across clinical stages and histological types

To further investigate the performance of the index-1 in discriminating lung cancer patients across different clinical stages and histological tumor types, the AUCs of the ROC for each stage and each histological type were compared (Fig. 3) and were found to be 0.70 (95% CI: 0.57–0.83) for the stages I and II patients, 0.89 (95% CI: 0.82–0.96) for the stage III patients and 0.79 (95% CI: 0.70–0.88) for the stage IV patients. In addition, this index demonstrated a similar performance for both adenocarcinoma and squamous cell

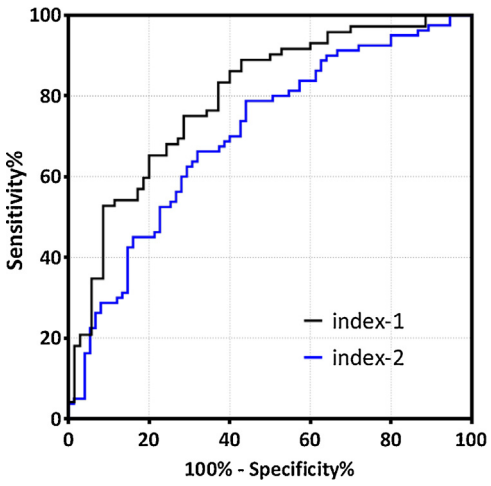

Fig. 2. The ROC curve of the index for discriminating lung cancer patients.
The ROC curve of the indices for distinguishing lung cancer patients from controls is shown. The AUCs of the ROC were 0.80 (95% CI: 0.72–0.87) and 0.72 (95% CI: 0.72–0.87) for index-1 and index-2, respectively.

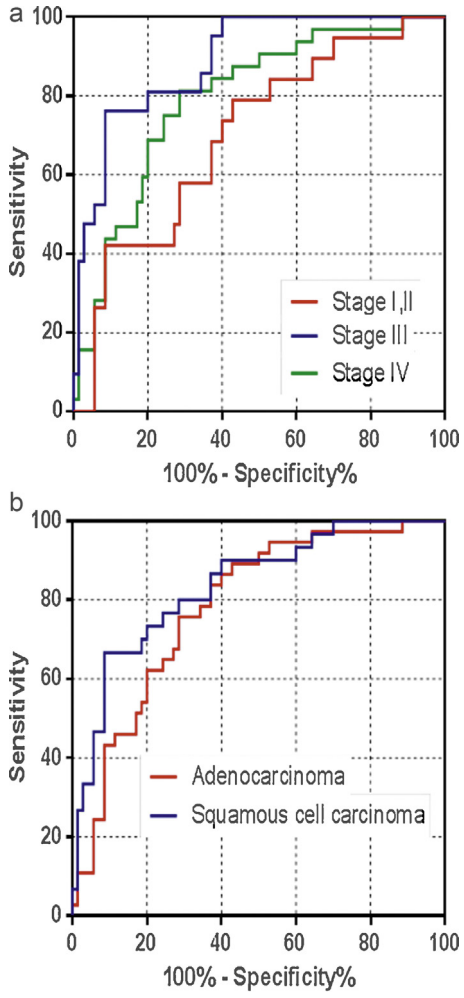

Fig. 3. ROC curves of the index subgrouped by cancer stage and histological type.
(A) ROC curves for each cancer stage for the dataset of this study. Stages I and II: AUC of the ROC = 0.70 (95% CI: 0.57–0.83); stage III: AUC of the ROC = 0.89 (95% CI: 0.82–0.96); and stage IV: AUC of the ROC = 0.79 (95% CI: 0.70–0.88). (B) ROC curves for the histological types for the dataset of this study. Adenocarcinoma: AUC of the ROC = 0.78 (95% CI: 0.70–0.87); and squamous cell carcinoma: AUC of the ROC = 0.84 (95% CI: 0.75–0.92).

carcinoma, with AUCs of the ROC of 0.78 (95% CI: 0.70–0.87) for adenocarcinoma and 0.84 (95% CI: 0.75–0.92) for squamous cell carcinoma.

4. Discussion

The present study was conducted to confirm the performance of a previously established amino acid multivariate index developed in Japan for use in the evaluation of lung cancer in Korean subjects. To confirm that differences between the datasets did not bias the results, the subjects' demographics were compared. The average age and BMI for the Japanese dataset used for the development of the index (control: 996 and cancer: 200) were 63.2 year for the controls and 65.0 year for the patients and 22.9 kg/m^2 for the controls and 22.5 kg/m^2 for the patients, respectively [13]. These characteristics were similar to those of the Korean subjects enrolled in the current study. However, the percentage of early stage patients (stages I and II) in this study was much lower (approximately 25%) compared with the Japanese study (approximately 60%).

Numerous clinical studies performed by other groups have also demonstrated cancer-related PFAA profiles, suggesting that the metabolic alterations in various cancers induce changes in PFAA levels; however, there are some discrepancies among results [7,12]. These differences may be due to the limited sizes of datasets or to differences in specimen processing procedures after blood samples were drawn. Several amino acids, such as Orn and Arg, undergo rapid *ex vivo* degradation at room temperature, and their levels significantly differ depending on whether blood samples are immediately cooled or are stored at room temperature for a period of time [28]. In the current study, the blood samples were cooled immediately within 1 min after blood collection and for at least 15 min before plasma separation so that the amino acids remained stable.

We found a global decrease in the following 13 amino acids in the lung cancer patients compared with the controls: Thr, Asn, Gln, Gly, Ala, Cit, Val, Met, Leu, His, Trp, Lys and Arg. We have previously reported that the overall PFAA concentration in patients with stage IV lung cancer is lower than that in stage I patients [13], indicating that the high percentage of later-stage cancers in the Korean dataset may have influenced the decreased PFAA levels. It is also known that protein malnutrition causes decreased PFAA concentrations [29]. In this study, approximately 60% of the patients had serum albumin levels of lower than 4.0 g/dL (standard value) (data not shown), suggesting that protein malnutrition may have influenced their lower PFAA levels. Since albumin data was available only for a limited number of subjects in this study, further studies are required to elucidate the mechanism behind the PFAA level decrease by studying PFAA changes in an albumin level matched study population.

To further evaluate the individual amino acid changes between the Korean and Japanese datasets, we compared the amino acid levels in terms of the total percentage of all amino acids. We detected significant decreases in the relative levels of Thr, Cit, His and Trp and increases in the relative levels of Pro, Ile, Phe and Orn. These changes are similar to those of the previously reported Japanese dataset [13]. Significant decreases in the Thr, Cit, His and Trp levels and decreases in the Pro and Ile levels were observed in the patients with stages I and II lung cancer, indicating that the PFAA profiles of patients with this type of cancer are altered from the early stages. The significant increases in the Phe and Orn levels observed only during the later stages (stages III and IV) have also been similarly reported in a previous Japanese study [13].

The mechanism behind the PFAA profile alterations observed in cancer patients is unknown. Indoleamine-2,3-dioxygenase (IDO), the first enzyme in the human kynurenine Trp metabolic pathway, has been reported to be overexpressed in various tumor types [30], potentially contributing to the decrease in the plasma Trp concentration. In addition, an *in vivo* study using tumor-bearing mice has demonstrated that some amino acids, such as Ala, Gly and Ser, may be produced by tumors [31]. Recently, a study has suggested that PFAA profiles can be influenced by metabolic changes occurring in various organs without tumor tissue. Our group has reported that High-mobility group box 1 protein (HMGB1), which is an autophagy-inducing stress protein secreted from tumors, induces muscles to supply glutamine to cancer cells as an energy source [32]. However, these results are not sufficient to explain why alterations in PFAA profiles can be observed during the early stages of cancer. Further clarification of the relationship between amino acid metabolism throughout the entire body and cancer development is required to understand these mechanisms more precisely.

In this study, the performance of the amino acid multivariate index for lung cancer, which was previously established and validated in a Japanese case-control dataset, was verified in an independent Korean dataset. The AUC of the ROC for the amino acid multivariate index in the Korean dataset reached 0.80. This value is similar to the AUC of the ROC previously reported for a Japanese dataset (0.777; 95% CI: 0.718–0.836) consisting of 421 controls and 85 lung cancer patients [17]. In addition, the AUC of the ROC for another index (index-2) that has been previously reported to discriminate lung cancer patients was 0.72 (0.731 for the Japanese dataset [17]), suggesting that the changes in the PFAA profiles of lung cancer patients are similar between the Korean and Japanese populations.

There is currently no test available that can detect all of the histological types of lung cancer. Although tumor markers are presently used to aid in the detection of lung cancer, these markers are not always useful due to their high histological specificity. For example, cytokeratin fragment (CYFRA) and squamous cell carcinoma antigen (SCC) are specific to squamous cell carcinoma, pro-gastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) are specific to small cell lung cancer, and carcinoembryonic antigen (CEA) is not specific to any particular histological type of cancer [33]. In addition, these tumor markers do not exhibit a sufficient ability to discriminate cancer patients at early stages. We have previously demonstrated that the amino acid multivariate index can discriminate lung cancer patients from controls regardless of the histological type or clinical stage, indicating that it is more sensitive than any of the currently used tumor markers [17]. We verified these results in the current study and found that this index has a significant ability to discriminate lung cancer patients from those in the early stages (stages I and II), independent of the histological type (adenocarcinoma or squamous cell carcinoma). The majority of the lung cancer patients in this study were either adenocarcinoma or squamous cell carcinoma, and the sample size for other histological types was too small to evaluate the performance of the index. Regarding other lung cancer types such as small cell lung cancer, we have previously reported that the performance of the index was similar to adenocarcinoma and squamous cell carcinoma [16]. Furthermore, the tendency for the AUC of the ROC for squamous cell carcinoma (0.84) to be higher than that for adenocarcinoma (0.78) was also observed in the Japanese dataset [17]. Collectively, these results suggest that this index has the potential to aid in the early detection of lung cancer of different histological types in both the Korean and Japanese populations.

In conclusion, this study demonstrated that there were significant differences in the PFAA profiles of the Korean lung cancer patients compared with the controls and that these differences are similar to those reported in a previous Japanese study. Our results also demonstrate that the amino acid multivariate index developed from the Japanese dataset can discriminate Korean lung cancer patients from healthy controls. Although additional prospective validation in a larger population with longitudinal follow-up to

evaluate the clinical utility of test such as improved outcomes is required, this technology may provide a simple and sensitive method for lung cancer screening in Korea.

Conflicts of interest

HN, NN, MM, YN are employees of Ajinomoto Co., Inc. HJK, SHJ, JSR, JEL, YCK, MKL, TWJ, SYL and KYL received research grants from Ajinomoto Co., Inc., and Hanmi Medicare, Inc. No other potential conflicts of interest relevant to this article are declared.

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References

[1] P. Felig, E. Marliss, J.L. Ohman, C.F. Cahill Jr., Plasma amino acid levels in diabetic ketoacidosis, Diabetes 19 (10) (1970) 727–729.
[2] J.E. Fischer, H.M. Rosen, A.M. Ebeid, J.H. James, J.M. Keane, P.B. Soeters, The effect of normalization of plasma amino acids on hepatic encephalopathy in man, Surgery 80 (1) (1976) 77–91.
[3] E. Holm, O. Sedlaczek, E. Grips, Amino acid metabolism in liver disease, Curr. Opin. Clin. Nutr. Metab. Care 2 (1) (1999) 47–53.
[4] S.Y. Hong, D.H. Yang, S.K. Chang, The relationship between plasma homocysteine and amino acid concentrations in patients with end-stage renal disease, J. Ren. Nutr. 8 (1) (1998) 34–39.
[5] G. Ravaglia, P. Forti, F. Maioli, G. Bianchi, M. Martelli, T. Talerico, et al., Plasma amino acid concentrations in patients with amnestic mild cognitive impairment or Alzheimer disease, Am. J. Clin. Nutr. 80 (2) (2004) 483–488.
[6] T.J. Wang, M.G. Larson, R.S. Vasan, S. Cheng, E.P. Rhee, E. McCabe, et al., Metabolite profiles and the risk of developing diabetes, Nat. Med. 17 (4) (2011) 448–453.
[7] A. Cascino, M. Muscaritoli, C. Cangiano, L. Conversano, A. Laviano, S. Ariemma, et al., Plasma amino acid imbalance in patients with lung and breast cancer, Anticancer Res. 15 (2) (1995) 507–510.
[8] D. Heber, L.O. Byerly, R.T. Chlebowski, Metabolic abnormalities in the cancer patient, Cancer 55 (Suppl. 1) (1985) 225–229.
[9] A. Kubota, M.M. Meguid, D.C. Hitch, Amino acid profiles correlate diagnostically with organ site in three kinds of malignant tumors, Cancer 69 (9) (1992) 2343–2348.
[10] H.S. Lai, J.C. Lee, P.H. Lee, S.T. Wang, W.J. Chen, Plasma free amino acid profile in cancer patients, Semin. Cancer Biol. 15 (4) (2005) 267–276.
[11] J.A. Norton, C.M. Gorschboth, R.A. Wesley, M.E. Burt, M.F. Brennan, Fasting plasma amino acid levels in cancer patients, Cancer 56 (5) (1985) 1181–1186.
[12] A.M. Proenza, J. Oliver, A. Palou, P. Roca, Breast and lung cancer are associated with a decrease in blood cell amino acid content, J. Nutr. Biochem. 14 (3) (2003) 133–138.
[13] Y. Miyagi, M. Higashiyama, A. Gochi, M. Akaike, T. Ishikawa, T. Miura, et al., Plasma free amino acid profiling of five types of cancer patients and its application for early detection, PLoS One 6 (9) (2011) e24143.
[14] H. Nakamura, H. Jinzu, K. Nagao, Y. Noguchi, N. Shimba, H. Miyano, et al., Plasma amino acid profiles are associated with insulin, C-peptide and adiponectin levels in type 2 diabetic patients, Nutr. Diabetes 4 (2014) e133.
[15] M. Yamakado, T. Tanaka, et al., Plasma amino acid profile is associated with visceral fat accumulation in obese Japanese subjects, Clin. Obes. 2 (1–2) (2012) 29–40.
[16] J. Maeda, M. Higashiyama, A. Imaizumi, T. Nakayama, H. Yamamoto, T. Daimon, et al., Possibility of multivariate function composed of plasma amino acid profiles as a novel screening index for non-small cell lung cancer: a case control study, BMC Cancer 10 (2010) 690.
[17] M. Shingyoji, T. Iizasa, M. Higashiyama, F. Imamura, N. Saruki, A. Imaizumi, et al., The significance and robustness of a plasma free amino acid (PFAA) profile-based multiplex function for detecting lung cancer, BMC Cancer 13 (2013) 77.
[18] K.W. Jung, Y.J. Won, H.J. Kong, C.M. Oh, D.H. Lee, J.S. Lee, Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011, Cancer Res. Treat.: Off. J. Korean Cancer Assoc. 46 (2) (2014) 109–123.
[19] M. Jung, National Cancer Screening Programs and Evidence-Based Healthcare Policy in South Korea, Health policy, Amsterdam, Netherlands), 2014.
[20] M. Sumitani, N. Takifuji, S. Nanjyo, Y. Imahashi, H. Kiyota, K. Takeda, et al., Clinical relevance of sputum cytology and chest X-ray in patients with suspected lung tumors, Intern. Med. (Tokyo, Japan) 47 (13) (2008) 1199–1205.
[21] D.R. Aberle, A.M. Adams, C.D. Berg, W.C. Black, J.D. Clapp, R.M. Fagerstrom, et al., Reduced lung-cancer mortality with low-dose computed tomographic screening, N. Engl. J. Med. 365 (5) (2011) 395–409.
[22] K. Shimbo, T. Oonuki, A. Yahashi, K. Hirayama, H. Miyano, Precolumn derivatization reagents for high-speed analysis of amines and amino acids in biological fluid using liquid chromatography/electrospray ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom.: RCM 23 (10) (2009) 1483–1492.
[23] K. Shimbo, S. Kubo, Y. Harada, T. Oonuki, T. Yokokura, H. Yoshida, et al., Automated precolumn derivatization system for analyzing physiological amino acids by liquid chromatography/mass spectrometry, Biomed. Chromatogr.: BMC 24 (7) (2010) 683–691.
[24] K. Shimbo, A. Yahashi, K. Hirayama, M. Nakazawa, H. Miyano, Multifunctional and highly sensitive precolumn reagents for amino acids in liquid chromatography/tandem mass spectrometry, Anal. Chem. 81 (13) (2009) 5172–5179.
[25] N. Okamoto, Use of aminoIndex technology for cancer screening, Ningen Dock 26 (6) (2012) 911–922.
[26] J.A. Hanley, B.J. McNeil, The meaning and use of the area under a receiver operating characteristic (ROC) curve, Radiology 143 (1) (1982) 29–36.
[27] K. Oishi, S. Yamamoto, N. Itoh, K. Miyazaki, T. Nemoto, Y. Nakakita, et al., Disruption of behavioral circadian rhythms induced by psychophysiological stress affects plasma free amino acid profiles without affecting peripheral clock gene expression in mice, Biochem. Biophys. Res. Commun. 450 (1) (2014) 880–884.
[28] J.S. Davis, C.J. Darcy, K. Piera, Y.R. McNeil, T. Woodberry, N.M. Anstey, Ex-vivo changes in amino acid concentrations from blood stored at room temperature or on ice: implications for arginine and taurine measurements, BMC Clin. Pathol. 9 (2009) 10.
[29] A. Polge, E. Bancel, H. Bellet, D. Strubel, S. Poirey, P. Peray, et al., Plasma amino acid concentrations in elderly patients with protein energy malnutrition, Age Ageing 26 (6) (1997) 457–462.
[30] S. Lob, A. Konigsrainer, D. Zieker, B.L. Brucher, H.G. Rammensee, G. Opelz, et al., IDO1 and IDO2 are expressed in human tumors: levo- but not dextro-1-methyl tryptophan inhibits tryptophan catabolism, Cancer Immunol. Immunother.: CII 58 (1) (2009) 153–157.
[31] J. Marquez, F. Sanchez-Jimenez, M.A. Medina, A.R. Quesada, I. Nunez de Castro, Nitrogen metabolism in tumor bearing mice, Arch. Biochem. Biophys. 268 (2) (1989) 667–675.
[32] Y. Luo, J. Yoneda, H. Ohmori, T. Sasaki, K. Shimbo, S. Eto, et al., Cancer usurps skeletal muscle as an energy repository, Cancer Res. 74 (1) (2014) 330–340.
[33] S. Ando, H. Kimura, N. Iwai, K. Kakizawa, M. Shima, M. Ando, The significance of tumour markers as an indication for mediastinoscopy in non-small cell lung cancer, Respirology (Carlton, Vic.) 8 (2) (2003) 163–167.