Application of "AminoIndex Technology" to cancer

Some authors have reported that plasma amino acid concentrations were altered in cancer patients because of various metabolic changes[10-14]. Furthermore, other clinical studies have demonstrated the possibility of using plasma amino acid concentrations as multivariate biomarkers in cancer screenings[15-18]. Furthermore, a particular clinical study on 5 types of cancer (gastric, lung, colorectal, prostate, and breast) was carried out to explore and validate the application of "AminoIndex Technology" to cancer screening[19].

The present multicenter study included the following institutions: Kanagawa Cancer Center; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; Osaka Medical Center for Cancer and Cardiovascular Diseases; Gunma Prefectural Cancer Center; Chiba Prefectural Cancer Center; Shizuoka Prefectural Cancer Center; Yokohama City University Medical Center; Yokohama Municipal Citizen's Hospital; Yokohama Minami Kyosai Hospital; Mitsui Memorial Hospital; Kameda Medical Center Makuhari and Kanagawa Health Service Association. It received institutional review board approval from all sites, and informed consent was obtained from all the patients. The present study used a new scoring system for calculation, known as AminoIndex® Cancer Screening (AICS), to analyze plasma samples from 2,043 cancer patients (**Fig. 3**) for screening purposes. Training and validation test datasets were used to establish an AICS score formula and to evaluate prediction accuracy, and all the results in this review are results in the validation test dataset. Amino acids included in the AICS formula for each cancer derived in the clinical research are shown in **Table 1**.

Some amino acids are commonly found in the AICS results for certain types of cancer: tryptophan (Trp) is seen in gastric, prostate, and breast cancers, whereas histidine (His) is seen in gastric, lung, and breast cancers. Similarly, particular amino acids may be cancer-specific, for example, threonine (Thr) in breast cancer or methionine (Met) in colorectal cancer. These data suggest that the AICS formula has plasma amino acid profiles that are common to several cancers or specific to a particular cancer.

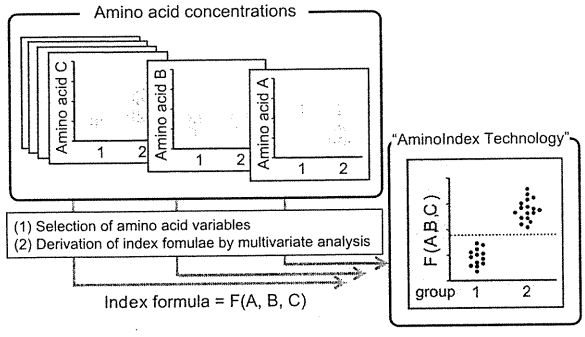


Fig. 2. Summary of "AminoIndex Technology"

AICS score and evaluation

AICS scoring involves evaluating multiple cancer types according to plasma amino acid concentrations on the basis of AICS values. As shown in **Fig. 4**, the minimum and maximum AICS values are 0.0 and 10.0, respectively, and the AICS values for specificities of 80% and 95% for each cancer are defined as 5.0 and 8.0, respectively. We presume that the higher the subject's AICS value, the greater the

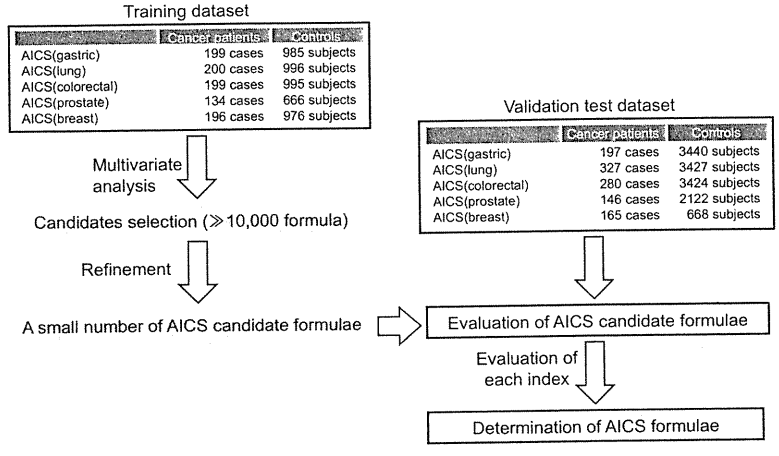
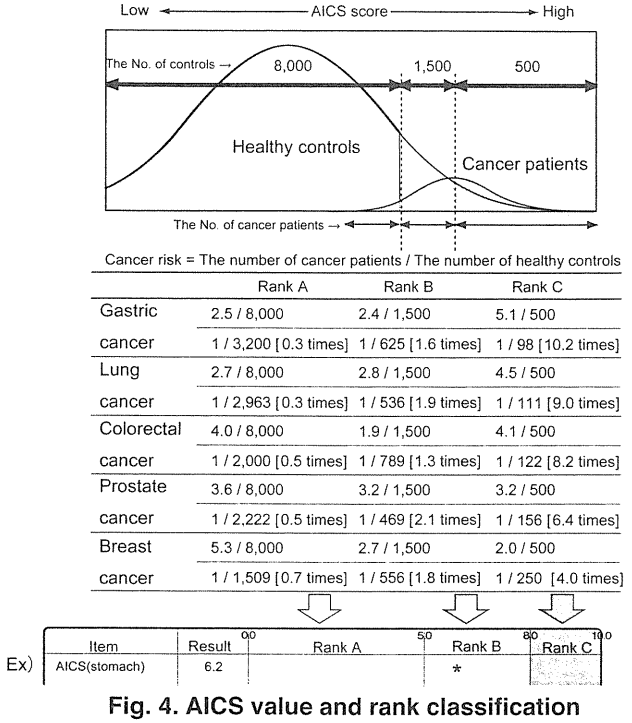


Fig. 3. Flow of AICS Derivation

Table 1. Amino acids included in AICS formulae

	Thr	Ser	Gln	Pro	Ala	Val	Met	Ile	Leu	His	Trp	Orn	Lys	Arg
AICS(gastric)					▼	▼			▼	▼	▼		▼	
AICS(lung)		▲	▼		■					▼		▲	■	
AICS(colorectal)		■		■		▼	▼	■					▼	
AICS(prostate)			▼		▲						▼	▲	▲	▼
AICS(breast)	▲				▲					▼	▼	▲		■

AICS: AminoIndex® Cancer Screening.
▲: Amino acids significantly increasing in cancer patients ($p < 0.05$)
▼: Amino acids significantly decreasing in cancer patients ($p < 0.05$)
■: Amino acids showing no significant difference in cancer patients



	Rank A	Rank B	Rank C
Gastric	2.5 / 8,000	2.4 / 1,500	5.1 / 500
cancer	1 / 3,200 [0.3 times]	1 / 625 [1.6 times]	1 / 98 [10.2 times]
Lung	2.7 / 8,000	2.8 / 1,500	4.5 / 500
cancer	1 / 2,963 [0.3 times]	1 / 536 [1.9 times]	1 / 111 [9.0 times]
Colorectal	4.0 / 8,000	1.9 / 1,500	4.1 / 500
cancer	1 / 2,000 [0.5 times]	1 / 789 [1.3 times]	1 / 122 [8.2 times]
Prostate	3.6 / 8,000	3.2 / 1,500	3.2 / 500
cancer	1 / 2,222 [0.5 times]	1 / 469 [2.1 times]	1 / 156 [6.4 times]
Breast	5.3 / 8,000	2.7 / 1,500	2.0 / 500
cancer	1 / 1,509 [0.7 times]	1 / 556 [1.8 times]	1 / 250 [4.0 times]

Fig. 4. AICS value and rank classification

*The table shows the approximate percentage of incidence for each cancer by rank. The results for each cancer are indicated from 2 different perspectives on 2 levels. The upper level of each row is the cancer risk calculated as the proportion of 10,000 examinees, as mentioned previously. The lower level is the cancer risk when the numerator is 1. Thus, the values in parentheses are the mean risk ratios when the prevalence of cancer is 1 (approximately 1/1,000).

likelihood that the subject is suffering from cancer. AICS values are divided into 3 categories: rank A, <5.0; rank B, 5.0–8.0; and rank C, ≥8.0. The rank B or C cutoff and rank C cutoff are defined as 5.0 and 8.0, respectively. Thus, if the specificity is 95%, then 5% of the healthy controls are assessed as rank C (a false-positive rate of 5%), whereas if the specificity is 80%, then 20% of the healthy controls are assessed as rank B or C (a false-positive rate of 20%).

The prevalence of cancer is approximately 0.1%, that is, 10 of 10,000 people. Based on the present multicenter clinical research, in the case of gastric cancer, for every 10,000 people going for an AICS test, the rank A, B, and C groups have approximately 2.5, 2.4, and 5.1 cancer patients in them, respectively. Thus, the percentages of cancer patients in the rank A, B, and C groups are 0.03% (2.5/8,000), 0.16% (2.4/1,500), and 1.02% (5.1/500), respectively. Therefore, when the cancer risk in each group is compared with the whole population (i.e., a prevalence of 0.1), the rank A, B, and C groups have approximately 0.3-, 1.6-, and 10-fold cancer risks, respectively. However, it should be emphasized that if a person is evaluated as rank B or C, they do not necessarily have cancer. Similarly, if a person is evaluated as rank A, they are not necessarily free of cancer.

Table 2. Rank classification and specificity, sensitivity, and positive predictive value of AICS values for various cancers

AICS	Incidence rate	AICS value ≥ 5.0 (Rank B or C)			AICS value ≥ 8.0 (Rank C)		
		Specificity	Sensitivity	Positive predictive value	Specificity	Sensitivity	Positive predictive value
AICS(gastric)	0.0917	80	75	0.34	95	51	0.93
AICS(lung)	0.0657	80	73	0.24	95	45	0.59
AICS(colorectal)	0.0820	80	60	0.25	95	41	0.67
AICS(prostate)	0.0690	80	64	0.22	95	32	0.44
AICS(breast)	0.0775	80	47	0.18	95	20	0.31

All data are presented as percentages (%). To calculate the positive predictive value, the estimated incidence rate in the national predicted prevalence by age group, which was derived from 15 population-based cancer registries in the monitoring of cancer incidence in Japan (1975–2005)[20], was used instead of the prevalence rate.
AICS: AminoIndex® Cancer Screening

Male

Item		Rank A	Rank B	Rank C
		0.0	5.0	8.0 10.0
AICS(gastric)	Cancer patients	33%	23%	44%
	Healthy controls	86%	11%	3%
AICS(lung)	Cancer patients	27%	27%	46%
	Healthy controls	81%	14%	5%
AICS(colorectal)	Cancer patients	39%	18%	43%
	Healthy controls	80%	14%	6%
AICS(prostate)	Cancer patients	36%	32%	32%
	Healthy controls	80%	15%	5%

Female

Item		Rank A	Rank B	Rank C
		0.0	5.0	8.0 10.0
AICS(gastric)	Cancer patients	8%	26%	66%
	Healthy controls	70%	22%	8%
AICS(lung)	Cancer patients	25%	32%	43%
	Healthy controls	79%	16%	5%
AICS(colorectal)	Cancer patients	42%	19%	39%
	Healthy controls	80%	17%	3%
AICS(prostate)	Cancer patients	53%	27%	20%
	Healthy controls	80%	15%	5%

Fig. 5. AICS test result distribution

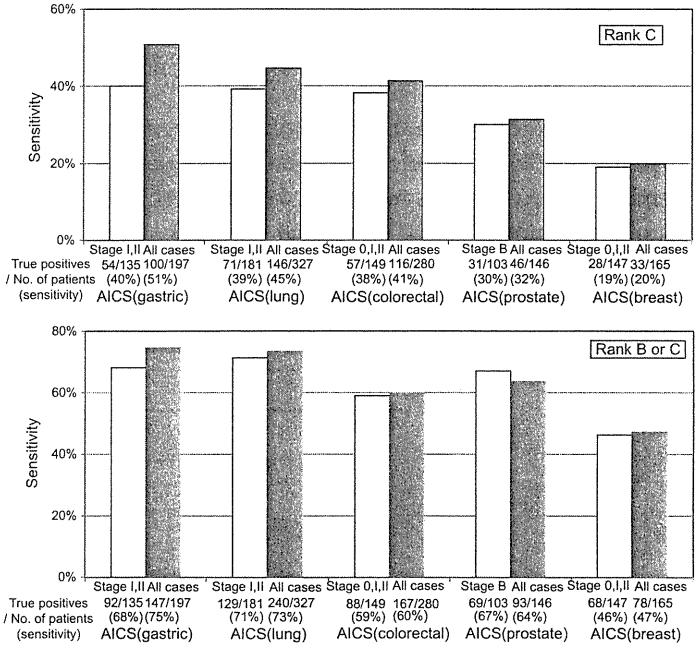


Fig. 6. Sensitivity of AICS for each type of cancer

Table 2 shows the AICS rank classifications and results (specificity, sensitivity, and positive predictive value). The sensitivities for gastric, lung, colorectal, prostate, and breast cancers at the rank B or C cutoff are 75%, 73%, 60%, 64%, and 47%, respectively, and those at the rank C cutoff are 51%, 45%, 41%, 32%, and 20%, respectively. **Fig. 5** compares the test results for the cancer patients and the healthy controls to demonstrate how the rank classification works, showing that 46% of the male patients with lung cancer and 5% of the male healthy controls were evaluated as rank C. The sensitivity for each cancer in patients with stage II (stage B) or earlier cancer is shown in **Fig. 6**. It shows that sensitivity for stage II (stage B) or earlier cancer is similar to that for cancer at any stage.

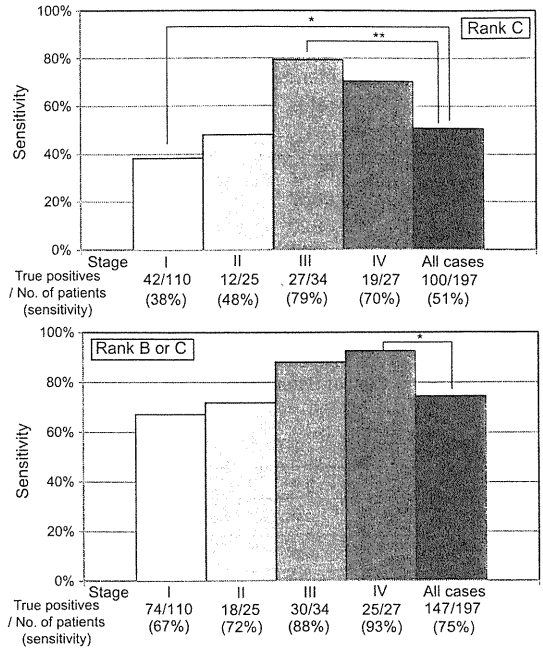
Evaluation of gastric cancer

Approximately 50,000 Japanese people died of gastric cancer in 2009, making it the second and third leading cause of death in men and women, respectively[20]. Although the pathogenesis of gastric cancer is still uncertain, *Helicobacter pylori* is thought to play a role. To use AICS for gastric cancer screening, an AICS

(gastric) score was derived from plasma amino acid levels in 199 gastric cancer patients using "AminoIndex Technology". Using this score, 197 patients in the validation test dataset, which was independent of the training dataset, were compared by either tumor stage, tissue type stratified analyses, or pepsinogen (PG) test results (**Fig. 7**).

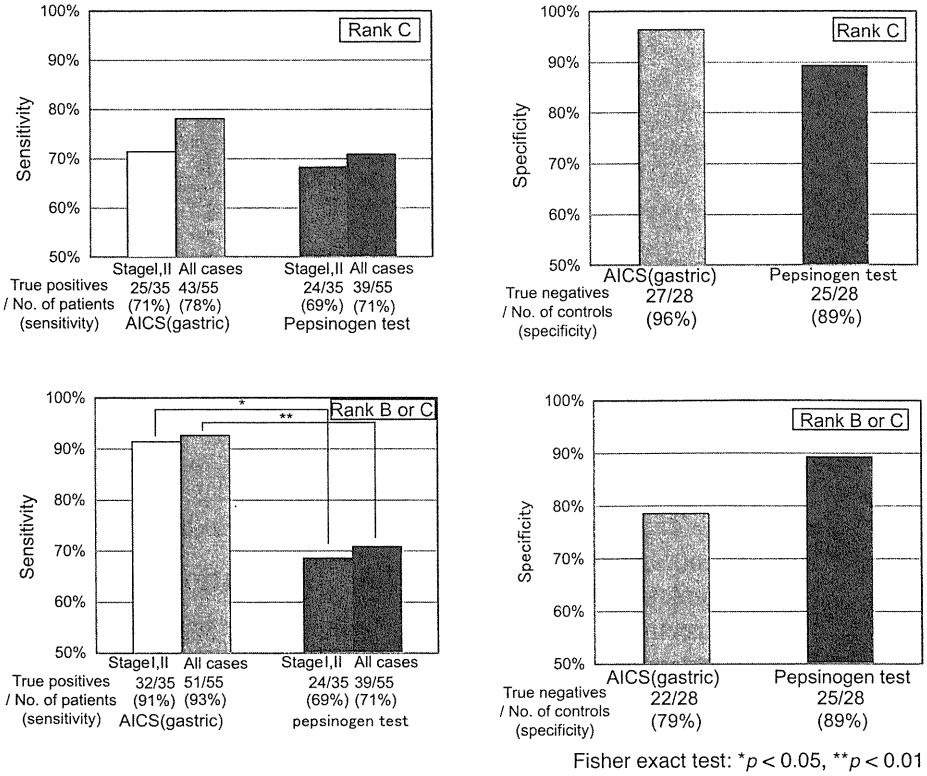
Early detection of gastric cancer is a major factor for a good prognosis. The sensitivity at each tumor stage is shown in **Fig. 7**. At the rank C cutoff, although there was a significant difference in sensitivity between stage I and all cases, the sensitivity was still 38% at stage I. There was no significant difference in sensitivity (48%) between stage II and all cases. At the rank B or C cutoff, there were no significant differences in sensitivity between stage I or II and all cases, and the sensitivities at stage I or II gastric cancer were high (stage I = 67%; stage II = 72%).

We compared the specificity and sensitivity of the PG test and AICS (gastric) in 55 patients with cancer and 28 healthy controls who underwent PG testing in a clinical study (**Fig. 8**). When PGI was ≤70 ng/mL and the PGI/II ratio was ≤3, the PG test result was defined as positive. When the sensitivities of PG testing and AICS (gastric) for gastric cancer were compared,



Fisher exact test: $^{*}p < 0.05$, $^{**}p < 0.01$

Fig. 7. Sensitivity of AICS (gastric) by stage



Fisher exact test: $^{*}p < 0.05$, $^{**}p < 0.01$

Fig. 8. Sensitivity and specificity for gastric cancer - AICS versus pepsinogen test

AICS (gastric) was significantly more sensitive ($p < 0.01$) than PG testing at the rank B or C cutoff.

As atrophic gastritis may produce a positive PG test, we compared the positive rate in atrophic gastritis patients using AICS (gastric) and PG testing (**Fig. 9**). In rank C patients, the positive rate for atrophic gastritis using the AICS (gastric) value was lower than that using PG testing ($p < 0.1$), suggesting that AICS (gastric) is a more accurate test for gastric cancer than the PG test.

We classified gastric cancer into several tissue types, which included poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and tubular adenocarcinoma. Among them, poorly differentiated adenocarcinoma and signet-ring cell carcinoma are difficult to detect using PG testing. The results of differential analysis by tissue type are shown in **Fig. 10**. For AICS (gastric), there was no significant difference in sensitivity between tissue types at the rank B or C cutoff, although there was a significant difference in sensitivity among tissue types at the rank C cutoff. The lowest sensitivity for all cases was 43%. This was for tubular adenocarcinoma, which had the lowest sensitivity among the 3 tissue types. These data indicate that AICS (gastric) can be used as a screening method for at least 3 tissue types (poorly differentiated adenocarcinoma, signet-ring cell carcinoma, tubular adenocarcinoma).

Evaluation of lung cancer

Approximately 67,000 Japanese died of lung cancer in 2009, making it the leading and second leading cause of death in men and women, respectively[20]. Early detection of lung cancer is important because patient survival dramatically decreases as the disease progresses. To apply AICS to lung cancer screening, we used "AminoIndex Technology" to derive an AICS (lung) score from the plasma amino acid concentrations of 200 lung cancer patients. Using this score, 327 patients in the validation test dataset, which was independent of the training dataset, were stratified by stage or tissue type (**Fig. 11**). When AICS (lung) scores were stratified by stage (**Fig. 11**), we found no significant difference in sen-

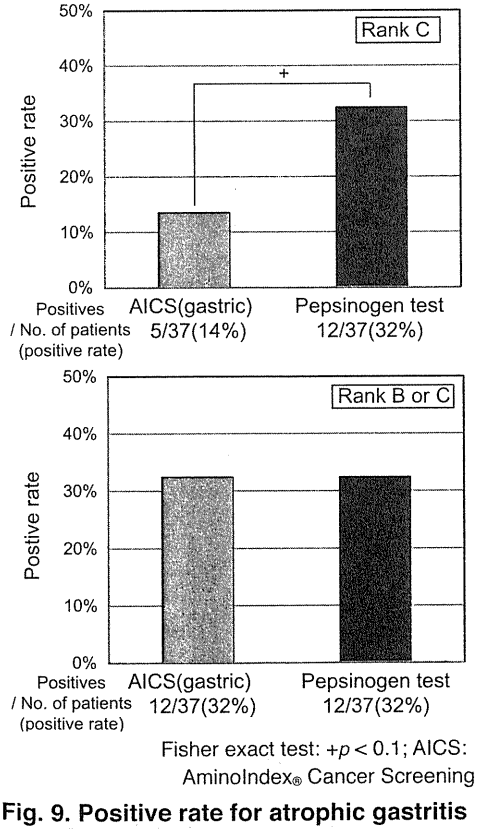


Fig. 9. Positive rate for atrophic gastritis

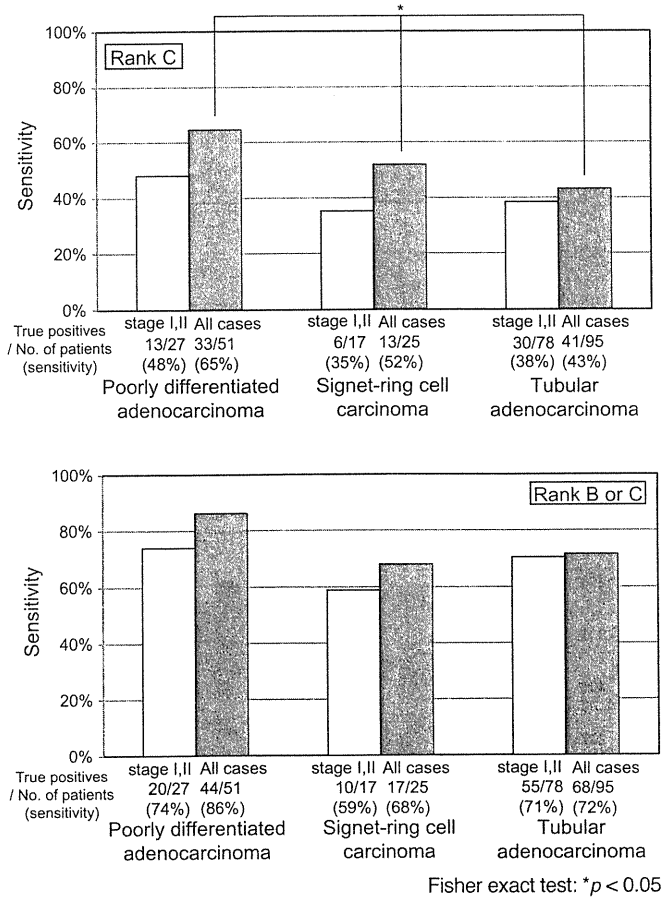


Fig. 10. Sensitivity of AICS (gastric) by tissue type

sitivity between stages. The sensitivity of stage I was 38% at the rank C cutoff and 70% at the rank B or C cutoff, suggesting that AICS (lung) can be used to detect early (stage I) lung cancer.

As lung cancer has a wide variety of tissue types and existing tumor markers are highly tissue-specific, it is difficult to determine tissue types other than squamous cell carcinoma using sputum cytology. To examine whether AICS (lung) scores were also dependent on tissue type, we stratified the AICS scores by tissue type (**Fig. 12**) and found no differences in sensitivities for adenocarcinoma, squamous cell carcinoma, or small cell carcinoma at the rank C cutoff and at the rank B or C cutoff. The sensitivities for all tissue types were more than 40% in rank C patients and more than 70% at the rank B or C cutoff.

Evaluation of colorectal cancer

Approximately 43,000 Japanese died of colorectal cancer in 2009, making it the third and leading cause of death in men and women, respectively[20]. Patient survival decreases as colorectal cancer progresses and therefore early detection of colorectal cancer is highly important. To apply AICS to colorectal cancer screening, "AminoIndex Technology" was used to derive an AICS (colorectal) score from the plasma amino acid concentrations in 199 patients with colorectal cancer. Using this score, 280 patients in the validation test dataset, which was independent of the training dataset, were stratified by stage and tissue type as shown below. When we stratified AICS (colorectal) sensitivity by stage (**Fig. 13**), we found no significant difference in sensitivity among stages. The sensitivity for stage 0 at the rank C cutoff was 55%, and that at the rank B or C cutoff was 64%. The positive rate of AICS (colorectal) for colonic polyps was significantly lower than that for colorectal cancer at the rank C cutoff and at the rank B or C cutoff (**Fig. 14**). This suggests that AICS (colorectal) is more specific for colorectal cancer than colonic polyps.

Evaluation of prostate cancer

Approximately 10,000 Japanese men died of prostate cancer in 2009[20]. Patient survival decreases as prostate cancer progresses and therefore early detection of prostate cancer is highly important. To apply AICS to prostate cancer screening, an AICS (prostate) score was derived from the plasma amino acid concentrations in 134 patients with prostate cancer using "AminoIndex Technology". Using this score,

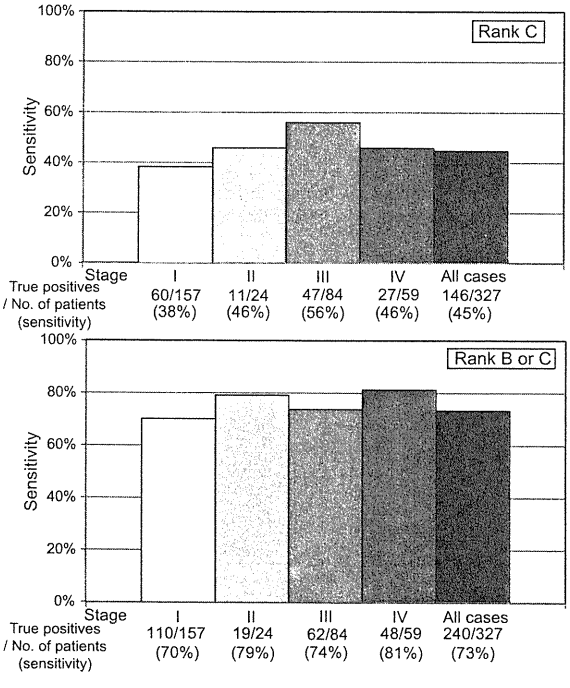


Fig. 11. Sensitivity of AICS (lung) by stage

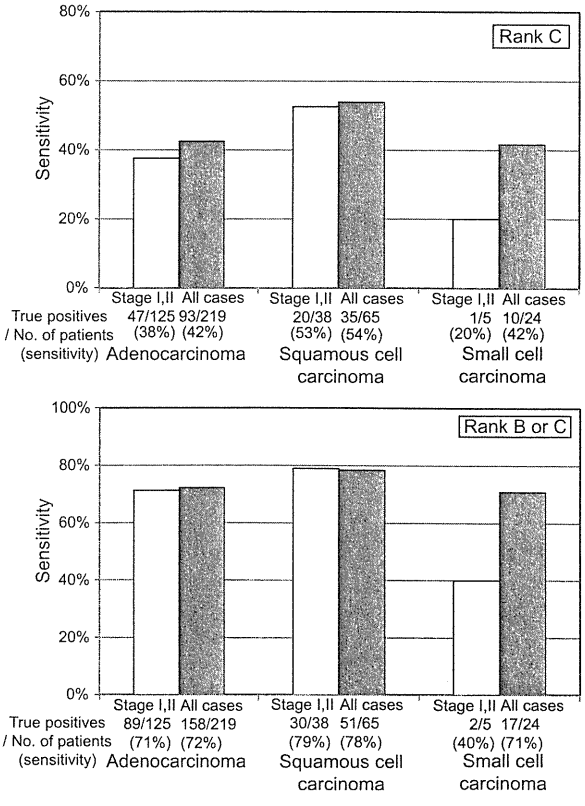


Fig. 12. Sensitivity of AICS (lung) by stage

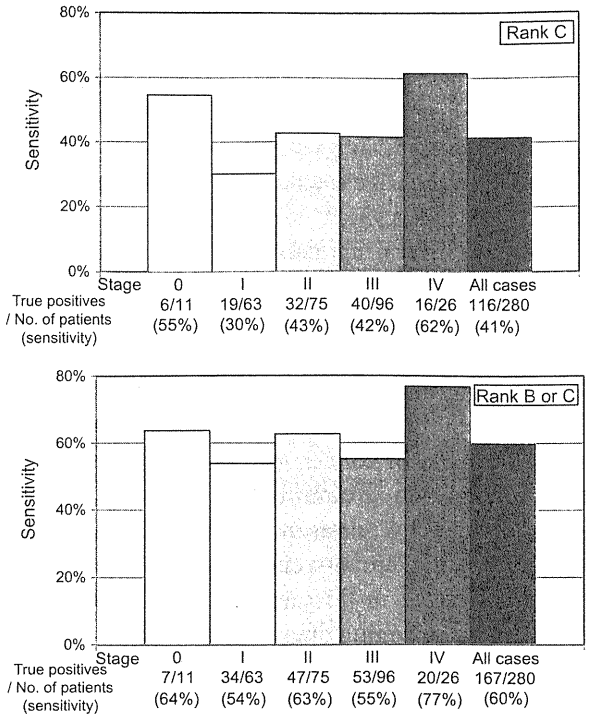
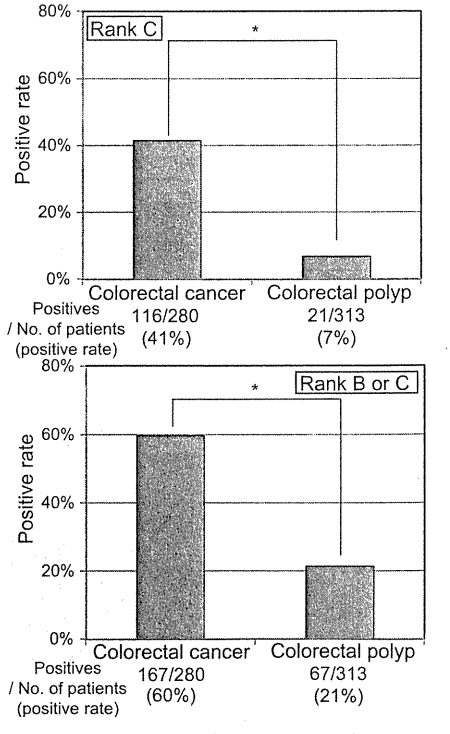


Fig. 13. Sensitivity of AICS (colorectal) by stage

146 patients in the validation test dataset, which was independent of the training dataset, were stratified by stage. The performance of AICS (prostate) in the "gray zone" of prostate-specific antigen (PSA) testing results was demonstrated.

When we stratified the AICS (prostate) scores by stage (**Fig. 15**), we found no significant difference in sensitivity among stages. The sensitivities at the rank C cutoff and at the rank B or C cutoff were 30% and 67%, respectively.

We also investigated a relationship between AICS (prostate) scores and PSA test values, which are commonly used for early detection of prostate cancer. The reference value for PSA in healthy individuals is ≤4.0 ng/mL, and further examination is needed when this value is exceeded. However, PSA scores of 4–10 ng/mL (the "gray zone") are not sufficiently predictive, making a better clinical test for such patients highly desirable. In patients with prostate cancer with PSA scores in the "gray zone," the sensitivity of AICS (prostate) was 35% at the rank C cutoff and 67% at the rank B or C cutoff (**Fig. 16**).



Fisher exact test: $*p < 0.001$

Fig. 14. Positive rate for colorectal polyps

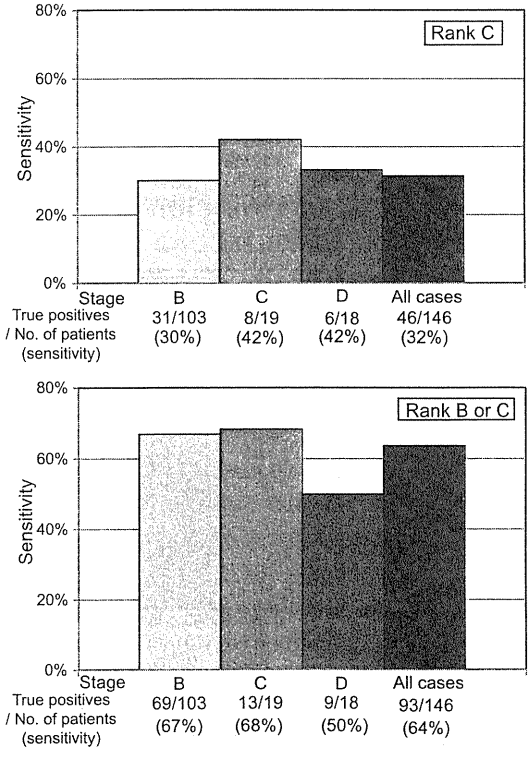


Fig. 15. Sensitivity of AICS (prostate) by stage

Evaluation of breast cancer

Approximately 12,000 Japanese died of breast cancer in 2009[20]. Early detection of breast cancer is very important, as the survival rate decreases with every stage. To apply AICS to breast cancer screening, an AICS (breast) score was derived from plasma amino acid concentrations in 196 patients with breast cancer using "AminoIndex Technology". Using this score, 165 patients in the validation test dataset, which was independent of the training dataset, were stratified by stage (**Fig. 17**). When stratified by stage (**Fig. 17**), the sensitivity of AICS (breast) was not significantly different between the stages. The sensitivity at stage 0 was 42% at the rank B or C cutoff.

Use of AICS

I would now like to summarize the results for each type of AICS test and describe its characteristics on the basis of the validation test dataset results (**Table 3**). AICS enables simultaneous testing for multiple cancers regardless of cancer or tissue type. Furthermore, because AICS can detect stage II (stage B) or earlier cancers and can easily be performed on a plasma sample, it can be carried out in conjunction with a comprehensive medical examination or regular health check-up.

There are several applications of AICS in clinical practice. First, it can be used as an alternative to existing cancer screening tests. Several well-known examination techniques are currently in use in cancer screening, for example mammography and ultrasonography in breast cancer screening. Depending on the type of cancer, various other screening tools, such as x-ray examinations, endoscopy, computed tomography, ultrasonography, and fecal occult blood testing are also currently used. The AICS method reviewed in this article can be applied to many cancer screening areas. AICS requires only a blood sample, making it more convenient and less invasive than several other screening methods. In addition to the screening methods mentioned above, genetic testing based on genetic polymorphisms is also used. However, one caveat regarding such genetic tests is that they cannot evaluate the contribu-

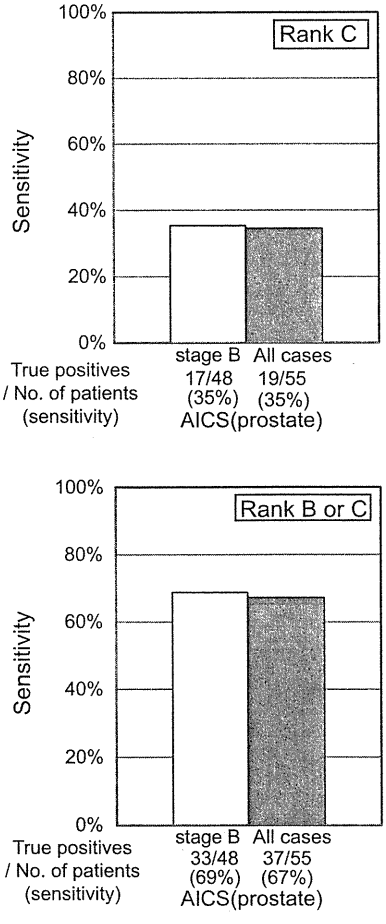


Fig. 16. Sensitivity of AICS (prostate) in PSA gray zone (4–10 ng/m)
PSA: prostate specific antigen; AICS: AminoIndex® Cancer Screening

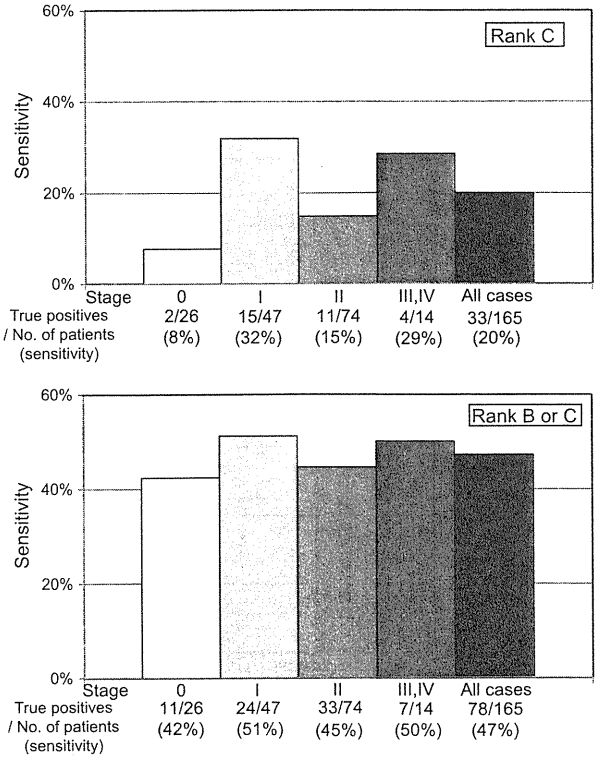


Fig. 17. Sensitivity of AICS (breast) by stage

Table 3. Characteristics of individual AICS tests

Test item	Characteristics
AICS(gastric)	1. High sensitivity for stage I and II gastric cancer 2. Higher sensitivity than pepsinogen testing in rank B or C 3. Lower positive rate for atrophic gastritis than pepsinogen testing in rank C 4. Equivalent sensitivity to tissue type, which is difficult to detect (poorly differentiated adenocarcinoma, signet-ring cell carcinoma)
AICS(lung)	1. High sensitivity for stage I and II lung cancer 2. Equivalent sensitivity for various tissue types of lung cancer
AICS(colorectal)	1. High sensitivity for stage 0, I, and II colorectal cancer 2. Low positive rate for colorectal polyps
AICS(prostate)	1. High sensitivity for stage B prostate cancer 2. High sensitivity for prostate cancer falling within the PSA gray zone (4–10 ng/mL)
AICS(breast)	1. High sensitivity for stage 0, I, and II breast cancer

AICS: AminoIndex® Cancer Screening; PSA: prostate-specific antigen

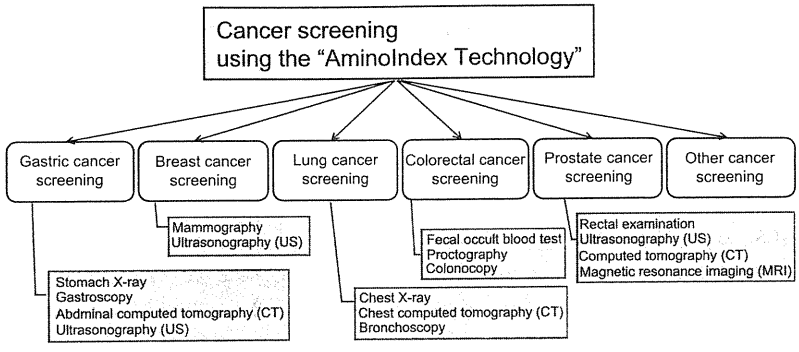


Fig. 18. Cancer screening using "AminoIndex Technology"

tion of environmental factors and lifestyle to overall risk. In contrast, as it is based on amino acid metabolites, AICS covers the influences of genetic and environmental factors and is therefore an alternative to genetic testing.

In addition, AICS can be used as a prescreening tool for specific cancers (**Fig. 18**). Existing screening tools have many drawbacks, such as exposure to radiation, cost, and inconvenience, reasons that can make people hesitant to undergo screening using them. With AICS, screening for gastric, lung, colorectal, prostate, and breast cancers can be conducted using a single blood sample, so AICS scores could be used to help a person decide whether to receive additional cancer screening.

In this article, we classified individuals whose AICS scores were ≥95% as rank C and those with AICS scores of ≥80% as rank B or C. However, it may be appropriate to use different classification thresholds according to the clinical context. After an AICS cutoff value is established based on appropriate specificity, it may be possible to apply it in practice to cancer screening.

Points to remember with AICS and issues to be addressed

This clinical research on AICS was conducted on Japanese subjects aged 25–90 years (for prostate cancer, 40–90 years). At present, it is not clear whether there are ethnic differences in AICS scores and therefore further studies are required. In addition, similar to regular medical examinations, blood must be collected in the morning after an 8 h fast because plasma amino acid levels are affected by dietary proteins and carbohydrates, as is the case of blood glucose and triglyceride levels. Not only solid food but also amino acid supplements (including liquids), amino acid preparations, and beverages containing protein and sugar (such as milk, soft drink and fruit juice) may influence results, if taken within 8 h before sampling. Also, as plasma amino acid concentrations differ during pregnancy, it may be

difficult to derive AICS values for pregnant women.

Conclusion

In this review, we have discussed the use of "AminoIndex Technology" in cancer screening. We expect that will be used as an alternative to current screening examinations or prescreening examinations for a variety of cancers. In the future, we hope that the effectiveness of AICS in practical cancer screening (e.g. cancer screening provided by local governments in Japan) will be clarified through clinical research, including longitudinal cohort studies.

Although we only discussed the application of "AminoIndex Technology" to cancer, clinical research on its use for other diseases is ongoing. If the results of such research verifies its usefulness, this technology will be established as a method of blood analysis using a single blood sample that can screen for multiple cancers while simultaneously evaluating the risk of developing many other diseases. In addition to evaluating disease risk, "AminoIndex Technology" could be used to promote dietary and exercise interventions. As mentioned previously, amino acid metabolomics research can be applied to many different clinical areas and we expect that novel applications for it will be created through such research.

Conflict of interest

I have no conflict of interest to declare for this review.

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がん登録の来し方～歴史を知る

The historical background of population-based cancer registries in Japan - Where we have been

岡本　直幸*

疾病登録システムとしての「がん登録」は、主に院内がん登録及び地域がん登録として遂行されている。前者は診療所や病院内での「診療録の記載」という形で古くより実施されていると思われるが、統計的なデータとしての扱いは近年になってであろう。後者の場合、1728年にLondonで初めて「cancer census」が行われたが、失敗に終っている[1,2]。その後1900年ごろになると、EnglandやGermanyにおいて、「がん」の予防や原因究明のためには集団内での「がん」統計が必要との認識からがん罹患調査の必要性が叫ばれ、1900年Germanyにおいて治療中の「がん」患者データの登録の試みが行われたが、十分な結果は得られていない。1902-1908年には、この試みがNetherlands、Spain、Portugal、Hungary、Sweden、Denmark、Icelandにまで広がりをみせたが、いずれも失敗に終わっている[1,2]。これらの調査の失敗の原因は、主として医師の協力が不十分であったとの認識から、USAのWoodは、「がん」を届出の必要な病気として全がん患者を法に基づいて登録すべきであるとの主張を行っている[3]。

このような情勢の中、1929年よりHamburgにおいて確立した地域がん登録としての稼働が始まり、1940年代にはNew York State（USA）, Connecticut（USA）, Denmark, Saskatchewan（Canada）, England and Wales, New Zealandで開始されるようになり、現在まで継続して実施されている[2]。表1に1950年以前に立ちあげられた地域がん登録を示しているが、全8登録のなかで5登録がVoluntaryではなくCompulsoryになっていることに注目していただきたい。

わが国においては、1951-53年に宮城県において東北大学の瀬木三雄先生のもとで「がんの実態調査」が行われ、1959年より出張採録をベースとした地域がん登録が実施されるようになった。その間に、米国の協力のもとで広島市（1957）、長崎市（1958）において、被爆者フォローを目的としたがん（組織）登録が開始されている[4,5]。この登録も医師の届出方式ではなく出張採録方式による登録であった。1960年代に入って、医師のVoluntary

表1. Population-based cancer registries established before 1950

Country(region)	Year of establishment	Notification
FR Germany(Hamburg)	1929	Voluntary
USA(New York State)	1940	Compulsory
USA(Connecticut)	1941	Compulsory
Denmark	1942	Compulsory
Canada(Saskatchewan)	1944	Compulsory
England and Wales(SW)	1945	Voluntary
England and Wales(Liverpool)	1948	Voluntary
New Zealand	1948	Compulsory

注：文献2より一部抽出

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をベースにした地域がん登録が、大阪府、愛知県、兵庫県で開始されている。現在（2011年9月）、45道府県1市で行われているが、一部の登録室の出張採録を含め、すべてVoluntaryベースで遂行されている。このように、わが国の地域がん登録のスタートは、欧米の地域がん登録の開始年と比較して大きな隔たりはないものの、登録の精度（DCO%）に関しては、一部の県を除き、国際的評価に耐えられる数値ではないことが長年の課題となっている。2006年の罹患データのDCO%は、2.9%（福井県）から68.9%（愛媛県）で、平均26.1%であり、欧米の1ケタ台のDCO%には遠く及ばない状況である[6]。そのため、Voluntaryによる登録ではなくCompulsoryな登録への法的整備を求めて、2006年9月に地域がん登録全国協議会より、国民の皆様に地域がん登録への理解を求める声明文を発表し、2009年11月には地域がん登録の法制化を謳っている政党に対してその活動を支援する旨の要望書を提出している。また、全国がん（成人病）センター協議会からも2009年11月に厚生労働大臣、2011年7月には内閣総理大臣・総務大臣等へ宛てて要望書が提出されている。その他にも全国衛生部長会や地域がん診療連携拠点病院連絡協議会からも同様の要望書が出されている。しかし、未だ大きな動きはなく、これまでと変わらず「地域がん登録」の重要性の認識は高まりを見せず、西欧並みの精度への改善への道のりは遠いと思われる状況である。

何故にわが国では「地域がん登録」の重要性の認識が低いのであろうか？

がん疫学研究と地域がん登録に30年以上携わってきた立場からその要因に関して私的に考察を試みた。

疫学の嚆矢と言われているのは、19世紀中ごろ、コッホがコレラ菌を発見する前のロン

表2 欧州と日本における疫学関連の歴史事象

西暦	欧州		西暦	日本	
	事象	関係者		事象	関係者
1662	ロンドンの人口と死亡統計	ジョン・グラント			
1700頃	生命保険統計	エドモンド・ハレー			
1835	人間について	アドルフ・ケトレー			
1839	イングランド・ウエールズ統計	ウイリアム・ファー			
1854	ロンドン・ブロードストリートのコレラ対策	ジョン・スノー			
1854-56 1870-91	クリミア戦争 聖トーマス病院に看護教習所を設立	フローレンス・ナイチンゲール	1875-80	聖トーマス病院へ留学	高木兼寛
1866	ミュンヘン大学に衛生学講座	マックス・フォン・ペッテンコーヘル	1884-88	コッホとペッテンコーヘルの元へ留学	森林太郎
1883	コレラ菌発見	コッホ	1885-92	コッホの元へ留学	北里柴三郎
1886-91	コレラの原因・細菌説と土壌説の争い	コッホ(細菌説) ペッテンコーヘル(土壌説)	1883 1884	軍艦筑波で脚気 練習船筑波で脚気減少	高木兼寛
			1885-90	脚気論争(細菌説と栄養説)	森林太郎(細菌説) 高木兼寛(栄養説)
1892	コレラブイヨンの摂取	ペッテンコーヘル	1890	破傷風・ジフテリアの血清療法開発	北里柴三郎
			1899	人口静態・動態統計	内閣統計局
1901	自殺	ペッテンコーヘル	1910	オリザニン(ビタミンB1)発見	鈴木梅太郎
1929	地域がん登録	ハンブルグ	1951	がん実態調査	瀬木三雄

ドンで、コレラの流行があり、日々増大する死亡者を井戸の使用を禁止することによってくい止めたジョン・スノーの働きだと言われている。スノーはコレラによる死亡者の住所を手掛かりに地図上にプロットするという手法を用いて、ブロードストリートの井戸が問題であることを確信し、使用禁止によってコレラの流行を阻止したわけである。その卓見は素晴らしいことであるが、そもそもコレラ死亡者のデータが収集・管理されており、利用可能であったという実状が前提にあったと思われている。実際にロンドンでは 17 世紀にジョン・グラント、18 世紀にエドモンド・ハレー、19 世紀前半にウイリアム・ファーらによって人口の把握や死亡統計の整備がきちんと行われていたのである。

わが国に目を転じると、疫学の嚆矢として認識されているのは、脚気の原因としてビタミンB1が鈴木梅太郎にて発見される20年ほど前に、高木兼寛による「海軍や陸軍で猛威を振るった脚気問題の解決」であろうと思われる。当時の脚気は、わが国の死亡原因の上位を占めており、海軍・陸軍では重大な問題であった。陸軍の医務官であった森林太郎の考えは「脚気の細菌説」で、海軍の医務官であった高木兼寛は栄養説を採っていた。高木は練習船筑波で従来の食事を改善し（白米中心の食事から洋食や麦御飯中心の食事へ）、軍艦龍驤（前年の航海で脚気患者と死亡者を出した）と同じ航海を辿らせ、脚気の発症を食い止めることに成功している。このとき用いられた手法が疫学的方法に基づいていたのである。一方、陸軍の森林太郎は、海軍で疫学的手法により脚気を防止した高木兼寛の説や研究を否定し、白米中心の食事の改善を行わず、脚気細菌説に固執し、陸軍の脚気による死亡の防止に失敗してしまった。

ここで、何故森林太郎は細菌説を唱え、高木兼寛は栄養説を採ったのであろうか？

当時、森、高木、そして北里柴三郎らは同じ時期に欧州へ留学している（表 2）。森はドイツのコッホ、ペッテンコーヘルのもとへ[9,12,13]、北里もコッホのもとへ、そして高木はイギリスのセント・トーマス病院が留学先となっている[7,10]。この留学先の相違によって、森と高木の疾病に対する考え方や対応法が大きく異なってしまったのではないかと推測している。さらに、森はドイツでのコッホ（細菌説）とペッテンコーヘル（土壌説）のコレラ論争を知っており、ペッテンコーヘルが敗北するのを目の当たりにして、コッホの細菌説への信頼を大きく増幅したのではないかと思われる。セント・トーマス病院へ留学した高木は、病気の原因を究明するという研究的視点よりも、理由はともあれ現状を改善する手法あるいは疫学的視点による疾病への対応方法に磨きをかけたのではないかと推測される。留学当時のイギリスでは、統計的手法や疫学的手法が重要視されていたころで、これらの手法に造詣の深かったナイチンゲールが活発に活動を展開していたころであった[8,11]。そのため、イギリスの当時の雰囲気やナイチンゲールらの影響を強く受けていたのではないかと推測している（図 1）。高木の脚気の研究は、対象集団（population at risk）

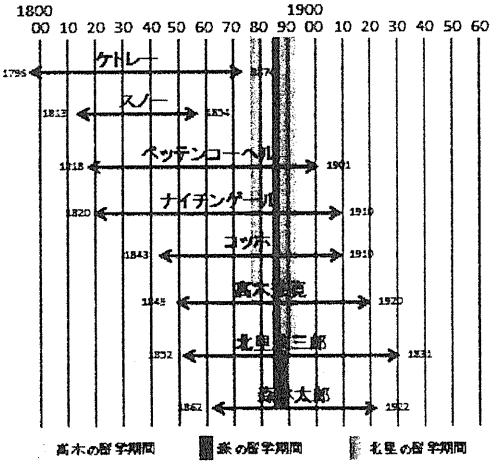

図 1　高木、森、北里の留学時期と同時代人の年表

を設定し、疾病や事件の頻度を計算するという基本的な疫学的手法に則っていたことからも、そのベースに歴史的なイギリスの考え方が反映していたことが窺われる。

ここで、わが国の疾病対策は、高木兼寛に代表される統計的手法や疫学的な研究ベースのもとで行われる疾病対策の道を中心として進むのではなく、森林太郎が支持する細菌説に代表されるように疾病の原因究明を目指す研究ベースの道を中心に据えて進むことになり、その流れが今日まで蜿蜒と引き継がれているのではないだろうか。森がペッテンコーヘルの考えに同調していたにも関わらずコッホの細菌説へ傾いていなければ、わが国の疾病対策は異なった道を歩んだかもしれない。というのは、ペッテンコーヘルはコレラ論争では土壌説を採って失敗に終わっているが、ミュンヘンにおいて下水道の導入等によって衛生環境状態の改善を図り、感染症の防止対策に大きな貢献をした研究者で、現在でもミュンヘンでは偉人として絶大な尊敬を勝ち得ており、ミュンヘン大学にはその名を翳した研究所も設立されるほどの研究者だったのである[14]。

「地域がん登録」に 30 年以上携わってきた思いが以上のような考えに結びついてしまった。今後の地域がん登録は、法的根拠に基づいて Compulsory な届出とすべきであろうし、社会一般の方々や衛生行政に携わる方々に、疫学的・公衆衛生学的な疾病対策の重要性を認識していただき、その基本的なデータとしてがん罹患、死亡のデータが不可欠であるという理解が人口に膾炙することを期待したいと思っている。積極的に「がん登録の有効性」を訴えることが必要で不可欠なことですが、無理に理解を進めるのではなく、実績の積み上げをみていただいて、理解が熟すのをじっくり時間をかけて待つのも１つの方策ではないかと感じている。そのためには、日々、粛々とがんデータの収集・蓄積・管理・保存を継続して行い、罹患データや生存率データを定期的に報告するとともに、誰もが利用可能であり、いつでもデータの公表ができるような状態にしておくことが肝要であると思っている。その意味で、「がん登録」は、われわれ人類社会の変遷や興亡を健康面から長期的に観察することを可能にする記録を作成していると考えることができる。

近年、過去の資料や記録が現在や未来にとって不可欠な情報を提供するという思想も生まれてきていることに大いなる期待を寄せるとともに、地域がん登録もその流れに寄与できるように準備と実践を重ねておくべき時期が来ているのではないだろうか[15]。

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Summary

Population-based cancer registries are currently receiving attention more than ever before. The first and oldest registration in the world was started in 1929, in Hamburg. The first registries in Japan were in Miyagi Prefecture, Hiroshima City, and Nagasaki City in the 1950s. Today, 45 out of 47 Japanese prefectures practice cancer registration. Though Japanese cancer registries have a 50 year-old history, their accuracy is far inferior to that of registries overseas. Which factors constitute an obstacle to improve accuracy of the cancer registries in our country? We considered some possible reasons from epidemiological and statistical viewpoints.

The governments and the bureaucrats of the West have developed statistics on population and death rates systematically since ancient times. With the advance of statistical methods, record-keeping of fundamental data came to be seen as a routine duty for administrators. Although our country has gathered useful information on infectious diseases and illness prevention, the techniques for identifying "population at risk" statistics were underdeveloped for many years. In this way, the registration of chronic diseases such as "cancer" has been insufficient. This historical background still affects the current cancer registries in our country, and accuracy does not improve easily.

Compulsory cancer registration is legally mandated in many European countries, and Japan needs to follow suit. The periodic data collection and compilation of basic information, indispensable to cancer control programs, will be a key to improving accuracy of our cancer statistics.

The archived data from a cancer registry can describe the history of public health administration and cancer control programs. This data should be recognized as a foundation for the future.

Plasma Free Amino Acid Profiling of Five Types of Cancer Patients and Its Application for Early Detection

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Abstract

Background: Recently, rapid advances have been made in metabolomics-based, easy-to-use early cancer detection methods using blood samples. Among metabolites, profiling of plasma free amino acids (PFAAs) is a promising approach because PFAAs link all organ systems and have important roles in metabolism. Furthermore, PFAA profiles are known to be influenced by specific diseases, including cancers. Therefore, the purpose of the present study was to determine the characteristics of the PFAA profiles in cancer patients and the possibility of using this information for early detection.

Methods and Findings: Plasma samples were collected from approximately 200 patients from multiple institutes, each diagnosed with one of the following five types of cancer: lung, gastric, colorectal, breast, or prostate cancer. Patients were compared to gender- and age- matched controls also used in this study. The PFAA levels were measured using high-performance liquid chromatography (HPLC)–electrospray ionization (ESI)–mass spectrometry (MS). Univariate analysis revealed significant differences in the PFAA profiles between the controls and the patients with any of the five types of cancer listed above, even those with asymptomatic early-stage disease. Furthermore, multivariate analysis clearly discriminated the cancer patients from the controls in terms of the area under the receiver-operator characteristics curve (AUC of ROC >0.75 for each cancer), regardless of cancer stage. Because this study was designed as case-control study, further investigations, including model construction and validation using cohorts with larger sample sizes, are necessary to determine the usefulness of PFAA profiling.

Conclusions: These findings suggest that PFAA profiling has great potential for improving cancer screening and diagnosis and understanding disease pathogenesis. PFAA profiles can also be used to determine various disease diagnoses from a single blood sample, which involves a relatively simple plasma assay and imposes a lower physical burden on subjects when compared to existing screening methods.

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Introduction

Several minimally-invasive, easy-to-use cancer diagnostic methods using peripheral blood or urine samples have recently been developed to ease the physical burden on patients and to reduce the costs and time involved [1,2,3,4,5,6,7,8]. Rapid advances have been made in cancer diagnosis and prognosis methods based on metabolome analysis [3,9,10,11,12,13,14], which frequently involves the use of multivariate analysis techniques, such as computer-aided, machine-learning systems for data mining.

Although metabolome analysis is a promising approach in screening for diseases such as cancer, some practical limitations remain. These include the necessity to measure a huge number of metabolites [15,16,17], data-redundancy problems, including the false-discovery rate (FDR) and overfitting, and cost constraints. One approach to overcoming these problems is "focused metabolomics", which limits the objects of the analysis to those that play roles in general metabolism and share physical similarities.

Amino acids are among the most suitable candidates for focused metabolomics as they are either ingested or synthesized endogenously and play essential physiological roles both as basic metabolites and metabolic regulators. To measure amino acids, plasma free amino acids (PFAAs), which abundantly circulate as a medium linking all organ systems, would be the most favorable target because their profiles have been known to be influenced by metabolic variations in specific organ systems induced by specific diseases [18,19,20,21]. Additionally, plasma samples can be collected easily from patients.

Several investigators have also reported changes in PFAA profiles in cancer patients [22,23,24,25,26,27,28]. However, despite evidence of a relationship between PFAA profiles and some types of cancer, few studies have explored the use of PFAA profiles for diagnosis because, although PFAA profiles differ significantly between patients, the differences in individual amino acids do not always provide sufficient discrimination abilities by themselves [24,29,30]. To address this issue, we previously constructed and tested a diagnostic index based on PFAA concentrations, known as the "AminoIndex technology" [29,30,31,32,33], to compress multidimensional information from PFAA profiles into single dimension and maximize the differences between patients and controls (Figure 1). We obtained preliminary data on the efficacy of the "AminoIndex technology" for the early detection of colorectal, breast, and lung cancers in approximately 150 samples from a single medical institute [29,30].

Moreover, technologies have recently been developed to analyze amino acids with high accuracy. For example, we developed a method to measure PFAA profiles using high-performance liquid chromatography (HPLC)–electrospray ionization (ESI)–mass spectrometry (MS) [34,35,36].

The present study aimed to determine the possibility of PFAA profiling for cancer diagnosis using a large number of samples from multiple medical institutes. We measured the PFAA profiles of approximately 200 cancer patients from three different institutes each with one of the following five types of cancer: lung, gastric, colorectal (CRC), breast, or prostate cancer. Patients were compared to five times sizes of gender- and age-matched controls also used in this study. We then compared the alterations in the

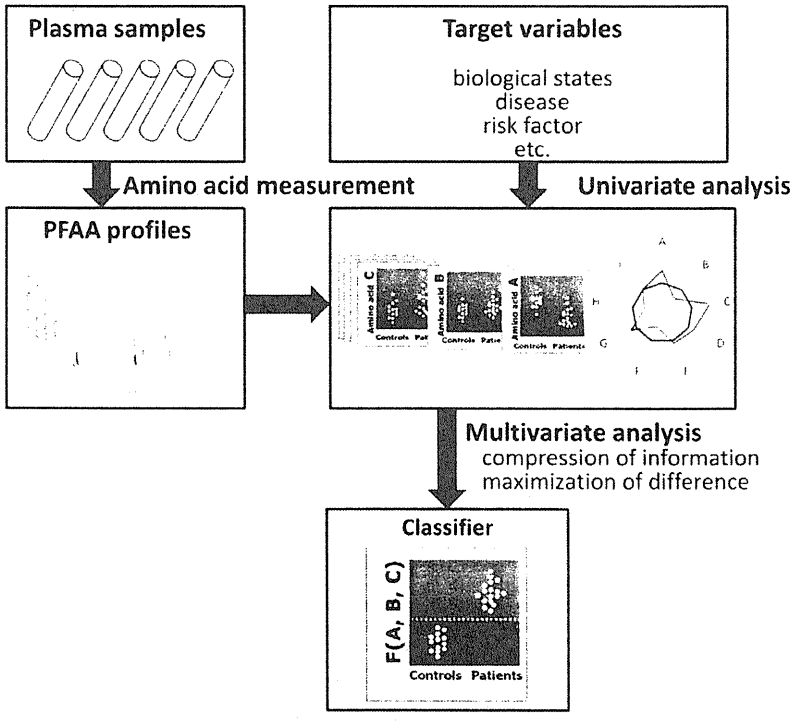

Figure 1. Concept of the generation of "AminoIndex technology". At the top of the diagram, PFAA concentrations are measured for each subject. In the middle, target variables and univariate analysis of PFAA profiles are represented. At the bottom, an estimation of the classifier with optimized discriminating power using multivariate analysis is presented.
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PFAA profiles between the cancer patients and the controls using univariate and multivariate analyses. As a result, significant alterations in PFAA profiles were observed in cancer patients in comparison to control subjects. We demonstrated two types of alterations in PFAA profiles in cancer patients: some differences reflected the metabolic changes common to many cancers, while others were specific to each type of cancer. We also found that both common and cancer type-specific alterations in PFAA profiles were observed even in the patients with early stage cancer. Furthermore, using a large number of samples allowed us to verify the robustness of PFAA profiling for the early detection of various cancers.

Materials and Methods

Ethics

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committees of the Kanagawa Cancer Center, the Osaka Medical Center for Cancer and Cardiovascular Diseases, the Okayama University Hospital, the Yokohama City University Medical Center, the Gunma Prefectural Cancer Center, the Shizuoka Prefectural Cancer Center, the Chiba Prefectural Cancer Center, the Yokohama Municipal Citizen's Hospital, the Yokohama Minami Kyosai Hospital, the Kanagawa Health Service Association, the Kameda Medical Center Makuhari, and the Mitsui Memorial Hospital. All subjects gave their written informed consent for inclusion before they participated in the study. All data were analyzed anonymously throughout the study.

Subjects

Data from Japanese patients with lung cancer (LC), gastric cancer (GC), colorectal cancer (CRC), breast cancer (BC), and prostate cancer (PC) were analyzed in this study. The patients had been histologically diagnosed with primary cancer at various Japanese medical institutes between 2006 and 2009. The LC patients were recruited from the Osaka Medical Center for Cancer and Cardiovascular Diseases, the Chiba Prefectural Cancer Center, the Kanagawa Cancer Center, and the Gunma Prefectural Cancer Center. The GC patients were recruited from the Okayama University Hospital, the Gunma Prefectural Cancer Center, and the Shizuoka Prefectural Cancer Center. The CRC patients were recruited from the Kanagawa Cancer Center, the Shizuoka Prefectural Cancer Center, and the Gunma Prefectural Cancer Center. The BC patients were recruited from the Yokohama City University Medical Center, the Kanagawa Cancer Center, and the Gunma Prefectural Cancer Center. The PC patients were recruited from the Kanagawa Cancer Center, the Yokohama Municipal Citizen's Hospital, the Yokohama Minami Kyosai Hospital, and the Gunma Prefectural Cancer Center. Control subjects with no apparent cancer were chosen from among those undergoing comprehensive medical examinations at three different Japanese medical institutes (the Center for Multiphasic Health Testing and Services of the Mitsui Memorial Hospital, the Kameda Medical Center Makuhari, and the Kanagawa Health Service Association) between 2008 and 2009.

Colonic polyp patients were recruited from among those undergoing endoscopic polypectomy at the Kameda Medical Center Makuhari between 2006 and 2008.

For the purposes of data analysis, the patients were assigned to five groups based on their primary cancer diagnoses (~140–200 patients per group), and five age- and gender-matched control groups were also established (Table 1). Data sets for all of the cancer patients and controls, as well as all cancer patients stratified by gender, were also analyzed.

PFAA measurement

Blood samples were collected from the controls and the patients prior to any medical treatment. Blood samples (5 ml) were collected from forearm veins after overnight fasting in tubes containing ethylenediaminetetraacetic acid (EDTA; Termo, Tokyo, Japan) and were immediately placed on ice. Plasma was prepared by centrifugation at 3,000 rpm at 4°C for 15 min and then stored at −80°C until analysis. After the plasma collection, all samples were stored and processed at the Institute for Innovation of the Ajinomoto Co., Inc. (Kawasaki, Japan). To reduce any bias

Table 1. Demographic and clinical characteristics of subjects.

Data set		LC		GC		CRC		BC		PC	
		Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Size	Total	200	996	199	985	199	995	196	976	134	666
	M/F	125/75	635/371	126/73	626/359	114/85	570/425	0/196	0/976	134/0	666/0
Age	Mean	65.0[a]	63.2	64.8[a]	62.9	63.7	62.4	55.3	54.5	69.4[c]	65.8
	(SD)	(10.0)	(9.2)	(10.8)	(9.7)	(9.5)	(9.5)	(12.6)	(11.1)	(6.7)	(6.1)
BMI	Mean	22.5	22.9	22.7	22.8	23.0	22.8	22.4	22.0	23.4	23.4
	(SD)	(3.8)	(3.0)	(3.2)	(3.0)	(3.7)	(3.0)	(3.4)	(3.5)	(2.7)	(2.5)
Stage	0	-		-		8		26	-	-	
	I(A)	29		120		63		75		0	
	II(B)	16		29		48		73		95	
	III(C)	54		26		59		13		19	
	IV(D)	28		24		19		0		15	
	Uncharacterized	1		0		2		9		5	

[a]$p<0.05$,
[c]$p<0.001$.
For LC, GC, CRC, and BC, cancer stages were determined according to the International Union Against Cancer TNM Classification of Malignant Tumors, 6th edition [38], and for PC, cancer stages were determined according to Jewett staging system [39].
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introduced prior to analysis, samples were analyzed in random order. The plasma samples were deproteinized using acetonitrile at a final concentration of 80% before measurement. The amino-acid concentrations in the plasma were measured by HPLC–ESI–MS, followed by precolumn derivatization. The analytical methods used were as described previously [34,35,36].

Among the 20 genetically-encoded amino acids, glutamate (Glu), aspartate (Asp), and cysteine (Cys) were excluded from the analysis because they are unstable in blood. Citrulline (Cit) and ornithine (Orn) were measured instead because they are relatively abundant in blood and are known to play important roles in metabolism. The following 19 amino acids and related molecules were therefore measured and analyzed: alanine (Ala), arginine (Arg), asparagine (Asn), Cit, glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), Orn, phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val).

Two metrics were made for each of the 19 amino acids including the absolute concentration of each amino acid, which directly reflected its availability and consumption, and the ratios associated with the specific metabolic status in each organ. The concentrations of the amino acids in the plasma were expressed in μM, and the ratios of the amino acid concentrations were expressed by the follow equation:

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

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

Statistical analysis

Two types of metric were used for each data set for analysis using either the amino-acid concentration or the ratio as explanatory variables.

Mean and SD. The mean amino-acid concentrations ± standard deviations (SDs) were calculated to determine summarized PFAA profiles for both patients and controls.

Mann-Whitney U-test. The Mann-Whitney *U*-test was used to assess significant differences of the plasma amino-acid concentrations between the patients and the controls.

ROC analysis. Receiver-operator characteristic (ROC) curve analyses were performed to determine the abilities of uni- and multi-variate analyses to discriminate between patients and controls. The patient labels were fixed as positive class labels. Therefore, an area under the ROC curve (AUC of ROC) value of <0.5 indicated that the amino acid level was lower in the patients than the controls, whereas an AUC of ROC value of >0.5 indicated that it was higher. The 95% confidence interval (95% CI) of AUC of ROC for the discrimination of patients based on amino acid concentrations and ratios was also estimated as described by Hanley and McNeil [37].

Two-way analysis of variance (ANOVA). The two-way ANOVA was used to evaluate the effects of gender, age, and smoking status as potential confounding factors. The presence of cancer and gender were assumed to be independent factors, age was treated as a continuous predictor rather than a categorical predictor, and the interaction term between the presence of cancer and smoking status was analyzed.

Two-class linear discrimination analysis (LDA). Linear discrimination analysis (LDA) with stepwise variable selection was performed to distinguish patients with each type of cancer from the control subjects, in which both the maximum and the minimum p-values for a term to be added or removed were set at 0.001.

Multi-class LDA for discrimination. LDA with stepwise variable selection was also performed to distinguish patients with a specific cancer from the complete data set containing all cancer patients stratified by gender (four kinds of cancer patients in each data set). Because the size of each group was smaller than that of two-class LDA, the maximum p-value for a term to be added was set at 0.05 and the minimum p-value for a term to be removed was set at 0.10. The Mahalanobis distance was used as a metric of classification. The accuracy was defined as the ratio of the correctly discriminated patients to the total number of patients with each cancer instead of AUC of ROC because ROC analysis could be applied only for two-class discrimination.

Leave one out cross—validation (LOOCV). LOOCV was performed to correct potential over-optimization for obtained LDA models. Briefly, one sample was omitted from the study data set, and the LDA model was calculated for the remaining samples to estimate coefficients for each amino acid. The function values for the left-out sample were calculated based on the model. This process was repeated until every sample in the study data set had been left out once.

Conditional logistic-regression (c-logistic) analysis. C-logistic analysis was also performed to verify the effects of age and gender, potential confounding factors, on the discriminatory abilities of obtained LDA models to differentiate patients with each type of cancer from the controls.

Subgroup analysis. To assess the effects of cancer stage, each data set was divided into a sub-data set according to disease stage and including corresponding controls, and analyzed using the ROC analysis in each data set.

Software

MATLAB (The Mathworks, Natick, MA) was used for the calculations of mean and SD, the Mann-Whitney *U*-test, ROC analysis, two-way ANOVA, LDAs, and LOOCV. GraphPad Prism (GraphPad Software, La Jolla, CA) was also used for the ROC curve analysis. LogXact (Cytel, Cambridge, MA) was used for the c-logistic analysis.

Results

Characteristics of subjects

Table 1 summarizes the characteristics of the subjects in this study. The data sets comprised 200 LC patients and 996 controls, 199 GC patients and 985 controls, 199 CRC patients and 995 controls, 198 BC patients and 976 controls, and 134 PC patients and 666 controls (Table 1). The sample size for each cancer type was greater than those in previous reports [25] and provided sufficient statistical power to test the robustness of the PFAA profiles for cancer diagnosis.

There were no significant differences in body mass index (BMI) among the data sets (Table 1). Weight loss due to malnutrition was therefore not expected to influence the results. Although significant differences in average age were observed among the data sets (LC, $p<0.05$; GC, $p<0.05$; and PC, $p<0.001$), the effects appeared to be relatively minor because the absolute values of these differences were small (Table 1).

For LC, GC, CRC, and BC, disease stages were determined according to the Sixth Edition of the International Union Against Cancer (UICC) Tumor–Node–Metastasis (TNM) Classification of Malignant Tumors [38]. For PC, the stage was determined

according to the Jewett staging system [39]. For all types of cancer, a large proportion of the patients had early-stage disease. The fractions of patients at each stage according to type of cancer were as follows: ~50% stage I, ~10% stage II, ~25% stage III, and ~15% stage IV for LC; ~60% stage I, ~15% stage II, ~13% stage III, and ~12% stage IV for GC; ~35% stages 0 and I, ~25% stage II, ~30% stage IV, and ~10% stage IV for CRC; ~5% stage 0, ~25% stage I, ~25% stage II, and ~7% stage III for BC; and ~75% stage B, ~13% stage C, and ~12% stage D for PC (Table 1).

The patients with each type of cancer could be further subdivided based on histological type (for LC, GC, CRC, and BC) or Gleason score (for PC), as is summarized in Table S1. The characteristics of 34 colonic polyp patients as well as the smoking status of patients are also summarized in Table S1.

Shared PFAA profiles among cancers

Univariate analysis was used to compare the PFAA profiles of the cancer patients and controls. The differences in the significance levels of each amino acid between the patients and the controls are shown in Figure 2A. The results of the ROC analysis are depicted in Figure 2B because the levels of significance depend on sample size. The concentrations and ratios of each amino acid profile for both patients and controls are shown in Tables S2. And the AUCs of ROC and their CIs of each amino acid are shown in Table S3 (concentration) and Table S4 (ratio), respectively.

Two-way ANOVA was used to evaluate the potential confounding effects of gender, age, and smoking status. Correcting for these factors did not greatly affect the significance levels of each amino acid, suggesting that their effects on the PFAA profiles were minor (Table S5).

The plasma concentrations of Gln, Trp, and His were significantly decreased in all of the cancers except PC, and none of the amino acids showed increased concentrations across all types of cancer ($p<0.05$). The ratios of Trp and His were significantly decreased, while those of Pro and Orn were increased, in all cancers ($p<0.05$) (Figure 2).

To further examine the shared traits among cancer patients, the PFAA profiles were compared using a pooled data set including all cancer patients and controls. Notably, the amino acids that were affected by this type of analysis had significant differences in both concentration and ratio: 11 amino acids (Asn, Gln, Cit, Val, Met, Leu, Tyr, Phe, His, Trp, and Arg) showed decreases, while four amino acids (Ser, Pro, Gly, and Orn) exhibited increases (Figure 2). Changes in Gln, Trp, His, Pro, and Orn were detected in the analysis for all types of cancer. Alterations in these amino acids might therefore reflect characteristic changes in metabolism that are common to all cancers.

Specific PFAA profiles for each cancer

In addition to the changes that were common to all of the cancers, we detected alterations in PFAA profiles that were specific to each disease type (Figure 2). Overall, the concentrations of most amino acids were decreased in GC and CRC patients, whereas no clear trends in amino acid concentrations were observed in the other groups (Figure 2). Furthermore, some of the amino acids showed opposite trends in different types of cancer. For example, the concentrations of Thr were decreased in GC and CRC patients, but increased in BC patients (Figure 2). These variations in the PFAA profiles might reflect specific characteristics of each cancer, in contrast to the limited set of amino acids that are responsible for the metabolic changes shared by all cancers.

Changes in PFAA profiles in early-stage cancers

Although alterations in the PFAA profiles of cachexic patients with advanced cancer have been well documented, few reports have considered early-stage patients. However, a large fraction of the cancer patients in the current data set were in the early stages of disease (Table 1). The differences in PFAA profiles according to disease stage were therefore examined for each cancer (Figure 3, Figure S1, Table S3, Table S4).

Notably, alterations in the PFAA profiles were detected in all patients, including those in the early stages of disease, in the current study. All amino-acid concentrations and ratios were drastically decreased in early stage disease patients, regardless of the subsequent progression. In particular, significant decreases of each amino acid concentration were observed in GC and CRC patients (Figure 3A), and changes in each ratio were notable in all of the cancer patients (Figure 3B).

Early-stage cancer patients are generally asymptomatic. Moreover, most of the subjects in the present study did not show significant weight loss (a symptom typical of cachectic patients) (Table 1), anorexia, or decreases in serum albumin concentrations (data not shown). The changes in the PFAA profiles in cancer patients therefore appeared to be independent of any effects caused by poor nutrition resulting from tumor progression.

Discriminating cancer patients and controls by PFAA profiles

The results of the univariate analyses suggested that cancer patients and controls could be discriminated using multivariate analysis. By assuming that the presence of cancer and the concentrations or ratios of the PFAA profiles were objective and explanatory variables, respectively, LDA was able to distinguish cancer patients from the corresponding controls with variable selection. The results of variable selection are indicated in Table 2 (concentration) and Table S6 (ratio), respectively.

The discrimination abilities for each cancer patient were evaluated using the AUC of ROC of the discriminate score and were found to be >0.75 in all cases (Table 3 and Table S7). In concrete analysis, AUCs for the discrimination of patients based on the amino acid concentrations and ratios, respectively, were also estimated as follows: 0.802 (95% CI: 0.766~0.838) and 0.802 (95% CI: 0.767~0.837) for LC; 0.849 (95% CI: 0.816~0.882) and 0.816 (95% CI: 0.780~0.852) for GC; 0.874 (95% CI: 0.842~0.906) and 0.881 (95% CI: 0.851~0.910) for CRC; 0.778 (95% CI: 0.741~0.815) and 0.778 (95% CI: 0.741~0.815) for BC; and 0.783 (95% CI: 0.740~0.826) and 0.779 (95% CI: 0.740~0.819) for PC (Table 3 and Table S7). The discriminate analysis was therefore able to adequately distinguish between different types of patient cancer.

Variable selection was also performed for each cancer patient. Eight amino acids were selected in more than two of the five kinds of cancers: Gln, Ala, Val, Ile, His, Trp, Orn, and Lys for the concentrations (Table 2A); and Ser, Gln, Val, Met, His, Trp, Lys, and Arg for the ratios (Table S6). Four of the amino acids (Gln, Val, His, and Trp) among each set were selected for both explanatory variables (Table 2 and Table S6). These amino acids were similar to those associated with all types of cancer as indicated by the univariate analysis (Gln, Trp, His, Pro, and Orn).

On the other hand, some amino acids incorporated into the LDA model were not identified as significant amino acids by the univariate analysis. For example, the Val concentration did not show a significant alteration in the univariate analysis (Figure 2A), but it was incorporated into the LDA model (Table 2). Because plasma concentrations of each amino acid are metabolically connected to each other, there might be a potential correlation that cannot be