Clinical diagnostic value of amino acids in laryngeal squamous cell carcinomas

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Background. Early diagnosis and treatment can improve the survival rates of patients with laryngeal squamous cell carcinoma (LSCC). Therefore, it is necessary to discover new biomarkers for laryngeal cancer screening and early diagnosis.

Methods. We collected fasting plasma from LSCC patients and healthy volunteers, as well as cancer and para-carcinoma tissues from LSCC patients, and performed quantitative detection of amino acid levels using liquid chromatography-mass spectrometry. We used overall analysis and multivariate statistical analysis to screen out the statistically significant differential amino acids in the plasma and tissue samples, conducted receiver operating characteristic (ROC) analysis of the differential amino acids to evaluate the sensitivity and specificity of the differential amino acids, and finally determined the diagnostic value of amino acids for laryngeal cancer. Additionally, we identified amino acids in the plasma and tissue samples that are valuable for the early diagnosis of laryngeal cancer classified according to the tumor-node-metastasis (TNM) classification.

Results. Asparagine (Asp) and homocysteine (Hcy) were two amino acids of common significance in plasma and tissue samples, and their specificity and sensitivity analysis showed that they may be new biomarkers for the diagnosis and treatment of LSCC. According to the TNM staging system, phenylalanine (Phe) and isoleucine (Ile) were screened out in the plasma of LSCC patients at early (I and Ⅱ) and advanced (Ⅲ and Ⅳ) stages; ornithine hydrochloride (Orn), glutamic acid (Glu), and Glycine (Gly) were selected in the tissue. These dysregulated amino acids found in LSCC patients may be useful as clinical biomarkers for the early diagnosis and screening of LSCC.

1 **Clinical diagnostic value of amino acids in laryngeal squamous cell carcinomas**

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17 **ABSTRACT**

18 **Background.** Early diagnosis and treatment can improve the survival rates of patients with

19 laryngeal squamous cell carcinoma (LSCC). Therefore, it is necessary to discover new biomarkers

20 for laryngeal cancer screening and early diagnosis.

21 **Methods.** We collected fasting plasma from LSCC patients and healthy volunteers, as well as

22 cancer and para-carcinoma tissues from LSCC patients, and performed quantitative detection of

23 amino acid levels using liquid chromatography-mass spectrometry. We used overall analysis and

24 multivariate statistical analysis to screen out the statistically significant differential amino acids in

25 the plasma and tissue samples, conducted receiver operating characteristic (ROC) analysis of the

26 differential amino acids to evaluate the sensitivity and specificity of the differential amino acids,

27 and finally determined the diagnostic value of amino acids for laryngeal cancer. Additionally, we

28   identified amino acids in the plasma and tissue samples that are valuable for the early diagnosis of

29   laryngeal cancer classified according to the tumor-node-metastasis (TNM) classification.

30   **Results.** Asparagine (Asp) and homocysteine (Hcy) were two amino acids of common significance

31   in plasma and tissue samples, and their specificity and sensitivity analysis showed that they may

32   be new biomarkers for the diagnosis and treatment of LSCC. According to the TNM staging

33   system, phenylalanine (Phe) and isoleucine (Ile) were screened out in the plasma of LSCC patients

34   at early (I and II) and advanced (III and IV) stages; ornithine hydrochloride (Orn), glutamic acid

35   (Glu), and Glycine (Gly) were selected in the tissue. These dysregulated amino acids found in

36   LSCC patients may be useful as clinical biomarkers for the early diagnosis and screening of LSCC.

37   **Keywords:** Laryngeal cancer, Staging, Amino acid, Biomarker, LC-MS

38   **INTRODUCTION**

39   Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck tumors,

40   accounting for more than 90% of laryngeal cancer cases (Megwalu & Sikora 2014). In the last 40

41   years, the incidence of LSCC has increased significantly, and most of the patients are 40-60 years

42   old with a male to female ratio of (7-9):1 (Li 2014; Siegel et al. 2019). Some patients are diagnosed

43   with distant metastasis or advanced stage laryngeal cancer due to untimely diagnosis or treatment

44   (Smith et al. 2016), which could lead to unsatisfactory treatment results and serious psychological

45   and economic burdens to patients (Guibert et al. 2011). Therefore, the early identification of

46   laryngeal cancer is very necessary. Although some current imaging methods (such as magnetic

47   resonance imaging, positron emission computed tomography, and computed tomography) are

48   frequently used to screen and diagnose laryngeal cancer, their effectiveness depends on the ability

49   of medical imaging workers to obtain image information (Yu & Wang 2021). Hence, it is highly

50   important to find more effective, simple, and less invasive diagnostic techniques that can be used

51   for laryngeal cancer screening or early detection.

52   A possible way to improve the screening or early detection of LSCC is the use of biomarkers.

53   LysoPC 16:0 and PAF might be diagnostic biomarkers for laryngeal cancer based on serum

54   metabolomics from patients and healthy individuals(Zhang et al. 2018). Six metabolites (D-

55 pantothenic acid, palmitic acid, myristic acid, oleamide, sphinganine, and phytosphingosine) could

56 be used as diagnostic biomarkers for laryngeal cancer according to urine metabolomic data(Chen

57 et al. 2019). Lysophospholipids and phospholipids might serve as lipid biomarkers for the

58 diagnosis of laryngeal cancer(Yu & Wang 2021). Amino acid metabolomics is an effective method

59 for identifying biomarkers of disease-related metabolites that contribute to the early diagnosis of

60 diseases (Siminska & Koba 2016). It is widely used in lung cancer (Maeda et al. 2010), gastric

61 cancer (Li et al. 2022), colorectal cancer (Leichtle et al. 2012), head and neck cancer (Cadoni et

62 al. 2020), and multiple myeloma (Yue et al. 2022). Currently, there are three main kinds of amino

63 acid detection methods: high performance liquid chromatography (HPLC), amino acid analysis,

64 and liquid chromatography-mass spectrometry (LC-MS). The poor resolution of amino acids and

65 the difficult separation of isomers make the results of HPLC unreliable. Amino acid analyzers take

66 a long time to analyze blood samples and cannot be used as a high-throughput analysis method.

67 Due to its high-efficiency separation ability, high sensitivity, high accuracy, and short detection

68 time, LC-MS has become the preferred detection method for metabolomics (Theodoridis et al.

69 2012). As far as we know, no laryngeal cancer-associate amino acid profiling of blood and tissue

70 samples has yet been performed.

71   In order to find reliable clinical biomarkers for laryngeal cancer screening, we used LC-MS to

72 analyze the amino acid levels in the plasma of 20 LSCC patients and 15 healthy volunteers, as well

73 as LSCC patients' cancer tissues and adjacent tissues. The different amino acids in the plasma and

74 tissue samples were determined and amino acids with diagnostic values were screened out. Our

75 study provides new strategies for identifying reliable clinical biomarkers for the early diagnosis

76 and screening of LSCC.

77   The tumor-node-metastasis (TNM) staging system based on anatomical study contributes to

78 cancer treatment selection, control activities, and outcome prediction (Huang & O'Sullivan 2017).

79 According to the eighth edition of the TNM staging system, we performed TNM staging for LSCC

80 patients and analyzed the differential amino acids in the plasma and tissues of LSCC patients at

81 early (Ⅰ and Ⅱ) stages and advanced (Ⅲ and Ⅳ) stages. Our study provides further evidence for the

82 early prognostic prediction of LSCC.

83

84 **MATERIALS & METHODS**

85 **Clinical subjects**

86 This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou

87 University (2021-KY-0417-002). Tissue samples and blood samples were obtained from patients

88 admitted to the First Affiliated Hospital of Zhengzhou University from October 2021 to February

89 2022. In this study, fasting blood samples were collected from 20 LSCC patients and 15 healthy

90 individuals (with no past or current history of cancers), as well as laryngeal cancer tissues and

91 para-carcinoma tissues from 20 LSCC patients (one patient with missing para-carcinoma tissue).

92 The inclusion criteria for this study were as follows: a diagnosis of LSCC was reported by

93 pathological examination, with or without cervical lymph node metastasis. The exclusion criteria

94 were: 1) patients who had previously or currently suffered from other tumors; 2) patients who had

95 received chemotherapy or radiotherapy prior to surgery; 3) patients with severe blood, metabolic,

96 respiratory, or other system diseases; and 4) patients suffering from infectious diseases such as

97 hepatitis B, syphilis, and human immunodeficiency virus.

98 **Blood sample preparation and metabolite extraction**

99 All selected subjects were required to fast for more than 8 hours before blood collection, and blood

100 from LSCC patients was collected before surgery. The blood samples were placed in a vacuum

101 anticoagulant tube containing lithium heparin, centrifuged at 4°C, 3,000 rpm for 10 min, and

102 centrifugation was completed within 1 h after the blood samples were collected. The plasma was

103 stored at -80 °C. An appropriate amount of plasma samples were pipetted accurately into 2 mL EP

104 tubes, 400 μL of 10% formic acid methanol-H_2O (1:1, V/V) solution was added, and then

105 centrifuged at 12,000 rpm, 4 °C for 5 min. Following that, 10 μL of the supernatant was taken and

106 added to 90 μL of 10% formic acid methanol-H_2O (1:1, V/V) solution. We took 100 μL of the

107 diluted sample and added it to 100 μL of the isotope internal standard with a concentration of 100

108 ppb. After being vortexed for 30 s, the supernatant was filtered through a 0.22 μm membrane, and

109 collected in a detection bottle. Content (μg/mL) =C (ng/mL)*0.5*2*10/sampling amount (μL).

Tissue sample preparation and metabolite extraction

111 The LSCC tissue and para-carcinoma tissue of patients were collected during the operation,

112 immediately put into a liquid nitrogen tank, and then stored at -80°C. An appropriate amount of

113 tissue samples were pipetted accurately into 2 mL EP tubes, added to 600 μL of 10% formic acid

114 methanol solution-H_2O (1:1, V/V) solution, and two steel balls were added, put into a tissue

115 grinder, 60 Hz grinding for 90 s, and then centrifuged at 12,000 rpm, 4 °C for 5 min. We took 10

116 μL of the supernatant and added it to 190 μL of 10% formic acid methanol-H_2O (1:1, V/V)

117 solution. The remaining sample pretreatment steps were the same as blood sample. Diluted 20-

118 fold content (μg/g) =C (ng/mL)*0.5*2*10/sampling amount (mg).

LC-MS analysis

120 Amino acid metabolism was performed on Waters ACQUITY UPLC (Waters, Milford, MA, USA)

121 coupled with the AB5000 mass spectrometer (AB SCIEX, Framingham, MA, USA) system. We

122 separated 5μL of the diluted sample on the ACQUITY UPLC®BEH C18 column (2.1×100 mm,

123 1.7 μm, Waters, USA). The LC-MS conditions were mainly optimized on the basis of the literature

124 (Liyanaarachchi et al. 2018; Thiele et al. 2019). The column temperature was maintained at 40 °C.

125 The mobile phase A in HPLC consisted of 10% methanol water, and the mobile phase B was 50%

126 methanol water, both containing 0.1% formic acid. The gradient profile was as follows: 10 ~ 30

127 % B in 0 ~ 6.5 min; 30 ~100 % B in 6.5 ~ 7 min; 100 % B in 7~ 14 min; and 100 ~ 10 % B in 14

128 ~ 17.5 min. In MS detection, a multiple reaction monitoring mode was used with an electronic

129 spray ion source operating in positive ion mode. The ion source temperature was 500 °C and the

130 spray voltage was 5,500 V. The collision gas was 6 psi, curtain gas was 30 psi, and both the

131 atomizing gas and auxiliary gas were 50 psi.

Statistical analysis

133   Unit variance scaling was performed to normalize the data before multivariate statistical analysis

134   for more reliable and intuitive results. Partial least squares discriminant analysis (PLS-DA) was

135   conducted by the Ropls package in R to distinguish different groups. The heatmap, box plots and

136   receiver operating characteristic (ROC) curves were performed using the pheatmap, ggpubr and

137   pROC packages in R, respectively. Nonparametric Mann-Whitney test was conducted to identify

138   the levels of differential amino acids between the LSCC and NC/ParaLSCC groups.

139

140   **RESULTS**

141   **Clinical characteristics of the subjects**

142   The detailed clinical characteristics of the subjects were summarized in Table 1. LSCC was

143   classified according to the eighth edition American Joint Committee on Cancer (AJCC) Staging

144   Manual (Amin et al. 2017). There was no statistical difference in age between the LSCC and NC

145   groups, and both included only male participants.

146   **Amino acid signatures of plasma and tissue samples from laryngeal carcinoma**

147   Amino acid profiling was performed to analyze the plasma and tissue samples from LSCC patients

148   and NC subjects. The levels of amino acids in the plasma and tissue samples were shown in Table

149   S1 and S2. PLS-DA model was used to explore the differences in amino acid fingerprinting

150   between LSCC patients and NC subjects in plasma samples. Both the R^2 and Q^2 of the established

151   PLS-DA model were greater than 0.5, indicating that this model had good explanatory and

152   predictive ability. The scores shown in Fig. 1A, and we found that the LSCC group plasma samples

153   were significantly different from those of the NC group. The reliability of the PLS-DA model was

154   further verified by the permutation test (*n*=100). The R^2 cutoff was 0.25 and the Q^2 cutoff was -

155   0.59 in the permutation test, indicating that this model was not over-fitting (Fig. 1B). Heatmap

156   visualized the alterations of amino acids in LSCC patients and NC subjects (Fig. 1C). The amino

157   acid signatures of tissue samples from laryngeal carcinoma were shown in Fig. 2.

158 **Potential biomarkers of amino acids in laryngeal cancer**

159 Variable importance in projection (VIP) is the variable weight value of the PLS-DA model, and

160 can be used to measure the impact strength and explanatory power of the differences in the

161 accumulation of each metabolite on the classification and discrimination of each group of samples

162 (Liu et al. 2021). VIP≥1 was considered as one of the screening standards for potential

163 biomarkers. Combined with the standard of VIP>1 in the PLS-DA model and $P< 0.05$ in t-test

164 (Table S3 and S4), six differential amino acids were finally screened out in the plasma, namely

165 asparagine (Asn), serine (Ser), homocysteine (Hcy), histidine (His), ornithine hydrochloride (Orn)

166 and tryptophan (Trp); eight differential amino acids were screened in the tissue, namely tyrosine

167 (Tyr), Asn, isoleucine (Ile), methionine (Met), threonine (Thr), aspartic acid (Asp), glutamic acid

168 (Glu), and Hcy. There were two amino acids of common significance in plasma and tissue samples,

169 Asn and Hcy (Fig. 3). These two amino acids may have important significance for the early clinical

170 diagnosis of LSCC. Receiver operating characteristic (ROC) analysis was employed to evaluate

171 the diagnostic performance of these two biomarkers for LSCC. In plasma samples, the area under

172 curve (AUC) values of Hcy and Asn were 0.765 and 0.896, respectively (Fig. 4A and 4B); in tissue

173 samples, the AUC valus of Hcy and Asn were 0.807 and 0.980, respectively (Fig. 4D and 4E).

174 When Hcy and Asn were chosen as the 'combined biomarker', the AUC value was 0.833 in

175 plasma samples, and 1.000 in tissue samples (Fig. 4C and 4F).

176 **Amino acid profiling between stage Ⅰ+Ⅱ patients and stage Ⅲ+Ⅳ patients according to the**

177 **TNM staging system**

178 LSCC patients were divided into stage Ⅰ+Ⅱ and stage Ⅲ+Ⅳ patients according to the eighth

179 edition of TNM staging system. The PLS-DA model was used to identify differential levels of

180 amino acids in plasma and tissue samples between these two groups. In Fig. 5A and 5B, a clear

181 distinction between LSCC stage Ⅰ+Ⅱ and stage Ⅲ+Ⅳ patients was determined in the plasma

182 and tissue samples. Heatmap visualized the alterations of amino acids in LSCC stage Ⅰ+Ⅱ and

183   stage Ⅲ+Ⅳ patients (Fig. 5C and 5D). Combined with the standard of VIP>1 in the PLS-DA

184   model and $P< 0.05$ in the *t*-test (Table S5 and S6), two differential amino acids were finally

185   screened out in the plasma, namely Phe and Ile; three differential amino acids were selected out in

186   the tissue, namely Orn, Glu and Gly. In plasma samples, the AUC of Phe and Ile were 0.781 and

187   0.792, respectively (Fig. 6A and 6B); in tissue samples, the AUC of Orn, Glu, and Gly were 0.85,

188   0.867, and 0.944, respectively (Fig. 6C-E).

189

190   **DISCUSSION**

191   Researchers have paid increasingly more attention to the important roles of amino acid

192   metabolomics in tumor pathogenesis (Sivanand & Vander Heiden 2020). However, there have

193   been few reports on the amino acid metabolomics of LSCC. In this study, we collected the fasting

194   plasma of LSCC patients and healthy volunteers, cancer and para-carcinoma tissues of LSCC

195   patients, and used LC-MS to quantitaively determine the levels of amino acids in plasma and tissue

196   samples. The different amino acids were screened out and the amino acids with high sensitivity

197   and specificity were identified as new biomarkers for LSCC. In the plasma samples, we found six

198   amino acids with statistical differences, which were Asn, Ser, Hcy, His, Orn and Trp; in tissue

199   samples, eight amino acids were found with statistical differences, which were Tyr, Asn, Ile, Met,

200   Thr, Asp, Glu, and Hcy. In both plasma and tissue samples, Hcy and Asn were statistically

201   significant amino acids, and were more valuable as a combined biomarker than individual markers.

202   In addition, differential levels of amino acids in plasma and tissue samples between LSCC stage

203   Ⅰ+Ⅱ and stage Ⅲ+Ⅳ patients were screened out. Two differential amino acids were screened

204   out in the plasma, namely Phe and Ile; three differential amino acids were screened out in the

205   tissue, namely Orn, Glu and Gly.

206   Hcy level is one of the key factors associated with cancers (Hasan et al. 2019) such as colorectal

207   (Kato et al. 1999) and cervical cancer (Silva et al. 2020). Hcy can directly damage DNA by

208   generating reactive oxygen species, which may be carcinogenic (Oikawa et al. 2003; Zhou et al.

209   2016). In this study, Hcy was one differential amino acid found in plasma and tissue samples. In

210   the plasma samples, the level of Hcy in the LSCC patient group was significantly lower than that

211   in the healthy control group (*P*<0.001), with an AUC of 0.896, specificity of 0.9, and sensitivity

212   of 0.867. The level of Hcy in LSCC tissue was significantly higher than in the adjacent tissue

213   (*P*<0.001), with an AUC was 0.98, specificity of 0.895, and sensitivity of 1. Ultimately, Hcy was

214   the most significant differential amino acid in this study. Hcy may be closely related to the

215   pathogenesis of LSCC and may be a new biomarker for diagnosis of LSCC in the future.

216   Asn, a non-essential amino acid, plays an important role in cellular metabolism, and in tumor

217   cells, the extracellular source of Asn is critical for cell proliferation (Figueiredo et al. 2016; Wang

218   et al. 2016). Asn was significantly altered in patients with non-small cell lung cancer (Liu et al.

219   2022). Inhibition of Asn can trigger the protective autophagy of cancer cells (Ji et al. 2017). In this

220   study, the differential level of Asn in plasma and tissue samples was statistically significant. In

221   plasma samples, the level of Asn in the LSCC patient group was lower than that of healthy people,

222   with an AUC of 0.765, specificity of 0.85, and sensitivity of 0.6. The level of Asn in LSCC tissue

223   was higher than in adjacent tissue, with an AUC of 0.807, specificity of 0.737, and sensitivity of

224   0.882. This further confirmed that Asn played a pivotal role in the pathogenesis of tumors, may be

225   a potential biomarker of LSCC, and may be used for diagnosis or treatment of LSCC in the future.

226   The TNM staging system, an international staging standard established by AJCC and the Union

227   for International Cancer Control (UICC), is an important clinical basis for treatment guidance and

228   prognosis prediction of patients. The TNM staging system is classified on the basis of the primary

229   tumor, regional lymph node involvement, and distant metastasis. Early diagnosis and treatment of

230   LSCC are crucial and can save manpower, financial resources, and time. In this paper, amino acid

231   metabolism detection was applied to the LSCC staging system in order to better determine the

232   treatment and prognosis of LSCC patients. We found that Phe and Ile were the differential amino

233   acids in the plasma, and Orn, Glu, and Gly were the differential amino acids in the tissue between

234   stageⅠ+Ⅱ patients and stageⅢ+Ⅳ patients. These amino acids had a good predictive effect on

235   early (Ⅰ andⅡ) and advanced (Ⅲ and Ⅳ) LSCC stages, as shown by ROC curve. Our study

236   provided further insights into the early prognostic prediction of LSCC and the identification of

237   novel targets or a molecular basis for the occurrence and development of LSCC.

238   Notably, our study was single-center with a small sample size. Future studies with larger clinical

239   data are needed to verify our current findings, so that this tool can be used for the effective clinical

240   diagnosis and staging of LSCC, bringing hope for the diagnosis and treatment of LSCC patients.

241   **CONCLUSION**

242   In this paper, the amino acids in the plasma and tissues of LSCC patients were detected using LC-

243   MS, and Asn and Hcy were two amino acids of common significance in both plasma and tissue

244   samples. In addition, Phe and Ile were two differential amino acids screened out in the plasma of

245   LSCC patients at early (Ⅰ and Ⅱ) and advanced (Ⅲ and Ⅳ) stages, and Orn, Glu, and Gly were

246   three differential amino acids selected in the tissue. These dysregulated amino acids in LSCC

247   patients may be promising potential clinical biomarkers for the early diagnosis and screening of

248   LSCC.

249

250   **ADDITIONAL INFORMATION AND DECLARATIONS**

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253   (81903094, 81600812), and the Natural Science Foundation of Henan Province (222300420566).

254   **Competing Interests**

255   The authors declare that they have no competing interests.

256   **Author Contributions**

257   •   Shousen Hu and Chang Zhao performed the experiments, analyzed the data, prepared figures

258   and/or tables, drafted the work or revised it critically for important content.

259   •   Zi'an Wang and Zeyun Li analyzed the data, drafted the work or revised it critically for

260   important content.

261   •   Xiangzhen Kong conceived and designed the experiments, analyzed the data, drafted the work

262   or revised it critically for important content.

263 **Human Ethics**

264 The following information was supplied relating to ethical approvals (i.e., approving body and any

265 reference numbers):

266 The study was carried out in accordance with the Helsinki Declaration. The present trial had

267 approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University

268 (2021-KY-0417-002), and all patients provided written informed consent.

269 **Data Availability**

270 The raw data are available in the supplementary files 1 and 2. The raw data shows the levels of

271 amino acids in the plasma samples and the tissue samples.

272

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Clinical characteristics of the subjects for amino acids metabolism analysis

Table 1 Clinical characteristics of the subjects for amino acids metabolism analysis

Characteristics	Serum sample		Tissue sample	
	LSCC	NC	LSCC	ParaLC
No.	20	15	20	19
Sex(male/female)	20/0	15/0	20/0	19/0
Age(years)	62.75±7.83	58.47±4.67	62.8±10.04	63.63±9.58
TNM stage (AJCC 8)				
Stage Ⅰ+Ⅱ	12	/	10	/
Stage Ⅲ+Ⅳ	8	/	10	/

AJCC: American Joint Committee on Cancer; T:Tumor; N:Node; M:Metastasis.

Figure 1

Amino acid profiling of the plasma samples from LSCC patients and NC subjects.

(A) Partial least squares discriminant analysis (PLS-DA) scores between LSCC and NC groups in plasma samples. PC1 and PC2 were the first and second principal components, respectively, and the percentage values represented the proportion of the total variance by each component. (B) The permutation test of PLS-DA model between LSCC and NC groups (*n*=100). (C) Overall heatmap of amino acid metabolites in plasma samples. Each section's colour represents the significance of changes in metabolites (red, up-regulated; blue, down-regulated).
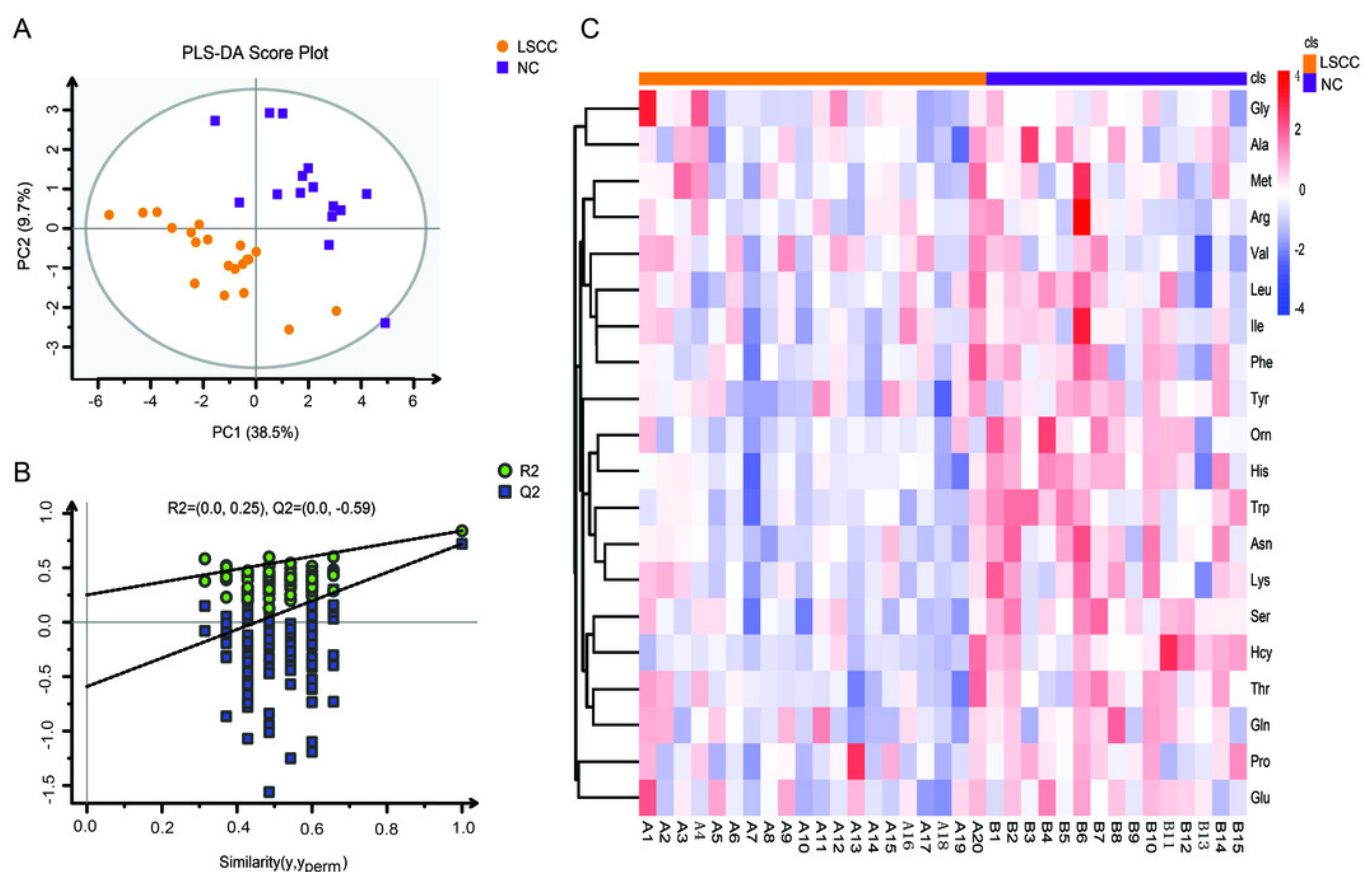
Figure 2

Amino acid profiling of the LSCC and ParaLSCC tissues from LSCC patients.

(A) PLS-DA scores and the permutation test of PLS-DA model between the LSCC and ParaLSCC tissues. (B) The permutation test of PLS-DA model between the LSCC and ParaLSCC tissues (n=100). (C) Overall heatmap of amino acid metabolites in tissue samples. Each section's colour represents the significance of changes in metabolites (red, up-regulated; blue, down-regulated).
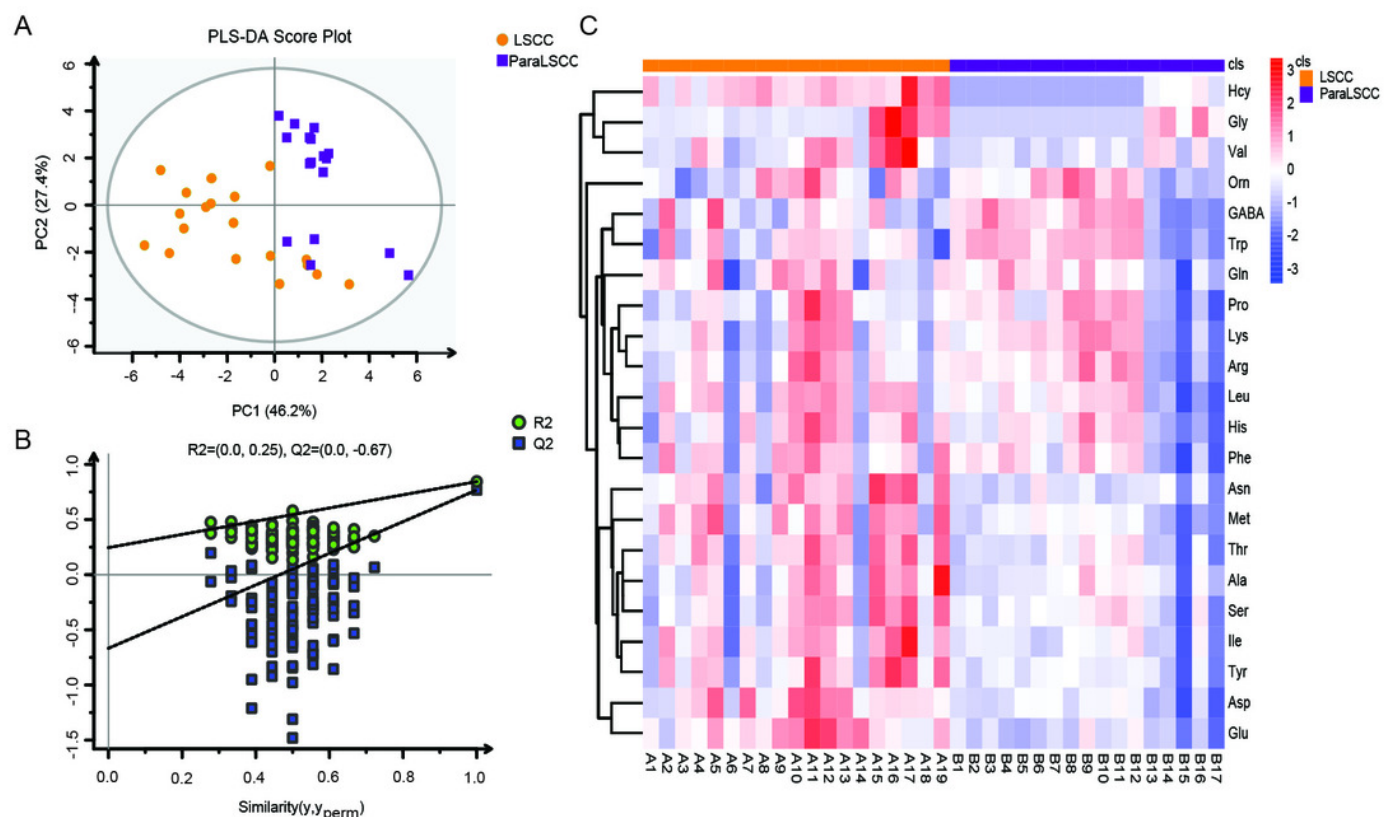
Figure 3

Commonly different amino acids in plasma and tissue samples.

The quantitative value of Asn and Hcy in plasma samples of LSCC and NC groups (A and B), and in the LSCC and ParaLSCC tissues from LSCC patients (C and D).
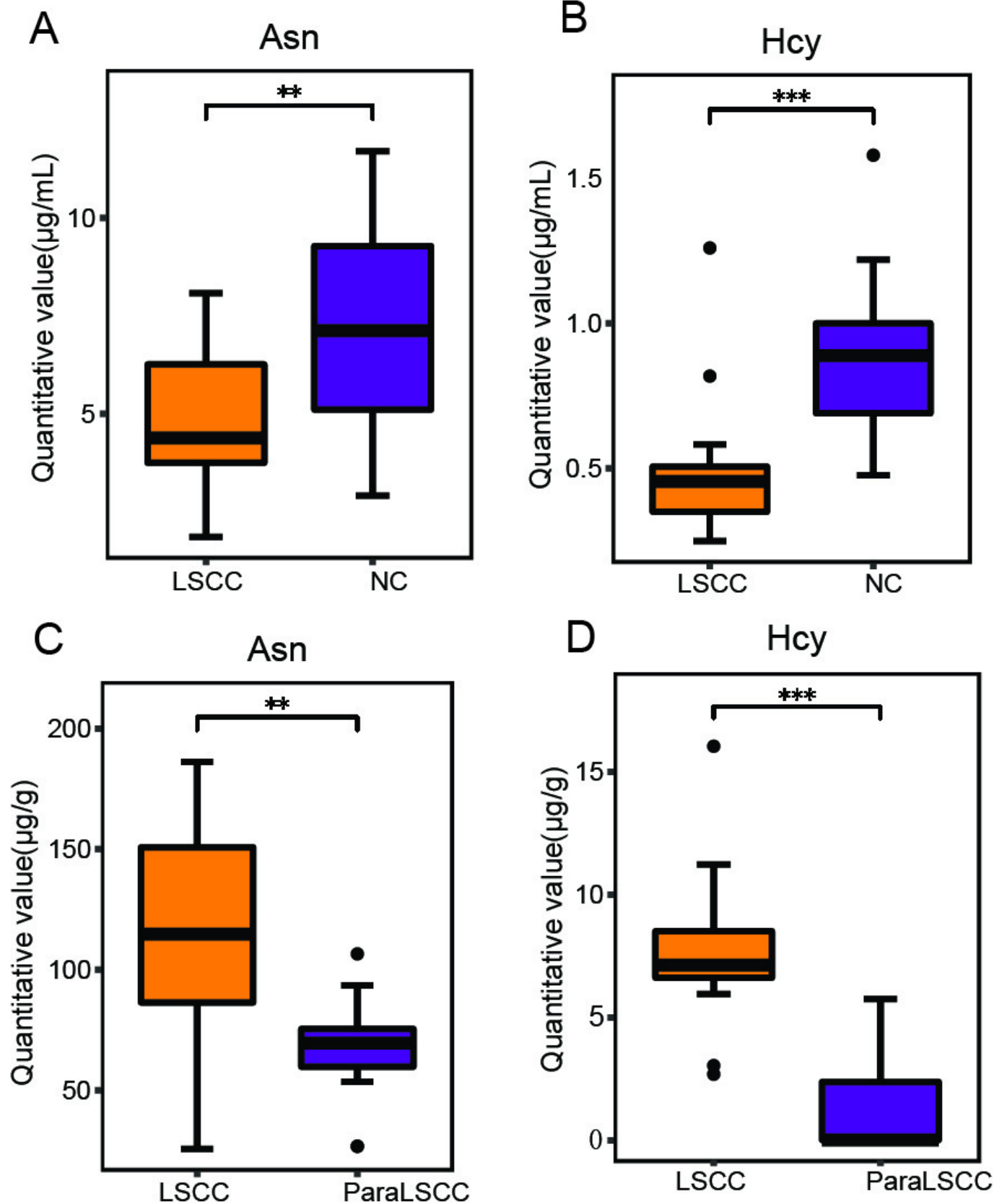
Figure 4

Receiver operating characteristic (ROC) analysis of two commonly different amino acids in plasma and tissue samples.

ROC analysis of Asn and Hcy, and their combination analysis in plasma samples (A-C) and in tissue samples (D-F). AUC, area under curve.
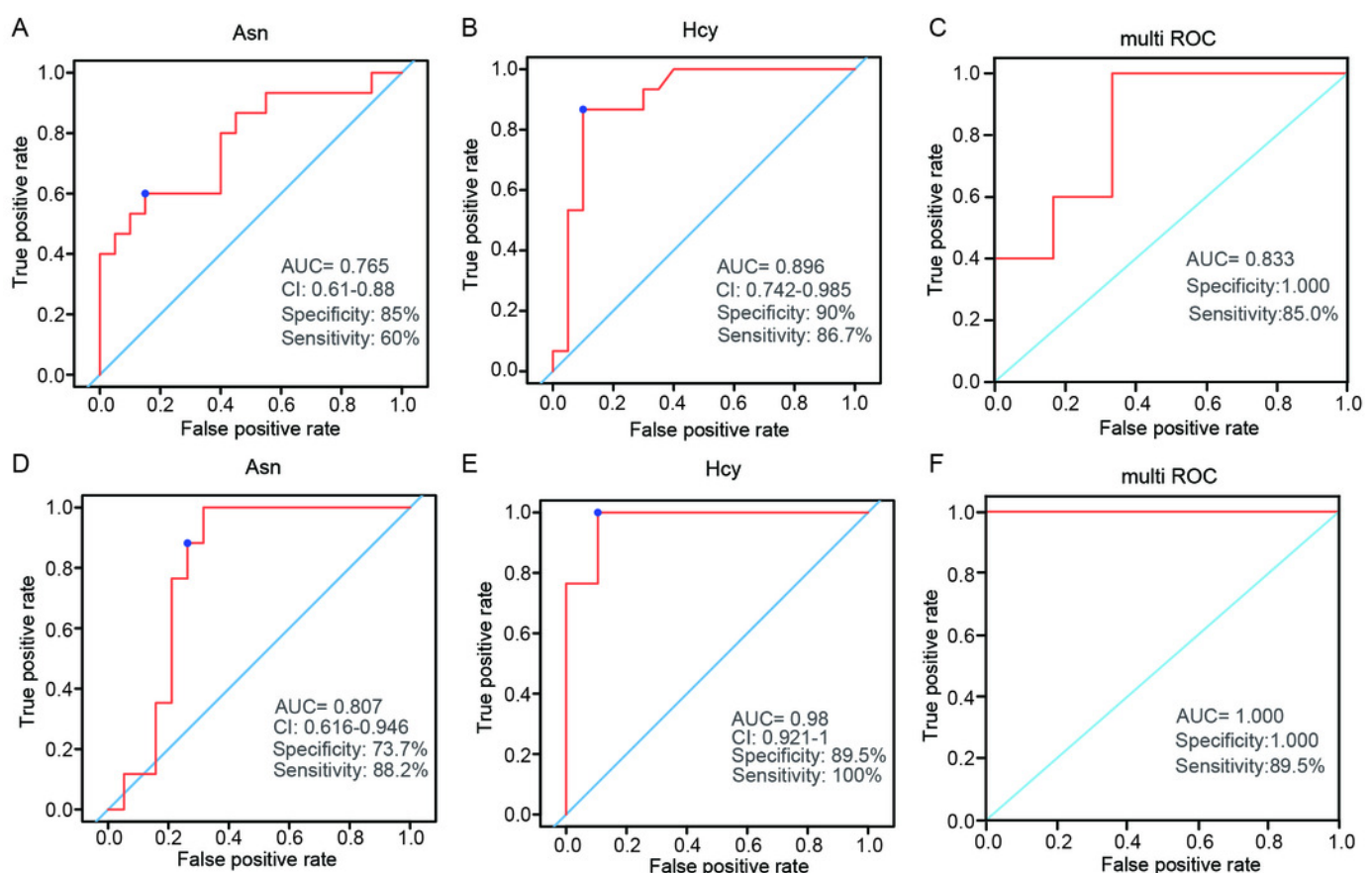
Figure 5

Amino acid profiling of the plasma and tissue samples between stage I+II and stage III+IV patients according to the TNM staging system.

PLS-DA scores of amino acids in plasma (A) and tissue (B) samples between LSCC stage I+II and stage III+IV patients. Overall heatmap of amino acids in plasma (C) and tissue (D) samples between LSCC stage I+II and stage III+IV patients.
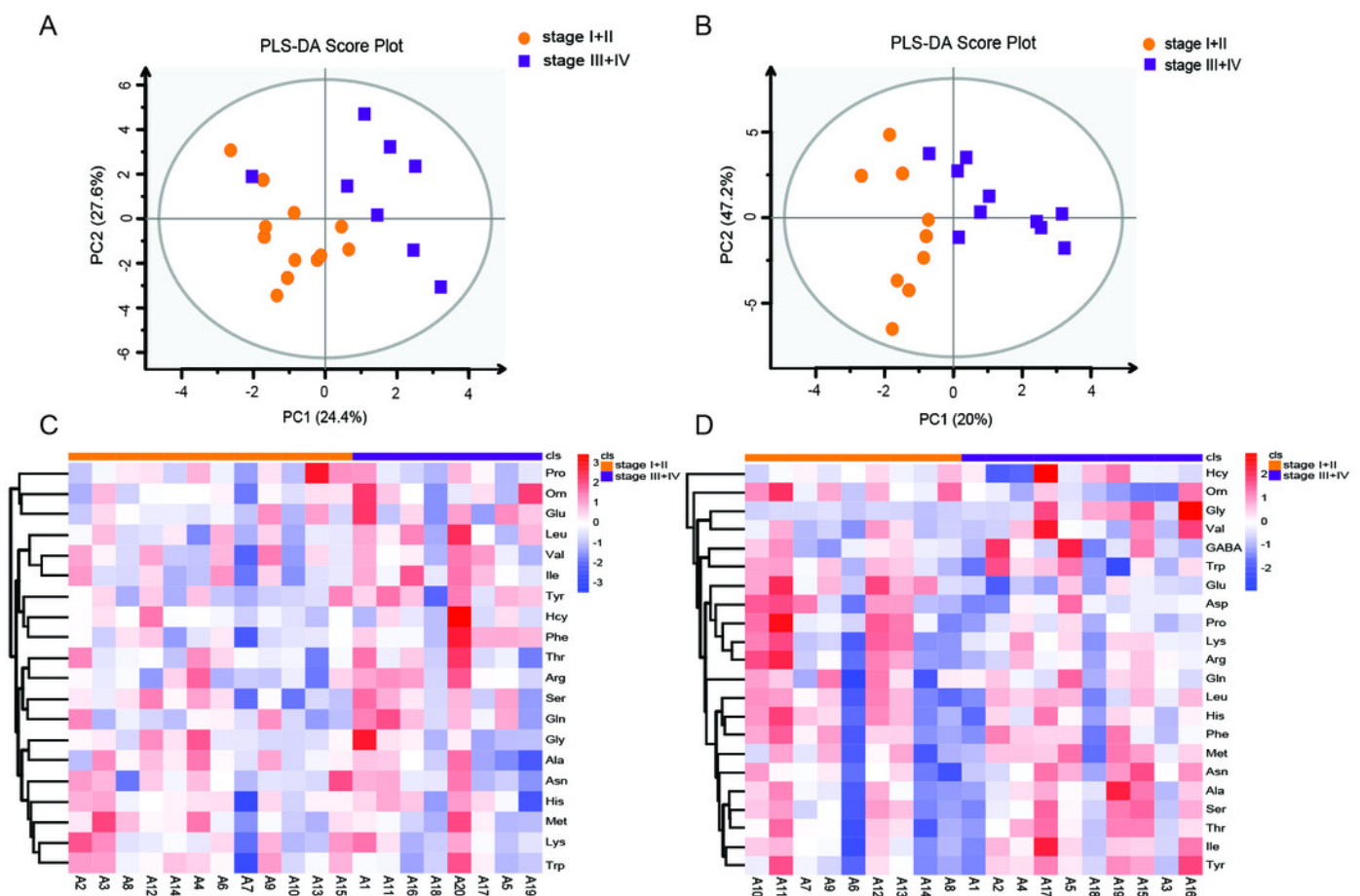
Figure 6

ROC analysis of the different amino acids in plasma and tissue samples between stage I+II and stage III+IV patients.

ROC analysis of Phe and Ile in plasma samples (A-B), and Orn, Glu and Gly in tissue samples (C-E).