# Plasma amino acid levels in individuals with bacterial pneumonia and healthy controls

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**Research article**

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# Abstract

Background: Amino acids play an important role in immune responses and as neurotransmitters. During the course of a bacterial pneumonia (BP) episode, from the onset to the recovery phase, immune responses dramatically change, as does the metabolism of amino acids, a concept referred to as immuno-nutrition. We investigated the differences in plasma amino acid levels between the acute and recovery phases in individuals with BP and healthy controls.

Methods: Two groups of participants were recruited: Healthy adults aged over 60 years who had undergone a medical health examination and patients, admitted to hospital with BP. Samples were collected on Day 0 (the day of admission for patients with BP) and Day 7.

Results: A total of 93 healthy adults and 60 patients with BP participated in the study. Of those with BP, 43 had their amino acids measured on Day 7. Patients with BP had markedly decreased plasma levels of 12 amino acids on Day 0. Histidine and tryptophan remained low, while aspartic acid, asparagine, ornithine, proline, and threonine were higher on Day 7 in both males and females. Phenylalanine increased at Day 0 and Day7, and returned to normal range at the post-recovery period.

Conclusions: The findings suggest that the host response against bacterial infection changed the plasma amino acid levels. Amino acid levels on Day 7 (representing convalescence) continued to display an amino acid profile distinct from that observed in healthy individuals. Based on these findings, providing amino acids to patients with BP should be modified depending on stage of BP from the perspective of immuno-nutrition.


# Background

In recent years, advances in analytical methodologies have paved the way for comprehensive detection of a wide range of chemical compounds; and metabolomics has allowed analysis of metabolites in various diseases [1]. Specifically, amino acids serve an important role in the immune response [2] and as neurotransmitters [3]. Improvements in liquid chromatography-mass spectrometry technology have enabled comprehensive analysis of plasma amino acids in general hospitals. The plasma levels of some amino acids, namely threonine, serine, asparagine, glutamine, citrulline, and histidine, have been reported to be markedly reduced in hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease and bacterial infection [4]. We speculated that individuals with bacterial pneumonia (BP) would have similar changes in their amino acid levels. In individuals with infections, the immune response associated with changes with amino acid metabolism transition, a concept referred to as immuno-nutrition [5, 6]. However, there have been no detailed studies of the changes in plasma amino acid levels over the course of an episode of BP. The aim of this study was to describe changes in plasma amino acid levels in the acute and recovery phases of BP in hospitalized patients, and to compare their amino acid profiles with individuals who had recovered from BP, and healthy controls.

# Methods

# Subjects and data collection

The BP group comprised patients admitted to our hospital between March 2016 and December 2019, who were diagnosed with BP according to the Japanese Respiratory Society guidelines [7] and the criteria of a previous community-acquired pneumonia study [8]. The diagnostic criteria were expectoration of purulent sputum [9, 10], an increase in C-reactive protein, and white blood cell count [11], the appearance of new opacities on thoracic radiography or computed tomography, and fever [12, 13]. All these criteria were required to be met for a diagnosis of BP. And the patients who already had medication with antibiotics within seven day or had any symptoms of BP more than two days before admission were excluded. The BP severity was scored according to the A-DROP scoring system [7, 14]. Patients with diabetes (hemoglobin Alc (HbA1c) > 6.1% or taking diabetes medication), chronic renal failure (estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 $m^2$), or a history of gastric resection were excluded. Sputum samples were screened by Gram staining and cultured on chocolate agar with sheep blood, mannitol salt agar with egg yolk, and modified Drigalski agar.

Samples were collected on admission (Day 0) on all BP patients. Of the patients with BP, 43 (26 males and 17 females) provided convalescent blood samples on Day 7. Fourteen male BP patients as also provided samples at outpatient more than one month after admission as post-recovery. Day 0 samples were collected within 24 hours after admission, 2–3 hours after lunch. Post-recovery samples were collected after 12 hours of fasting. Meal plans were planned under the guidance of a nutritional manager to contain 600 kcal, 25 g protein, and 80–100 g carbohydrates. Leftover food from each served meal was measured, and the percentage of the meal eaten was calculated.

The control group comprised healthy individuals aged > 60 years who received a health examination at our hospital between May 2018 and January 2019. Participants were selected to ensure that each 5-year interval of age above 60 years included ten males and ten females. Blood samples were collected in an EDTA-2Na tube in the morning following an overnight fast. Individuals were excluded if they had taken any medication or had diabetes (HbAlc > 6.1% or on diabetes medication), chronic kidney disease (eGFR < 60 mL/min/1.73 $m^2$), and/or malignancies.

Blood samples for amino acid analysis were immediately placed in a test tube containing EDTA-2Na and were transported on ice to the testing laboratory. The samples were then immediately centrifuged, and the obtained plasma was stored frozen at − 40ºC. Thirty-eight amino acids and taurine were subsequently analyzed by SRL Inc (Tokyo, Japan) using liquid chromatography-mass spectrometry (LC-MS). We obtained the plasma levels of the total amino acids, taurine, the nine essential amino acids, and 13 non-essential amino acids from the reports supplied by SRL Inc.

## Statistical Analysis

Amino acid levels between male and female participants in the control group, and age and body mass index (BMI) between the patient and control groups were compared using the Mann-Whitney U test. The correlation between the level of each amino acid with age and BMI in the control group was assessed using multiple linear regression analysis. The levels of amino acids in each group were compared using a nonparametric multiple comparison test (the Steel-Dwass method), and the severity of pneumonia and meal consumption were compared using the chi-squared test. P-values < 0.05 were considered significant.

## Results

A total of 93 healthy controls (50 males and 43 females) and 60 patients (36 males and 24 females) with BP participated in this study. The etiologic bacteria were identified by culture in 48 patients. The pathogens included Streptococcus pneumoniae (n = 13), Haemophilus influenzae (n = 11), Staphylococcus aureus (n = 6), Klebsiella pneumoniae (n = 4), and other bacteria (n = 14). The remaining 12 patients had either gram-positive cocci (n = 8) or gram-negative rods (n = 4) on microscopy. All patients had completed their courses of antibiotics by Day 7.

Figure 1 shows the age distribution of participants in the BP and control groups. The average ages of the males and female patients in the BP group were 79.3 and 82.2 years, respectively.

Among the healthy controls, the levels of amino acids were higher in males than in females (Table 1). Multiple regression analysis revealed that some amino acids were correlated with age and/or BMI. Therefore, these amino acid levels were adjusted for age and BMI in comparisons between the control and BP groups.

Table 1
Comparison of amino acid levels according to sex. SD, standard deviation.

| | **Male (N = 50) (mean ± SD, nmol/mL)** | **Female (N = 43) (mean ± SD, nmol/mL)** | **P** |
|---|---|---|---|
| Total amino acids | 2804 ± 2 67 | 2532 ± 198 | < 0.001 |
| Taurine | 69.2 ± 15.8 | 73.6 ± 18.2 | 0.15 |
| Alanine | 335.2 ± 72.5 | 288.5 ± 55.8 | < 0.001 |
| Arginine | 93.2 ± 17.7 | 83.5 ± 18.5 | < 0.001 |
| Asparagine | 45.1 ± 6.19 | 41.2 ± 4.74 | 0.003 |
| Aspartic acid | 3.23 ± 0.98 | 4.05 ± 6.29 | 0.52 |
| Citrulline | 33.8 ± 8.62 | 31.1 ± 6.16 | 0.12 |
| Cystine | 37.3 ± 9.23 | 34.2 ± 10.2 | 0.03 |
| Glutamic acid | 43.6 ± 14.5 | 37.7 ± 13.7 | 0.03 |
| Glutamine | 586.6 ± 71.2 | 570.7 ± 54.4 | 0.14 |
| Glycine | 209.8 ± 45.8 | 223.0 ± 55.8 | 0.36 |
| Histidine | 80.3 ± 11.6 | 74.9 ± 7.02 | 0.02 |
| Isoleucine | 64.3 ± 12.8 | 47.1 ± 9.19 | < 0.001 |
| Leucine | 125.2 ± 19.8 | 99.1 ± 16.1 | < 0.001 |
| Lysine | 200.1 ± 28.7 | 174.3 ± 22.5 | < 0.001 |
| Methionine | 25.6 ± 4.26 | 21.3 ± 2.36 | < 0.001 |
| Ornithine | 60.4 ± 9.72 | 52.7 ± 9.29 | < 0.001 |
| Phenylalanine | 57.8 ± 7.19 | 53.4 ± 7.41 | 0.002 |
| Proline | 157.3 ± 68.0 | 108.4 ± 21.1 | < 0.001 |
| Serine | 107.0 ± 19.2 | 107.4 ± 19.2 | 0.93 |
| Threonine | 127.1 ± 24.2 | 113.4 ± 18.7 | 0.002 |
| Tryptophan | 53.3 ± 7.94 | 48.1 ± 6.42 | < 0.001 |
| Tyrosine | 63.3 ± 11.0 | 57.2 ± 10.1 | 0.008 |
| Valine | 218.6 ± 37.6 | 184.6 ± 30.0 | < 0.001 |

Table 2 shows the result of blood test in BP patients. Both male and female BP patients had the A-DROP scores ranging from 0 to 3 with a median of 2. Among the patients with BP, the amino acid levels did not differ significantly according to the pathogen, or the BP severity (Table 3).

Table 2
Blood test in patients with bacterial pneumonia. SD, standard deviation.

| | Male (N = 36) (mean ± SD) | Female (N = 24) (mean ± SD) |
|---|---|---|
| White blood cell | 11613 ± 4222 (/μL) | 14065 ± 12863 (/μL) |
| C reactive protein | 13.4 ± 8.2 (mg/dL) | 12.6 ± 7.8 (mg/dL) |
| Blood urea nitrogen | 19.1 ± 6.1 (mg/dL) | 19.5 ± 12.4 (mg/dL) |

Table 3
Severity of pneumonia using the A-DROP scoring system according to sex

| Score | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Males | 2 | 11 | 16 | 6 | 0 | 0 |
| Females | 4 | 7 | 5 | 7 | 0 | 0 |
| All | 6 | 18 | 21 | 13 | 0 | 0 |

Twelve amino acids (alanine, arginine, asparagine, citrulline, glutamine, glycine, histidine, lysine, methionine, serine, threonine, and tryptophan) were significantly lower in the BP group than in the control group in both male and female participants on Day 0 (Tables 4 and 5) but phenylalanine was considerably higher. Of the amino acids that were decreased on Day 0, histidine, and tryptophan remained low on Day 7, but asparagine, aspartic acid, ornithine, proline, and threonine were significantly higher on Day 7 than in the controls in both males and females (Tables 4 and 5). On Day 7, most of the other amino acids in BP patients were similar to those of controls.

Table 4
Comparison of amino acid levels in patients with bacterial pneumonia and healthy controls: Males

| | Day 0 (N = 36) | | Day 7 (N = 26) | | P | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SD nmol/mL | Patients/ controls | Mean ± SD nmol/mL | Patients/ controls | Day 0 vs controls | Day 7 vs controls | Day 0 vs Day 7 |
| Total amino acids | 2275 ± 420 | 0.82 | 2954 ± 421 | 1.05 | < 0.001 | 0.17 | < 0.001 |
| Taurine | 107.5 ± 44.1 | 1.55 | 117.3 ± 61.2 | 1.70 | < 0.001 | < 0.001 | 0.94 |
| Alanine | 273.9 ± 66.3 | 0.82 | 368.5 ± 85.2 | 1.10 | < 0.001 | 0.18 | < 0.001 |
| Arginine | 56.0 ± 18.4 | 0.60 | 98.4 ± 25.7 | 1.00 | < 0.001 | 0.90 | < 0.001 |
| Asparagine | 39.3 ± 11.2 | 0.87 | 55.6 ± 13.2 | 1.23 | 0.003 | < 0.001 | < 0.001 |
| Aspartic acid | 5.55 ± 2.90 | 1.72 | 8.04 ± 5.44 | 2.49 | < 0.001 | < 0.001 | 0.28 |
| Citrulline | 21.4 ± 10.1 | 0.63 | 26.2 ± 9.63 | 0.77 | < 0.001 | < 0.0012 | 0.07 |
| Cystine | 36.5 ± 11.2 | 1.01 | 38.7 ± 10.3 | 1.08 | > 0.99 | 0.37 | 0.57 |
| Glutamic acid | 40.3 ± 20.4 | 1.13 | 556.4 ± 92.0 | 1.54 | 0.76 | 0.002 | 0.06 |
| Glutamine | 459.2 ± 128.7 | 0.78 | 556.3 ± 92.0 | 0.95 | < 0.001 | 0.17 | 0.002 |
| Glycine | 133.0 ± 36.0 | 0.63 | 214.8 ± 44.3 | 1.02 | < 0.001 | 0.88 | < 0.001 |
| Histidine | 57.4 ± 13.6 | 0.71 | 64.2 ± 13.9 | 0.80 | < 0.001 | < 0.001 | 0.16 |
| Isoleucine | 56.9 ± 21.0 | 0.88 | 71.8 ± 18.4 | 1.12 | 0.12 | 0.25 | 0.01 |
| Leucine | 115.8 ± 33.4 | 0.92 | 114.2 ± 29.2 | 0.91 | 0.17 | 0.13 | > 0.99 |
| Lysine | 146.2 ± 52.9 | 0.77 | 222.4 ± 54.4 | 1.17 | < 0.001 | 0.007 | < 0.001 |
| Methionine | 20.2 ± 5.77 | 0.79 | 24.3 ± 5.87 | 0.95 | < 0.001 | 0.38 | < 0.03 |
| Ornithine | 54.2 ± 16.9 | 0.90 | 73.3 ± 21.0 | 1.21 | 0.0546 | 0.02 | 0.001 |
| Phenylalanine | 92.8 ± 31.3 | 1.61 | 70.2 ± 13.9 | 1.22 | < 0.001 | < 0.001 | 0.001 |

| | Day 0 (N = 36) | | Day 7 (N = 26) | | P | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SD nmol/mL | Patients/ controls | Mean ± SD nmol/mL | Patients/ controls | Day 0 vs controls | Day 7 vs controls | Day 0 vs Day 7 |
| Proline | 125.9 ± 43.2 | 0.80 | 186.0 ± 51.2 | 1.18 | 0.05 | 0.007 | < 0.001 |
| Serine | 71.0 ± 17.7 | 0.66 | 101.0 ± 20.6 | 0.94 | < 0.001 | 0.32 | < 0.001 |
| Threonine | 78.7 ± 24.6 | 0.62 | 144.0 ± 28.1 | 1.13 | < 0.001 | 0.04 | < 0.001 |
| Tryptophan | 42.4 ± 10.3 | 0.79 | 45.9 ± 11.4 | 0.86 | < 0.001 | 0.01 | 0.25 |
| Tyrosine | 61.9 ± 14.1 | 0.98 | 69.3 ± 11.7 | 1.09 | 0.97 | 0.06 | 0.06 |
| Valine | 204.0 ± 46.9 | 0.93 | 214.9 ± 45.1 | 0.98 | 0.34 | 0.89 | 0.69 |

Table 5

Comparison of amino acid levels in patients with bacterial pneumonia and healthy controls: Females

| | Day 0 (N = 24) | | Day 7 (N = 17) | | P | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SD nmol/mL | Patients/Controls | Mean ± SD nmol/mL | Patients/Controls | Controls vs Day 0 | Controls vs Day 7 | Day 0 vs Day 7 |
| Total amino acids | 2056 ± 375 | 0.81 | 3000 ± 624 | 1.18 | < 0.001 | 0.04 | < 0.001 |
| Taurine | 89.1 ± 43.0 | 1.21 | 93.6 ± 46.7 | 1.27 | 0.59 | 0.27 | 0.98 |
| Alanine | 221.7 ± 75.6 | 0.77 | 380.0 ± 143.0 | 1.32 | < 0.001 | 0.03 | 0.002 |
| Arginine | 55.7 ± 21.9 | 0.67 | 108.7 ± 37.2 | 1.30 | < 0.001 | 0.04 | < 0.001 |
| Asparagine | 35.1 ± 10.5 | 0.85 | 53.0 ± 14.6 | 1.29 | 0.002 | 0.006 | < 0.001 |
| Aspartic acid | 4.56 ± 2.72 | 1.13 | 6.16 ± 3.82 | 1.52 | 0.15 | 0.002 | 0.25 |
| Citrulline | 23.6 ± 9.44 | 0.76 | 29.1 ± 11.2 | 0.94 | 0.001 | 0.48 | 0.14 |
| Cystine | 36.6 ± 12.8 | 1.07 | 39.4 ± 13.4 | 1.15 | 0.60 | 0.23 | 0.66 |
| Glutamic acid | 37.6 ± 18.2 | 1.00 | 47.4 ± 22.3 | 1.25 | > 0.99 | 0.24 | 0.32 |
| Glutamine | 444.6 ± 106.3 | 0.78 | 567.5 ± 103.7 | 0.99 | < 0.001 | > 0.99 | 0.005 |
| Glycine | 138.5 ± 29.6 | 0.62 | 253.0 ± 91.4 | 1.13 | < 0.001 | 0.22 | < 0.001 |
| Histidine | 50.1 ± 9.51 | 0.67 | 61.9 ± 13.7 | 0.83 | < 0.001 | 0.002 | 0.02 |
| Isoleucine | 44.2 ± 12.9 | 0.94 | 75.6 ± 26.3 | 1.60 | 0.26 | < 0.001 | < 0.001 |
| Leucine | 94.3 ± 23.3 | 0.99 | 113.5 ± 40.0 | 1.19 | 0.76 | 0.47 | 0.42 |
| Lysine | 119.9 ± 39.3 | 0.69 | 195.3 ± 51.4 | 1.12 | < 0.001 | 0.39 | < 0.001 |
| Methionine | 18.4 ± 6.44 | 0.86 | 26.7 ± 10.5 | 1.25 | 0.01 | 0.04 | 0.007 |
| Ornithine | 45.8 ± 15.4 | 0.87 | 73.5 ± 15.5 | 1.40 | 0.08 | < 0.001 | < 0.001 |
| Phenylalanine | 80.9 ± 23.4 | 1.52 | 74.2 ± 19.7 | 1.39 | < 0.001 | < 0.001 | 0.84 |

| | Day 0 (N = 24) | | Day 7 (N = 17) | | P | | |
|---|---|---|---|---|---|---|---|
| | **Mean ± SD nmol/mL** | **Patients/Controls** | **Mean ± SD nmol/mL** | **Patients/Controls** | **Controls vs Day 0** | **Controls vs Day 7** | **Day 0 vs Day 7** |
| Proline | 117.5 ± 47.8 | 1.08 | 210.0 ± 68.4 | 1.94 | 0.93 | < 0.001 | < 0.001 |
| Serine | 73.5 ± 20.0 | 0.77 | 115.9 ± 29.8 | 1.21 | < 0.001 | 0.03 | < 0.001 |
| Threonine | 70.6 ± 21.1 | 0.68 | 146.1 ± 52.2 | 1.42 | < 0.001 | 0.001 | < 0.001 |
| Tryptophan | 35.2 ± 8.64 | 0.73 | 43.9 ± 13.3 | 0.91 | < 0.001 | 0.37 | 0.08 |
| Tyrosine | 58.5 ± 14.1 | 0.92 | 73.8 ± 20.0 | 1.16 | 0.24 | 0.11 | 0.04 |
| Valine | 157.5 ± 27.0 | 0.85 | 202.1 ± 48.1 | 1.09 | 0.001 | 0.62 | 0.01 |

Among patient with BP, meal consumption was higher on Day 7 than on Day 0 (Table 6), showing recovery of the participants' appetites.

Table 6
Meal consumption by patients with bacterial pneumonia on Days 0 and 7 according to sex

| | **Males** | | **Females** | |
|---|---|---|---|---|
| Number of meals eaten | Day 0 (N = 36) | Day 7 (N = 26) | Day 0 (N = 24) | Day 7 (N = 17) |
| < 1 | 8 | 1 | 9 | 2 |
| 1–2 | 12 | 2 | 8 | 3 |
| > 2 | 16 | 23 | 7 | 12 |

Fourteen male BP patients provided follow-up blood samples after their recovery (mean ± SD: 12.4 ± 10.6 months). After recovery levels of most amino acids were similar to those of the control group, but the level of alanine was significantly higher and the level of serine was significantly lower than that of the control group (Table 7).

Table 7
Comparison of amino acid levels of 14 males with bacterial pneumonia with those of controls on Day 0 and after recovery

| | **Day 0** | | | **Post-recovery** | | |
|---|---|---|---|---|---|---|
| | Mean ± SD nmol/mL | Patients/ controls | P patients vs controls | Mean ± SD nmol/mL | Patients/ controls | P patients vs controls |
| Total amino acids | 2241 ± 357 | 0.82 | < 0.001 | 2934 ± 240 | 1.05 | 0.12 |
| Alanine | 268.3 ± 70.1 | 0.80 | 0.01 | 388.9 ± 63.4 | 1.16 | 0.02 |
| Arginine | 48.2 ± 14.4 | 0.49 | < 0.001 | 91.3 ± 17.8 | 0.93 | 0.13 |
| Asparagine | 37.8 ± 9.87 | 0.84 | 0.04 | 49.9 ± 13.2 | 1.11 | 0.59 |
| Citrulline | 19.1 ± 9.37 | 0.56 | < 0.001 | 36.0 ± 10.2 | 1.06 | 0.47 |
| Glutamine | 424.8 ± 105.7 | 0.72 | < 0.001 | 633.3 ± 81.7 | 1.08 | 0.23 |
| Glycine | 123.8 ± 36.8 | 0.59 | < 0.001 | 212.2 ± 39.7 | 1.01 | 0.90 |
| Histidine | 53.6 ± 8.56 | 0.67 | < 0.001 | 76.4 ± 6.83 | 0.95 | 0.40 |
| Lysine | 127.7 ± 37.7 | 0.67 | < 0.001 | 177.9 ± 39.7 | 0.93 | 0.42 |
| Methionine | 19.0 ± 5.08 | 0.74 | < 0.001 | 25.0 ± 3.17 | 0.98 | 0.70 |
| Serine | 66.2 ± 18.1 | 0.62 | < 0.001 | 93.0 ± 16.8 | 0.87 | 0.03 |
| Threonine | 68.8 ± 19.0 | 0.54 | < 0.001 | 111.5 ± 29.4 | 0.88 | 0.09 |
| Tryptophan | 40.6 ± 9.79 | 0.76 | < 0.001 | 49.7 ± 11.0 | 0.93 | 0.55 |
| Phenylalanine | 100.6 ± 36.4 | 1.74 | < 0.001 | 58.1 ± 10.9 | 1.00 | 0.98 |

# Discussion

Differences were observed in the levels of 17 amino acids between patients with BP and healthy controls, and some amino acids correlated with the age or BMI. Previous reports of amino acid levels in the Japanese population have been based on healthy participants over a wide age range [15]. In previous studies, the levels of some amino acids have been reported to vary according to age and BMI. In our study the healthy controls were younger than the patients with BP, and matching patients and controls on comorbidities was not feasible.

In this study, amino acid levels were not related to the severity of BP. S. pneumoniae and H. influenzae were the most frequently identified etiologic agents, similar to the findings of a previous Japanese study [7, 13]. Cytokines and metabolites produced from the host during the bacterial infection are similar in some respects and differ in others, between gram-positive and gram-negative bacteria [16, 17]. However, we did not find any significant differences in amino acid levels according to Gram stain type, therefor, the changes in the amino acid levels among patients with BP in this study can be considered representative of those of patients with BP.

Except for alanine and serine, after recover the amino acid levels of patients with BP were similar to those of controls, which suggests that the differences in amino acids between the BP patients and the controls were driven by infection.

We excluded the patients who had any symptom of BP more than two days before admission. Therefore, we considered that Day 0 was the acute phase of BP. All patients in the BP group completed their antibiotics within 7 days, and none experienced a recurrence of pneumonia. Therefore, we considered all patients to be in convalescence on Day 7. Of the 12 amino acids that were significantly lower on Day 0 only three amino acids remained at a lower level on Day 7, but the levels of five amino acids were higher than those in the control group on Day 7. This may have been partly attributable to BP patients increased food consumption. As in previous studies, BP patients had higher phenylalanine levels than those of controls on Days 0 and 7 [18, 19]. The plasma amino acid levels of the BP patients differed from those of controls on Day 7, but most of the amino acid values of the BP patients were similar to those of the controls once they had fully recovered, indicating that amino acid metabolism takes a while to recover following infection.

In this study, the BP patients had lowered plasma levels of arginine and citrulline on Day 0, while their level of ornithine was not lowered. Arginine is a substrate for nitric oxide (NO) production and is known to be involved in inflammation. A previous report described the relationship between citrulline, arginine, and ornithine in the context of NO production [20]. During bacterial infection arginine and citrulline play important roles in disease pathogenesis [21], and arginine metabolism regulates host immunity [22]. These findings indicate that the urea cycle and NO production significantly changed during the acute phase of BP, modifying host immunity.

A previous study found that in patients with pneumonia due to influenza H1N1, glycine, histidine, and threonine decreased within 24 hours of admission to hospital [23]. In our study the amino acid levels of BP patients were significantly lower than those of healthy controls on Day 0, indicating the amino acid metabolism changes in response to bacterial infection as well as viral infection. Glycine, histidine, and threonine lie within the same metabolic pathway to produce pyruvic acid [3], thus glycogenesis may be enhanced through the tricarboxylic acid cycle via these amino acids; however, all three of these amino acids were decreased in BP patients on Day 0. Alanine, cystine, and tryptophan are also metabolized to pyruvic acid. Cystine was the only one of these amino acids that not decreased in BP patients on Day 0. This suggest a selective use of amino acids for glycogenesis in BP.

The metabolism of glycine, histidine, and threonine affects the efficiency of complement-mediated bacterial killing [24], while, glycine attenuates the inflammatory response induced by the glycine-gated chloride channel [25]. Therefore, a reduction in glycine levels at the early stage of BP may enhance the immune response. Oral administration of cystine may the decrease in glutamic acid caused by stress and may enhance immunity [26]. However, further studies are needed to understand the implications of changes in plasma amino acid levels in the host during BP.

Arginine, glutamine, histidine, and proline are metabolized to glutamic acid. In this study, glutamic acid and proline were not decreased on Day 0, and the proline level was higher than that of the controls on Day 7. This shows that in BP, different changes occur in plasma levels of amino acids which share metabolic pathways toward 2-oxoglutaric acid through glutamic acid. L-histidine augments the oxidative damage against gram-negative bacteria induced by hydrogen peroxide [27], and histidine is a precursor of histamine and contributes to inflammation with pneumonia [28]. Further studies are needed to evaluate the relationship between bacterial infections and these amino acids.

Each amino acid has been shown to be involved in inflammation and the immune response, leading to the concept of "immuno-nutrition" [5, 6]. This study showed that individual amino acid level differed significantly in the early and recovery phases of BP. In bacterial infection, the total plasma amino acid decreases before the onset of clinical symptoms such as fever [19]. However, there are amino acids, such as methionine, that are essential for bacterial proliferation [29]. Furthermore, L-alanine and L-lysin are the main constituent elements of the peptidoglycans in the bacterial wall [30]. Tryptophan starvation is a recognized antimicrobial defense mechanism, which mediates immunoregulatory effects [31]. Therefore, these amino acids are not only reduced owing to enhanced metabolism related to fever or antibody production, but also affected by active countermeasures against bacterial infection.

Plasma phenylalanine is known to increase with either bacterial or viral infection [32], and the details of this have been elucidated [19]. The explanation is that catabolism of muscular skeletal protein related to infection results in phenylalanine release, and the released phenylalanine is used in synthesis of inflammation-related substances in the liver. In the present analysis, phenylalanine increased at Day 0 and Day7 (Table 4, Table 5), and decreased at the post-recovery period (Table 7).

There is a potential to develop tools to assess the severity of BP, or to diagnose BP by untargeted metabolomics [33, 34]. The metabolism of arginine, proline, and pyruvate has been extracted for the discrimination of severity [33]. In our study, amino acids, which are metabolized into glutamic or pyruvic acid, decreased significantly in the early stage of BP but some amino acids such as proline and cystine that did not change. The interaction between individual amino acids and immunity are potential topics for future studies.

The levels of six amino acids (asparagine, citrulline, glutamine, histidine, serine, and threonine) have been shown to be decreased in patients with acute exacerbations of chronic obstructive pulmonary disease with bacterial infection [4]. These amino acids were reduced in the BP patients in this study. In this study, in the current study the changes of amino acid level changes varied by sex. The topic of sex-related differences in amino acid changes in response to bacterial infection needs to be studied further.

# Conclusions

The levels of 12 amino acids were significantly lower BP patients than in healthy controls in the acute phase of BP, and this is likely to have been attributable to the bacterial infection. During convalescence, the levels of some amino acids differed from those on admission and those of healthy controls, suggesting that there is a distinct amino acid metabolism profile during the convalescent phase following bacterial infection. On the basis of these results, nutritional intervention with amino acids should be modified according to the stage of BP from the perspective of immuno-nutrition.

# Abbreviations

BP: bacterial pneumonia; LC-MS: liquid chromatography-mass spectrometry; HbAlc: hemoglobin A1c; eGFR: Estimated glomerular filtration rate; BMI: body mass index; SD: standard deviation;

# Declarations

### Author contribution

HI had full access to the study data, takes full responsibility for the integrity of the data and the accuracy of the analysis, contributed to the study design, contributed to writing of the manuscript, the data analysis, drafting and revising the paper, and agree to be accountable for all aspects of the work.

### Competing interests

None


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### Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

### Consent for publication

Not applicable


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### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Sanyudo Hospital at 57th, 63th, and 64th committee meeting. Written informed consent was obtained from each participant in accordance with the tenets of the Declaration of Helsinki. These processes were performed in accordance with the Japan Ministry of Health, Labor, and Welfare ethics guidelines [35].

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## Figures

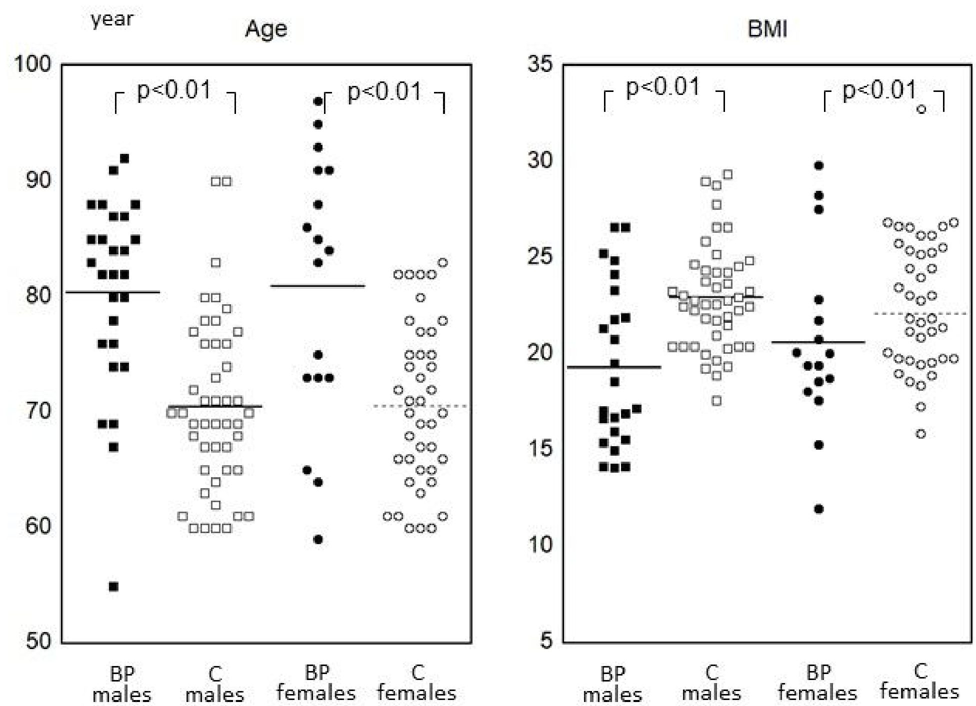


**Figure 1**

Age and body mass index in patients with bacterial pneumonia and healthy controls. The number of participants in each group were 50, 36, 43, and 24 for BP males, control males, BP females, and control females, respectively. The horizontal solid lines show the mean values in each group. Abbreviations: BP, bacterial pneumonia patient; C, healthy controls