Amino acids are usually considered protein subunits or nutrients. However, recent advances in the metabolomics of amino acids and high-throughput analytical techniques have shown that amino acids in the body (e.g. in the blood) can be used as biomarkers for evaluating disease risk or progression and for selecting proper treatment. For example, one study has reported that the risk of developing diabetes mellitus can be predicted from the metabolite profiles of a combination of 3–5 amino acids in the blood. These new biomarkers, which are derived from combinations of amino acid concentrations, allow prediction of the risk of developing diabetes mellitus even when adjusted for conventional insulin resistance-related indices such as fasting insulin levels, homeostatic model assessment of risk of insulin resistance, and a 75-g oral glucose tolerance test. Amino acid metabolism indices differ from conventional biochemical indices and therefore may be regulated by a different paradigm. I anticipate that further research into amino acid metabolism will provide new information about disease risk and progression.

Advances in analytical techniques are among the most important reasons why novel findings concerning amino acid metabolism, such as the ability to predict the risk of developing diabetes, have been reported over the last several years. Plasma amino acid concentrations have conventionally been measured using an amino acid autoanalyzer that combines ion-exchange chromatography and a ninhydrin reaction. However, this method is so time-consuming that it has been used only for specific purposes, such as clinical studies with limited blood samples or in the diagnosis of inborn errors of metabolism. Progress
in amino acid analysis techniques has led to using liquid chromatograph mass spectrometry (LC-MS) more frequently than the conventional method and analysis using LC-MS has enabled high-throughput measurement of plasma amino acids. Moreover, with the spread of this technology an enormous database of information is now available and it provides many clinically important insights into amino acid metabolism. As a result, many new amino acid metabolism findings have recently been reported.

In this review, I will first briefly discuss the role of plasma amino acids in the body and then summarize recent clinical research using novel plasma amino acid biomarkers for cancer screening.

**Functions of amino acids in the body**

Amino acids account for approximately 20% of an individual’s body weight so a 50 kg person has approximately 10 kg of amino acids, most of which exist as proteins (Fig. 1). Free amino acids, known as the amino acid pool, are found in cells, intercellular components, plasma, and other biological components. Amino acids, which are digested and absorbed from foods and then enter the amino acid pool, from which they are used for protein synthesis, and are later returned to the amino acid pool as a result of protein degradation or excreted in the urine or feces. In this turnover process, the body replaces its protein composition through metabolism every 2–3 months.

The concentration of free amino acids in the plasma, which is part of the amino acid pool, is precisely regulated by a variety of control mechanisms and is maintained at a constant level in healthy individuals. Many studies have shown that various disease states, such as hepatic or renal failure, Alzheimer’s disease, psychological disorders, and inflammatory bowel disease, may alter the plasma amino acid balance through aberrations in these regulatory mechanisms.

Also, as amino acid metabolism occurs actively in the muscle, the liver, the brain, the kidney, and the small intestine, changes in the amino acid metabolism balance in these organs are reflected as changes in the plasma amino acid concentrations.

**Metabolomics research and “AminoIndex Technology”**

Recent advances in analytical techniques have enabled disease states to be analyzed through the comprehensive measurement of metabolites. However, problems remain in the clinical setting with regard to the reproducibility, quantification, and cost of this type of analysis. The approach to such analysis using “AminoIndex Technology” is based on the assessment of diseases and physical conditions using plasma amino acid concentrations obtained through the measurement of a particular subset of metabolites. Measurement of amino acid metabolites is particularly useful for predicting various conditions because amino acid metabolism is closely related to many other metabolic pathways, such as glucose and lipid metabolism, and amino acids can therefore be considered as “hubs” to which many types of metabolites on the metabolic map are connected.

So the measurement of amino acid levels enables us to infer the statuses of various aspects of the overall metabolic map, such as those of glucose or lipid metabolism. In the previously mentioned study using metabolites as a measure of the risk of developing diabetes, an assay of 61 metabolites resulted in the identification of 3 or 5 metabolites associated with disease risk, all of which were amino acids. The underlying concept of “AminoIndex Technology” is to focus on these amino acids and to establish a reproducible and quantifiable method of measurement with applications in the clinical setting.

“AminoIndex Technology” is a technique in which multivariate analysis of the plasma amino acid concentration (Fig. 2) is used to compute disease or health condition scores. Through the use of statistical models, it can be used to predict the detection or severity of a single disease and clarify the status of multiple diseases or health conditions from a single blood sample. Recent research using “AminoIndex Technology” has been reported in other areas in addition to the cancer studies reported in this review, and their clinical utility has been summarized in another review.
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\(^\text{10-14}\). Furthermore, other clinical studies have demonstrated the possibility of using plasma amino acid concentrations as multivariate biomarkers in cancer screenings\(^\text{15-18}\). 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\(^\text{19}\).

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; Kamagata 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\(_c\) 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.

**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...
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

<table>
<thead>
<tr>
<th>AICS</th>
<th>Incidence rate</th>
<th>AICS value ≥ 5.0 (Rank B or C)</th>
<th>AICS value ≥ 8.0 (Rank C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>AICS(gastric)</td>
<td>0.0917</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>AICS(lung)</td>
<td>0.0657</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>AICS(colorectal)</td>
<td>0.0820</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>AICS(prostate)</td>
<td>0.0690</td>
<td>80</td>
<td>64</td>
</tr>
<tr>
<td>AICS(breast)</td>
<td>0.0775</td>
<td>80</td>
<td>47</td>
</tr>
</tbody>
</table>

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), was used instead of the prevalence rate.

AICS: AminoIndex, Cancer Screening

<table>
<thead>
<tr>
<th>Item</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AICS(gastric)</td>
<td>AICS(lung)</td>
</tr>
<tr>
<td>Cancer patients</td>
<td>Rank A 33%</td>
<td>Rank A 8%</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Rank B 23%</td>
<td>Rank B 23%</td>
</tr>
<tr>
<td>Cancer patients</td>
<td>Rank A 27%</td>
<td>Rank A 27%</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Rank B 27%</td>
<td>Rank B 27%</td>
</tr>
<tr>
<td>Cancer patients</td>
<td>Rank A 39%</td>
<td>Rank A 15%</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Rank B 80%</td>
<td>Rank B 13%</td>
</tr>
<tr>
<td>Cancer patients</td>
<td>Rank A 36%</td>
<td>Rank A 32%</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Rank A 80%</td>
<td>Rank A 15%</td>
</tr>
</tbody>
</table>

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. 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,
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-
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. 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. 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,
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).
Evaluation of breast cancer

Approximately 12,000 Japanese died of breast cancer in 2009. 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/mL)](image1)

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

![Fig. 17. Sensitivity of AICS (breast) by stage](image2)
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

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**Table 3. Characteristics of individual AICS tests**

<table>
<thead>
<tr>
<th>Test item</th>
<th>Characteristics</th>
</tr>
</thead>
</table>
| 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

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**Fig. 18. Cancer screening using “AminoIndex Technology”**
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.

**References**