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ORIGINAL ARTICLE

Identification of amino acids metabolomic profiling in human plasma distinguishes lupus nephritis from systemic lupus erythematosus

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Abstract

Lupus nephritis (LN) is an immunoinflammatory glomerulonephritis associated with renal involvement in systemic lupus erythematosus (SLE). Given the close relationship between plasma amino acids (AAs) and renal function, this study aimed to elucidate the plasma AA profiles in LN patients and identify key AAs and diagnostic patterns that distinguish LN patients from those with SLE and healthy controls. Participants were categorized into three groups: normal controls (NC), SLE, and LN. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was employed to quantify AA levels in human plasma. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were utilized to identify key AAs. The diagnostic capacity of the models was assessed using receiver operating characteristic (ROC) curve analysis and area under the ROC curve (AUC) values. Significant alterations in plasma AA profiles were observed in LN patients compared to the SLE and NC groups. The OPLS-DA model effectively separated LN patients from the SLE and NC groups. A joint model using histidine (His), lysine (Lys), and tryptophan (Trp) demonstrated exceptional diagnostic performance, achieving an AUC of 1.0 with 100% sensitivity, specificity, and accuracy in predicting LN. Another joint model comprising arginine (Arg), valine (Val), and Trp also exhibited robust predictive performance, with an AUC of 0.998, sensitivity of 93.80%, specificity of 100%, and accuracy of 95.78% in distinguishing between SLE and LN. The joint forecasting models showed excellent predictive capabilities in identifying LN and categorizing lupus disease status. This approach provides a novel perspective for the early identification, prevention, treatment, and management of LN based on variations in plasma AA levels.

Keywords Systemic lupus erythematosus · Lupus nephritis · Diagnose · UPLC-MS/MS · Amino acids · Metabolomics

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Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease characterized by a range of clinical manifestations. Lupus nephritis (LN) is a common renal complication associated with SLE. Research indicates that the majority of patients with SLE develop LN within five years of their initial diagnosis (Anders et al. 2020), and approximately 10% of those with LN progress to end-stage renal disease (Almaani et al. 2017).

At present, immunosuppressants, such as mycophenolate mofetil, azathioprine, and cyclophosphamide, combined with glucocorticoids are commonly used first-line therapy for LN patients. An increasing array of newly developed biological agents are also emerging in the treatment of SLE. Rituximab, the first biological agent approved for use in SLE, has shown some effectiveness in individual case reports. However, randomized controlled trials have not consistently met their primary endpoints (Rovin et al. 2012). Nonetheless, it may still provide benefits for patients with refractory SLE (46). The Phase III BLISS-LN study demonstrated that belimumab, a BAFF monoclonal antibody, significantly outperforms placebo in treating LN, effectively reducing organ damage while maintaining good tolerability (Furie et al. 2020). The post hoc analysis of the BLISS-LN study further revealed that the response rates were higher when belimumab was administered in conjunction with mycophenolic acid analog (MPAA) compared to cyclophosphamide. The 2024 KDIGO guidelines endorse belimumab as a supplementary immunosuppressive option, particularly recommending its combination with MPAA for LN patients at high risk of recurrence (Kidney Disease 2024). Furthermore, the 2023 EULAR guidelines advocate for the earlier application of biological agents to facilitate reductions in glucocorticoid dosages (Fanouriakis et al. 2024), reflecting a shift in the management strategies for SLE.

Despite this, the safety and efficacy of biological agents require further verification through larger-scale research and clinical practice. Side effects related to immunosuppressive therapy still remain a concern (Anders et al. 2020). Previous metabolomics studies have shown the potential of metabolic profiling in LN diagnosis (Bird et al. 2015). Biological fluid samples are readily available, and effective small changes in gene and protein expression are amplified on metabolites, thus making mass spectrometry detection easier and more sensitive, and reflecting the real pathophysiological state of organisms more directly and accurately (Zhou et al. 2022). Compared with traditional high performance liquid chromatography, the speed, sensitivity and separation efficiency of ultra-performance liquid chromatography (UPLC-MS/MS) are 9 times, 3 times and 1.7 times respectively, which shortens the analysis time, reduces the amount of solvent and the analysis cost (Zhou et al. 2022). The current gold standard for diagnosing LN in clinical practice is invasive renal biopsy. However, tissue collected by renal biopsy may be inadequate for pathological diagnosis, and the biopsy procedure can be further complicated by some complications such as bleeding, hematuria and perirenal hematoma (Manno et al. 2004). Undergoing a series of biopsies for monitoring disease progression and treatments is unlikely suitable for patients with LN. Investigating the specific metabolic profiles of SLE and LN patients may provide valuable insights for monitoring treatment effects and could potentially reduce the long-term reliance on high doses of glucocorticoids and immunosuppressants (2016).

Amino acid (AA) AAs can modulate immunity as well as regulate the T cell stress pathway (14; 15), and are involved in the pathogenesis of SLE through immunopathogenic pathways, which provide a variety of targets for therapeutic intervention (Sharabi and Tsokos 2020). AAs play crucial roles in immune cell proliferation, differentiation, and functional activation. For example, T cell activation upregulates several AA transporters, including SLC7A5. The deletion of SLC7A5 activates the mTOR signaling pathway and enhances MYC transcription factor expression, ultimately inhibiting T cell proliferation (Sinclair et al. 2014). T cell activation requires adequate levels of tryptophan (Trp) and arginine (Arg); when deprived of these AAs, activated T cells are unable to progress to the S phase of the cell cycle. Additionally, the depletion of leucine (Leu) and isoleucine (Iso) prompts T cells to enter the S-G1 phase, leading to cell cycle arrest and apoptosis (1820). Moreover, AA transporters, branched-chain AAs, glutamate (Glu), glutamine (Gin), glutathione (GSH), and serine (Ser), along with the catabolism of Trp and Arg, have been shown to modulate the generation and function of regulatory T cells (Tregs) (Yan et al. 2022). In summary, AA availability and metabolism are critical for regulating immune homeostasis and responses. Evidence suggests that some AAs, such as leucine, methionine, glutamine, arginine, and alanine, are essential in T cell metabolism (Wei et al. 2017). Besides, AA metabolic pathways, such as glutamine (Suthanthiran et al. 1990), tryptophan (Choi et al. 2020), and cysteine (Suwannaroj et al. 2001), can be used as potential therapeutic targets in lupus mouse models.

The specificity and sensitivity of the AA joint forecast models for identifying patients with early kidney disease are superior to the reported values of serum creatinine, urea nitrogen, and cystatin-C (Li et al. 2018). The composition of AAs in the diet has various effects on the evolution of chronic kidney disease in rats (Pillai et al. 2019). Aromatic AAs promote the recovery of renal function, while branched chain AAs quickly damage renal function and stimulate renal fibrosis (Pillai et al. 2019). However,

animal experiments suggested that Arg intake accelerates renal fibrosis and shortens the life span in LN (Peters et al. 2003). Taurine supplementation increases autoantibodies, increases albuminuria, and leads to more severe glomerulonephritis (Li et al. 2020). Hence, AA inhibitors or AA supplements are promising targeted treatments to prevent the deterioration of LN.

At present, the abnormal AA metabolism of LN patients are not completely understood. Therefore, we aim to utilize a metabolomic approach focused on 20 AAs through UPLC-MS/MS technology to investigate specific AA profiles and biomarkers that differentiate LN patients from SLE patients without renal involvement and healthy controls. Our objective is to establish diagnostic models for LN based on plasma AA profiles. These models may provide a novel method for the early diagnosis of LN and facilitate dynamic monitoring of disease progression through variations in plasma AA levels.

Materials and methods

Human subjects

All patients signed an informed consent form. The design principles and related sample collection scheme of the present study were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (ethical approval number: 2021-KY-0477-003), and they were also in line with the principles of the Helsinki Declaration. Grouping criteria: A total of 94 patients aged from 13 to 70 years old were enrolled. Patients in the LN group were diagnosed by renal puncture in the Department of Nephrology of the First Affiliated Hospital of Zhengzhou University from June 2018 to December 2020. The SLE group was newly diagnosed SLE patients treated in the Rheumatic Immunology Department of the same hospital in the same period. The participants of the healthy control group are volunteers in the physical examination center in the same hospital in the same period matching the sex and age of LN and SLE patients. The inclusion criteria: (1) The enrolled SLE patients were classified according to the 1997 classification criteria of the American College of Rheumatology (Hochberg 1997). In addition, all SLE patients had no renal involvement, with renal function within the normal range, urine protein quantification < 0.5 g/24 h, and centrifuged urine with less than 5 red blood cells under high power microscope. (2) In addition to meeting the diagnosis criteria of SLE (Hochberg 1997), LN patients need to have any of the following clinical manifestations of renal involvement: urine protein quantification ≥ 0.5 g/24 h, or centrifugal urine>5 red blood cells per high magnification field, or abnormal renal function. (3) All LN patients have undergone renal biopsy in our hospital. The pathological classification of LN was based on the pathological classification of LN by International Society of Nephrology (ISN)/Society of Nephrology (RPS) in 2003 (Weening et al. 2004). (4) All SLE and LN patients were newly diagnosed without drug treatment like glucocorticoid, immunosuppressant, hydroxychloroquine, cyclophosphamide, and monoclonal antibody drugs (such as Belliumab) before, some patients have taken angiotensin blocking agents before hospitalization in our hospital. The exclusion criteria: (1) Patients with obvious liver, brain, heart, lung, or blood damage were excluded. (2) Patients with hepatitis B virus-associated glomerulonephritis, henoch purpura nephritis, diabetic nephropathy, rheumatoid arthritis renal damage, vasculitis renal damage, membranous nephropathy, IgA nephropathy and other primary and secondary renal diseases assessed by clinical and pathological analysis were excluded. (3) Patients with stress factors, such as vigorous exercise within 24 h, infection, fever, significantly high blood sugar, pregnancy, tumor and severe hypertension, and patients with a history of alcoholism, smoking and oral contraception were also excluded.

Preparation of internal standard solution, standard solution and standard curve

A total of 20 AAs standard compounds (MedChemExpress, MonmouthJunction, NJ, UnitedStates) were diluted in water or dimethylsulfoxide at concentrations from 1 to 100mM. The internal standard (IS) was prepared from an isotope-labeled mix of 20 AAs (MilliporeSigma, Burlington, MA, UnitedStates) and acetonitrile (ACN, ThermoFisherScientific, Waltham, MA, UnitedStates) at a concentration of 100 nM.

All subjects were requested to fast overnight and abstain from any other medication for 24 h before sampling. The peripheral venous blood samples were collected in a blood vessel containing heparin sodium between 6:00 and 8:00 am, then centrifuged for 10 min at a rate of 1500 revolutions per minute in 4°C. After all, the blood samples were transferred to a 1.5 ml centrifuge tube and stored at -80°C.

Samples and standard curves were prepared by protein precipitation method (Zhou et al. 2021). For each sample, 50ul ACN and 50ul of thawed plasma and 150 µl of isotope internal standard working fluid (100 nM) were mixed into 1.5mL EP tubes. The standard curves were formulated by mixing 50ul of thawed plasma (from 30 normal control patients) with 50ul of AAs working fluid (10–100 nM), and 150ul of isotope internal standard working fluid (100 nM) in 1.5 ml Ep tubes. After eddy oscillation (1500RPM, 10 min) and centrifugation (12000 g, 4°C, 10 min), the supernatant

was filtered through a 0.2 mm polytetrafluoroethylene filter and stored in a clean injection bottle for mass spectrometry.

Data processing and statistical analysis

The chromatograms of AAs and isotope-labeled AAs were generated by OriginLab (Northampton, MA, USA). SIMCA-P software (v.14.1, Umetrics, Umeå, Sweden) was used to generate orthogonal partial least squares discriminant analysis (OPLS-DA) models to better understand the similarities and differences of metabolites among groups. The model validation and significance were determined from the R (Almaani et al. 2017) and Q (Almaani et al. 2017) values. The variable importance in projection (VIP) value of AAs in the models was calculated to indicate the contribution to the classification of samples. VIP value > 1.2 was considered statistically significant for intergroup differences. OPLS-DA with 200 permutations were used to further demarcate the groups(Permutation tests of OPLS-DA models was shown in Supplementary Figure S1). The corresponding shared and unique structures (SUS) plot was generated to provide information on the contribution of AAs to the group discrimination. The SUS-plot is a powerful analytical tool for identifying diagnostic markers. It enables the comparison and analysis of two OPLS-DA models, allowing for the examination of similarities and differences in compound trends within the models. Compounds that exhibit the same or opposite trends, as well as those unique to each model, are represented in different areas of the coordinate axes or quadrants. This facilitates the discovery, classification, and analysis of specific markers (Wiklund et al. 2008). The joint diagnosis model of AAs was established based on the logistic regression method. The receiver operating characteristic (ROC) curve was used to comprehensively evaluate the effect of the model and select the boundary value.

The measurement data are presented as the average ± standard deviation. Chi-square test, t test, and the Fisher's exact test were used for comparison among groups. P<0.05 was considered statistically significant.

Results

The metabolic profiles of 20 plasma AAs significantly differ in LN and SLE patients

94 patients were enrolled from June 2018 to December 2020. A total of 32 SLE patients were included in this study, including 2 males and 30 females, with an average age of (34.550 ± 15.273) (13~67) years old, 32 LN patients were enrolled, including 4 male patients and 28 female patients, the average age was (30.470 ± 10.417) (13~54) years old. The levels of plasma AAs of the three groups are shown in Supplementary Table S1. As shown in Supplementary Table S1, there were 12 significant different AAs in the LN group compared with NC group, and 10 differencial AAs between the LN and SLE groups ($P < 0.05$).

We established a supervised OPLS-DA method to visualize the separation trends of the three groups (Fig. 1A). SUS plots were utilized to compare the SLE and LN groups against the NC control group as a common reference. As shown in Fig. 1B, variables located in regions A1 or A2 were solely upregulated or downregulated in the LN group, reflecting the metabolic characteristics of this group. Conversely, variables found in regions B1 or B2 were specific to the SLE group. The variables situated on the diagonals (regions C1 or C2) were important for both groups. Consequently, amino acids including histidine (His), valine (Val), tryptophan (Trp), cysteine (Cys), and lysine (Lys) were identified as specific to the LN group, while asparagine (Asn) was identified as unique to the SLE group. Arginine (Arg) was shared by both the SLE and LN groups.

Plasma profiles of arg and Trp distinguish LN patients from healthy individuals

The OPLS-DA model of the LN and NC groups (Fig. 2A) was established to analyze the distribution difference of plasma AAs between the two groups. The S-plot (Fig. 2B), combined with the columnar scatter diagram (Fig. 3), showed that the decreased Trp levels (Fig. 3E) and increased Arg levels (Fig. 3F) were the main contributors to the intergroup distribution difference. In addition, the VIP values of Arg and Trp were both greater than 1.20 ($P < 0.05$), thus Arg and Trp may be the main differential AAs between LN patients and normal controls (Table 1).

Plasma profiles of arg and asn distinguish SLE patients from healthy individuals

In order to investigate the plasma AA spectrum differences among SLE patients with active disease activity and inactive disease activity and NC group, SLE patients ($n = 32$) were divided into active SLE group (SLEDAI ≥ 6, $n = 19$) and inactive SLE group (SLEDAI < 6, $n = 13$), and OPLS-DA models were established by pairwise among three groups (Fig. 4). Predictive performance of key AAs was shown in Supplementary Table S2. Arg (AUC = 0.983 ± 0.014, $P < 0.001$, Sensitivity = 89.50%, Specificity = 100.00%, Accuracy = 95.93%) and Asn (AUC = 0.956 ± 0.025, $P < 0.001$, Sensitivity = 100.00%, Specificity = 82.80%, Accuracy = 89.47%) may be the major differential AAs between the active SLE group and the NC group with excellent prediction performance (Fig. 4A-D,

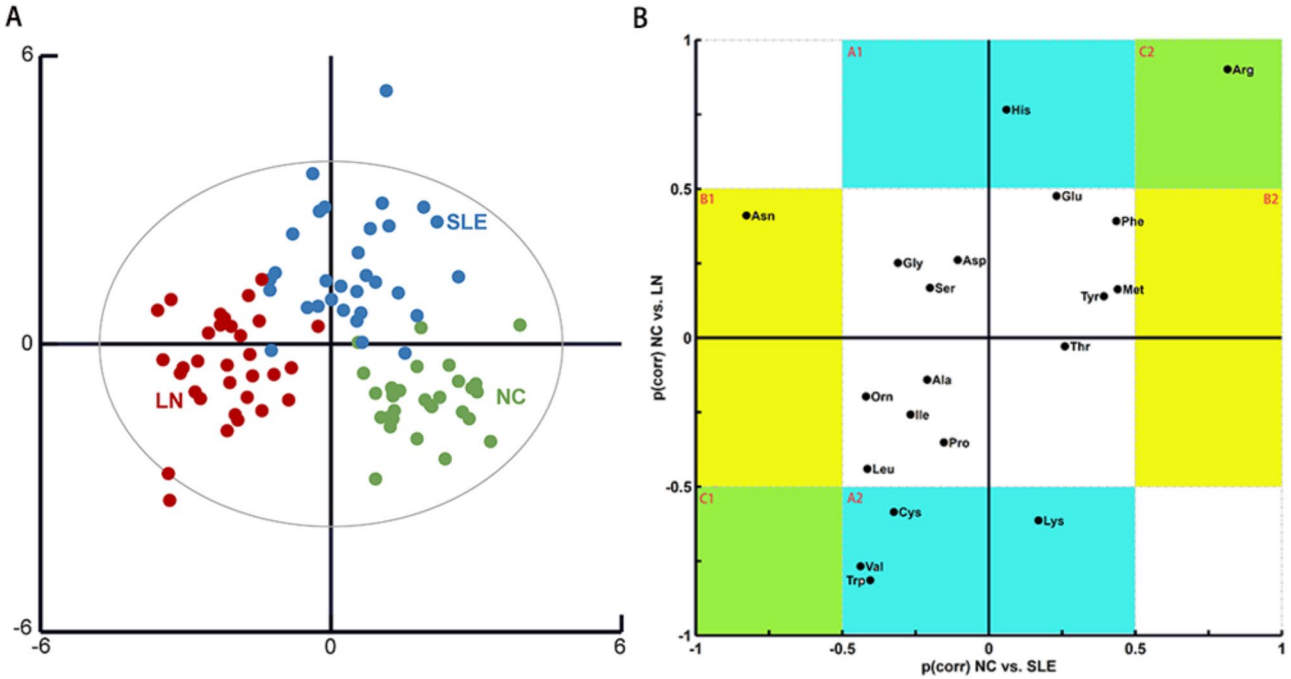

Fig. 1 Plasma profles of AAs for the healthy controls (NC) and the SLE patients and LN patients. (**A**) Orthogonal partial-least-squares discriminant analysis (OPLS-DA) score plot highlighted the difference in AA levels can distinguish the NC group (green spots), LN patients (red spots) and SLE patients (blue spots). $R^2X = 0.299$, $R^2Y = 0.662$, $Q^2 = 0.615$. (**B**) As the control group provided a common reference for comparing the SLE and LN groups, SUS plots were generated using the two OPLS-DA models with the control group as the reference

Supplementary Table S2). Similarly, The ability of Arg (AUC = 0.957 ± 0.028, $P < 0.001$, Sensitivity = 100.00%, Specificity = 86.20%, Accuracy = 90.37%) and Asn (AUC = 0.935 ± 0.037, $P < 0.001$, Sensitivity = 100.00%, Specificity = 72.40%, Accuracy = 80.74%) to discriminate the inactive SLE group and the NC group were also excellent (Fig. 4E-H, Supplementary Table S2). The R (Almaani et al. 2017)Y and Q (Almaani et al. 2017) values of the OPLS-DA model for active and inactive SLE were both less than 0.5, and the predictive performances of the key intergroup differencial AAs His and Ile were general (Fig. 4I-L, Supplementary Table S2), indicating that the model fitting degree and predictive ability were not ideal, the AA profiles of the two groups were not significantly different.

Lys, Trp, His, and Asn may be the main differential AA profiles in the Progression of SLE patients to LN

We next established an OPLS-DA model of the LN and SLE groups (Fig. 2C), which indicated a clear separation of the two groups. The S-plot (Fig. 2D) and columnar scatter diagrams (Fig. 3) showed that the decreased levels of Trp (Fig. 3E) and Lys (Fig. 3G)as well as the increased levels of His (Fig. 3H) and Asn (Fig. 3I) were the main differential AA profiles distinguishing SLE patients from LN patients, and the VIP values were all greater than 1.20 ($P < 0.05$; Table 1). Therefore, plasma Lys, Trp, His, and Asn may be the main differential AAs for distinguishing LN patients from SLE patients.

The plasma levels of Arg in SLE and LN patients may be related to lupus disease status

To compare the above key AAs for distinguishing lupus patients (SLE or LN) from normal controls, we regarded patients in a lupus state (SLE or LN) as a whole SLE + LN group and compared them to the NC group to establish an OPLS-DA model (Fig. 5A). The S-plot (Fig. 5B) and AUC curves (Fig. 5C and D) indicated that increased Arg levels, decreased Trp levels, and decreased Val levels distinguished lupus patients from normal controls.

We next constructed ROC curves of the selected plasma AAs to evaluate their predictive performances, the AUC values for 20 AAs in plasma of study participants were shown in Supplementary Table S3. The VIP values of Arg were all greater than 1.20 in the three pairwise comparisons (SLE vs. NC, LN vs. NC, and SLE + LN vs. NC) ($P < 0.05$; Table 1). Further analysis showed that the AUC values of Arg in the three pairwise comparisons were all greater than 0.95 ($P < 0.001$, Supplementary Table S3 and Fig. 3B、Figure 5D), and the sensitivity, specificity, and accuracy indexes were all above 85% (Table 2).

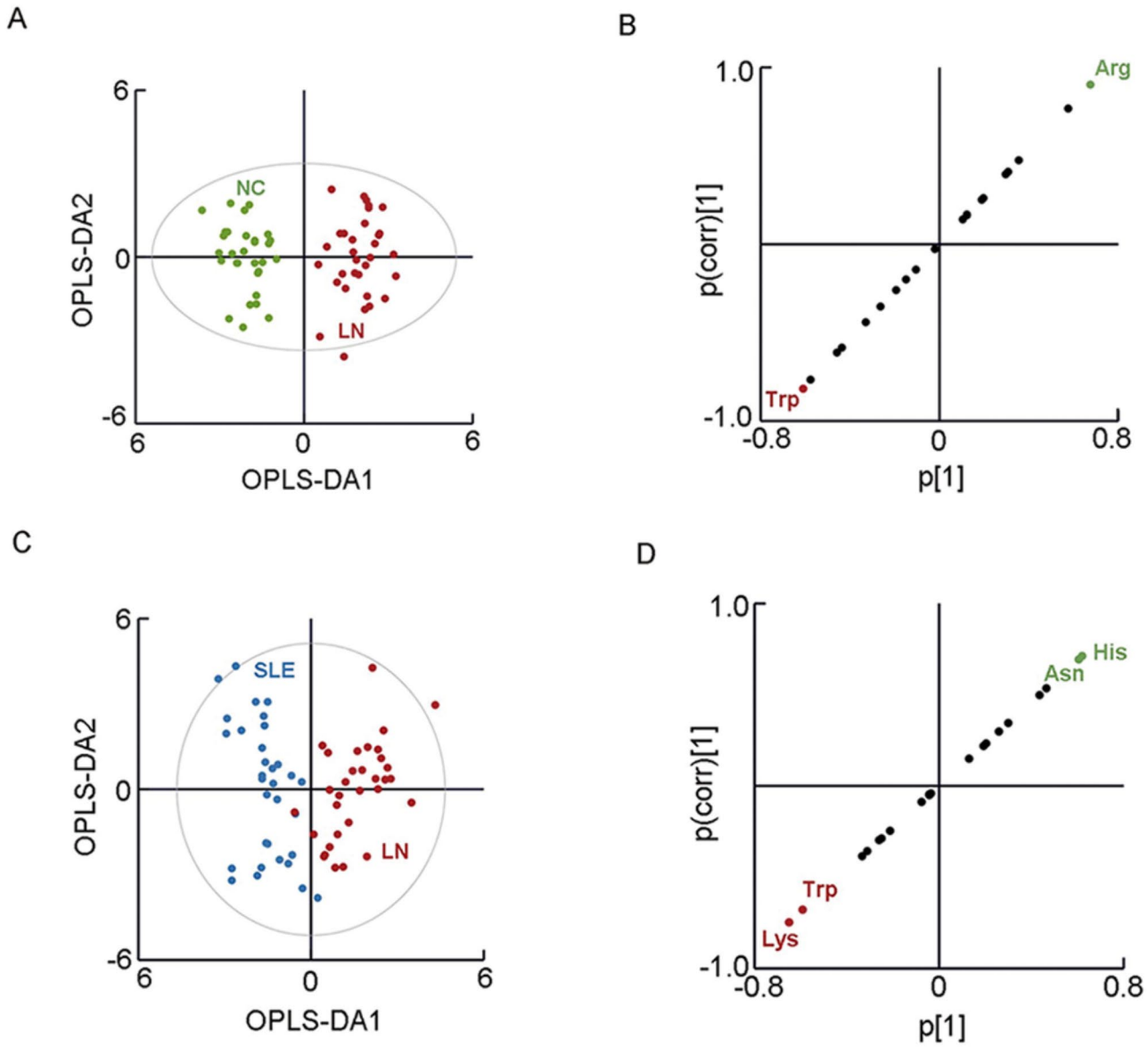

Fig. 2 Separated metabolomic analyses between the LN, SLE and NC groups. (**A**) OPLS-DA plot showing the visual separation between the LN group and NC group. $R^2X = 0.216$, $R^2Y = 0.858$, and $Q^2 = 0.726$. (**B**) The S-plot shows that the plasma levels of Arg and Trp were the major contributors to the separation between the LN group and NC group. (**C**) OPLS-DA plot showing the visual separation between the SLE and LN patients. $R^2X = 0.375$, $R^2Y = 0.739$, and $Q^2 = 0.671$. (**D**) The S-plot shows that the plasma levels of Lys, Trp, His, and Asn were the major AAs contributing to the difference between the SLE group and LN group

These results suggested that among the selected three AAs, increased plasma Arg expression had the best predictive efficacy of lupus disease status.

The Lys, His, and Trp Key AAs are significantly related to the progress of LN disease

To screen out the key AAs for the progression of LN patients, the patients in the SLE group and the NC group were regarded as an overall SLE + NC group and were compared to the LN disease group to establish an OPLS-DA model (Fig. 5E). The plasma profiling of AAs achieved a significant separation trend. Decreased Trp levels and increased His levels were the key differential AAs (Fig. 5F). The VIP values of Lys, Trp and His in the SLE group vs. LN group, LN group vs. NC group, and SLE + NC group vs. LN group comparisons were all greater than 1.2 (Table 1, $P < 0.05$).

Further analysis showed that Lys had AUC values greater than 0.95 for the LN vs. NC, LN vs. SLE, and LN vs. SLE + NC comparisons (Supplementary Table S3). Lys had good sensitivity, specificity and accuracy to distinguish the

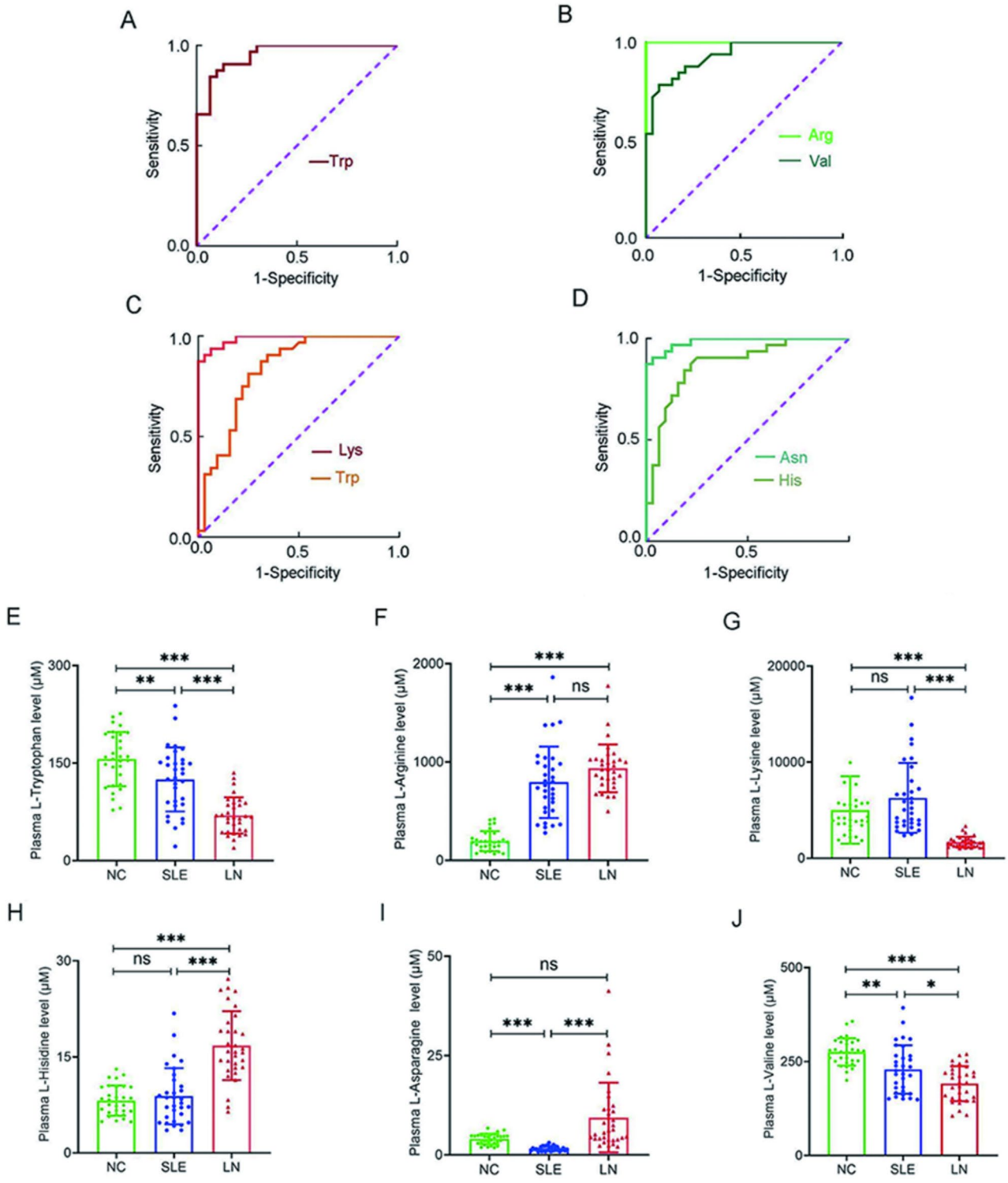

Fig. 3 Trp, Lys, and His can distinguish LN group from SLE and NC groups. Receiver-operating characteristic (ROC) curve demonstrates that the plasma levels of Trp (**A**) as well as Arg and Val (**B**) discriminated the LN group from the NC group with area under the ROC curve (AUC) values greater than 0.85. The plasma levels of Lys and Trp (**C**) as well as His and Asn (**D**) had excellent predictive ability to discriminate SLE patients from LN patients. Plasma levels of Trp (**E**), Arg (**F**), Lys (**G**), His (**H**), Asn (**I**), and Val (**J**). Data are presented as the mean ± SD. ns indicates no statistical significance. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were determined by ANOVA followed by Tukey's or Games-Howell post hoc comparison test

Table 1 Variable important in projection of 20 AAs in study participants

AA (μM)	SLE vs. NC		LN vs. NC		SLE vs. LN		SLE + LN vs. NC		NC + SLE vs. LN	
	VIP	*P*	VIP	*P*	VIP	*P*	VIP	*P*	VIP	*P*
Gly	0.853	0.023	0.910	0.040	1.135	0.000	0.500	0.994	0.952	0.000
Ala	0.490	0.150	0.499	0.423	0.491	0.563	0.256	0.199	0.425	0.899
Arg	1.917	0.000	1.751	0.000	0.760	0.072	1.877	0.000	1.200	0.000
Asn	1.943	0.000	0.840	0.002	1.452	0.000	0.674	0.123	1.425	0.000
Asp	0.479	0.736	0.576	0.085	0.624	0.054	0.389	0.370	0.607	0.051
Cys	0.856	0.021	1.149	0.000	0.772	0.060	1.149	0.000	1.069	0.000
Glu	0.879	0.068	0.946	0.001	0.457	0.105	0.882	0.004	0.731	0.004
His	0.320	0.439	1.501	0.000	1.496	0.000	1.218	0.000	1.688	0.000
Iso	1.020	0.046	0.637	0.058	0.623	0.832	0.854	0.054	0.808	0.204
Leu	0.968	0.003	0.866	0.001	0.878	0.463	1.035	0.001	0.987	0.005
Lys	0.571	0.171	1.209	0.000	1.563	0.000	0.900	0.178	1.322	0.000
Met	1.124	0.001	1.011	0.210	0.950	0.036	1.023	0.003	0.456	0.464
Orn	0.993	0.005	0.585	0.228	0.728	0.030	0.755	0.015	0.307	0.654
Phe	1.075	0.001	0.830	0.003	0.874	0.630	1.183	0.000	0.677	0.241
Pro	0.602	0.160	0.689	0.015	0.512	0.417	0.717	0.034	0.787	0.075
Ser	0.550	0.092	0.569	0.144	1.044	0.001	0.612	0.867	0.733	0.007
Thr	0.665	0.069	0.098	0.919	0.782	0.068	0.710	0.246	0.417	0.291
Trp	0.992	0.009	1.581	0.000	1.498	0.000	1.388	0.000	1.600	0.000
Tyr	0.941	0.001	0.340	0.195	1.051	0.009	1.060	0.003	0.632	0.135
Val	1.028	0.001	1.503	0.000	1.115	0.009	1.380	0.000	1.457	0.000

Legend: Data represent the mean ± standard deviation (range). P-values were determined by the Student's t test. $P < 0.05$ (two-tailed) was regarded as statistically different. Gly, glycine; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; His, histidine; Iso, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; NC, negative control; VIP, variable importance in projection.

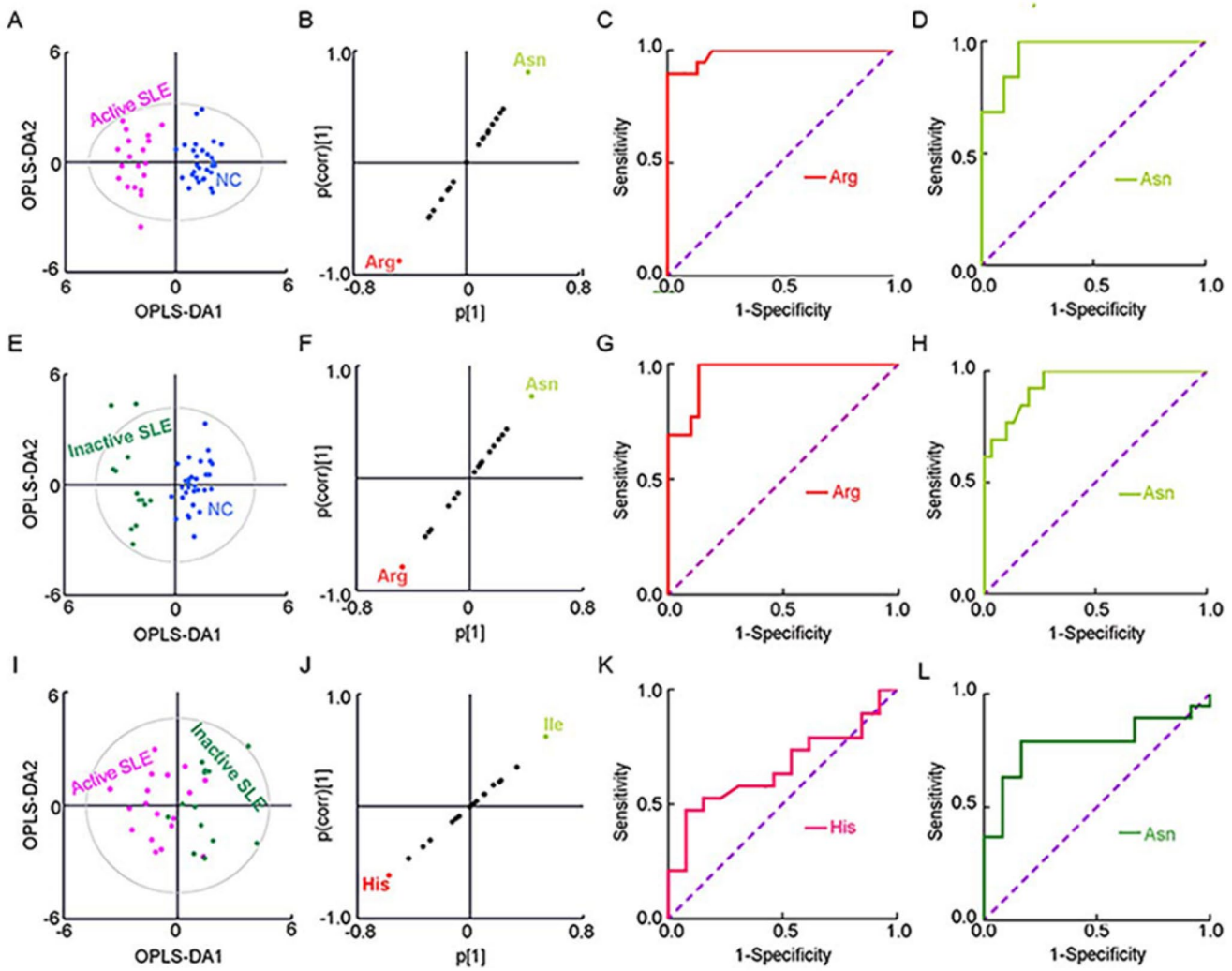

Fig. 4 Plasma profles of AAs for the active SLE patients (SLEDAI ≥ 6), inactive SLE patients(SLEDAI < 6)and the normal controls (NC). (A) OPLS-DA plot showing the visual separation between the active SLE group and the NC group. The ellipse indicates the Hotelling T2 (0.95) range for the model. $R^2X = 0.248$, $R^2Y = 0.896$, $Q^2 = 0.784$. (B) The S-plot identified plasma levels of Asn and Arg to be the major contributors to the separation between the active SLE group and the NC group. ROC curve demonstrates that the plasma level of Asn and Arg discriminated the active SLE group from the NC group (C and D) according to AUC values, which were greater than 0.95. (E) OPLS-DA plot showing the visual separation between the patients in the inactive SLE and NC groups. $R^2X = 0.381$, $R^2Y = 0.869$, $Q^2 = 0.699$. (F) The S-plot identified plasma levels of Asn and Arg as the major AAs contributing to the difference between the inactive SLE and NC groups. The plasma level of Asn and Arg showed excellent predictive ability to discriminate inactive SLE patients from the NC group (G and H). (I) OPLS-DA plot showing the visual separation between the active SLE patients and inactive SLE patients was not significant. $R^2X = 0.352$, $R^2Y = 0.475$, Q^2=-0.107. R (Almaani et al. 2017)Y and Q (Almaani et al. 2017) were less than 0.5, which meant that the modol fitting degree and predictive performance were not good enough. (J) The S-plot identified plasma levels of His and Ile as the major AAs contributing to the difference between the the active SLE and inactive SLE groups. (K) and (L) showed the predictive abilities of plasma His and Ile to discriminate active SLE patients from the inactive SLE group were not ideal (AUC < 0.8)

LN group from the NC group, SLE group, and SLE + NC group (Table 2).

In addition, the AUC value for Trp was greater than 0.90 for the LN vs. NC (Fig. 3A) and LN vs. SLE + NC (Fig. 5G) comparisons, but it was lower for the LN vs. SLE comparison (Fig. 3C, Supplementary Table S3). The corresponding sensitivity, specificity, and accuracy of Trp were higher for the LN vs. NC and LN vs. SLE + NC comparisons (Table 2).

The AUC value for His was greater than 0.85 for the LN vs. NC, LN vs. SLE + NC (Fig. 5H), and LN vs. SLE (Fig. 3D) comparisons (Supplementary Table S3). The corresponding sensitivity, specificity, and accuracy of His were greater than 80% in the LN group vs. NC group and LN group vs. SLE + NC group comparisons (Table 2).

In conclusion, the plasma levels of Lys, His, and Trp are the key AAs that may contribute to the progress of LN disease.

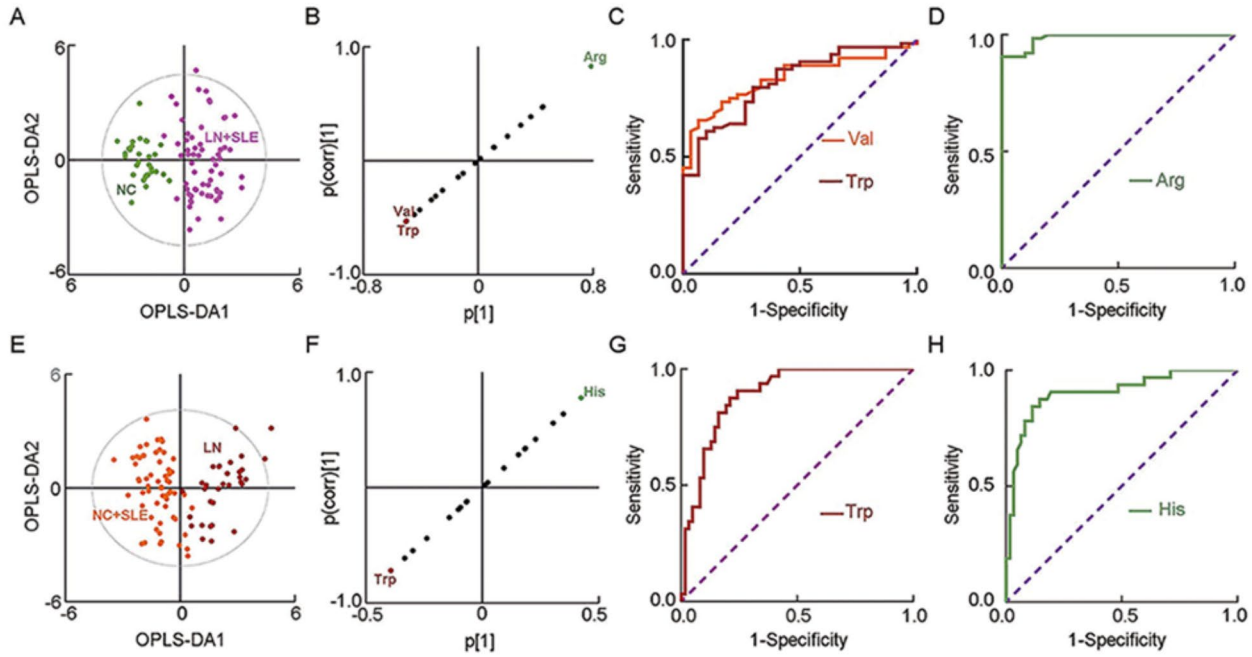

Fig. 5 Specific AA profiles of the LN + SLE and the SLE + NC groups. (**A**) OPLS-DA plot showing the visual separation between the LN + SLE group and the NC group. $R^2X = 0.304$, $R^2Y = 0.755$, and $Q^2 = 0.692$. (**B**) The S-plot identified plasma levels of Val, Trp, and Arg to be the major contributors to the separation between the LN + SLE group and the NC group. Receiver-operating characteristic (ROC) curve demonstrates that the plasma level of Val, Trp, and Arg discriminated the LN + SLE group from the NC group (**C** and **D**) according to AUC values, which were greater than 0.85. (**E**) OPLS-DA plot showing the visual separation between the patients in the SLE + NC and LN groups. $R^2X = 0.303$, $R^2Y = 0.752$, and $Q^2 = 0.706$. (**F**) The S-plot identified plasma levels of Trp and His as the major AAs contributing to the difference between the SLE + NC and LN groups. The plasma level of Trp and His showed excellent predictive ability to discriminate LN patients from the SLE + NC group (**G** and **H**)

The joint forecast models predict LN and lupus disease status patients with excellent predictive performance

We investigated and compared the predictive efficacy of individual and joint key AAs as forecast models. The predictive performance indicators and ROC curves of the models are shown in Supplementary Table S4 and Fig. 6.

For the LN vs. SLE comparison, the joint forecast model of plasma Asn, His, and Lys had a better predictive ability than the separate diagnostic efficiency of Asn, His, and Lys (Table 2; Fig. 6A) with a sensitivity of 100%, specificity of 96.90%, accuracy of 98.45%, and an AUC value of 0.998 (Supplementary Table S4 and Fig. 6A).

For the SLE + NC vs. LN comparison, the joint forecast model of plasma His, Lys, and Trp had a superior diagnostic performance (AUC = 1.0, sensitivity = 100%, specificity = 100%, and accuracy = 100%; Supplementary Table S4 and Fig. 6B).

For the LN + SLE vs. NC comparison, the joint forecast model of plasma Arg, Val, and Trp had a better predictive ability (AUC = 0.996, sensitivity = 93.80%, specificity = 100%, and accuracy = 95.78%; Supplementary Table S4 and Fig. 6C) than that of the single AA diagnostic models (Table 2 and Fig. 6C).

Discussion

The present study reported that the plasma profiles of AAs can distinguish LN patients from SLE patients and healthy controls. Further analyses suggest that a combined predictive model utilizing plasma His, Lys, and Trp, as well as a diagnostic model comprising plasma Arg, Val, and Trp, might provide promising predictions for LN and the disease status of lupus patients, demonstrating favorable diagnostic performance .

AAs can regulate immunity through a variety of mechanisms, including central energy metabolism, redox balance, and epigenetic modification (Kelly and Pearce 2020). Various AAs and their transporters are pivotal for T cell activation, differentiation, and effector function (Wang and Zou 2020). Previous studies have reported that the main AA metabolomic profiles of SLE patients are characterized by

Table 2 Predictive performance of the key AAs

	AUC	*P**	Cutoff value (μM)	Sensitivity (%)	Specificity (%)	Accuracy (%)
SLE vs. NC						
Arg	0.971 ± 0.017	0.000	330.625	96.90	86.70	91.97
Asn	0.946 ± 0.026	0.000	2.450	93.80	83.30	88.72
His	0.508 ± 0.078	0.919	13.150	18.80	100.00	58.09
Lys	0.645 ± 0.073	0.058	5785.000	46.90	83.30	64.51
Trp	0.669 ± 0.072	0.027	152.000	75.00	60.00	67.74
Val	0.738 ± 0.069	0.002	219.500	50.00	96.70	72.60
LNvs.**NC**						
Arg	1.000 ± 0	0.000	460.313	100.00	100.00	100.00
Asn	0.712 ± 0.067	0.004	7.120	43.80	100.00	70.99
His	0.938 ± 0.033	0.000	12.350	84.40	96.70	90.35
Lys	0.966 ± 0.019	0.000	2150.000	87.50	93.30	90.31
Trp	0.953 ± 0.024	0.000	93.800	84.40	93.30	88.71
Val	0.928 ± 0.030	0.000	230.500	78.10	93.30	85.45
LNvs.**SLE**						
Arg	0.655 ± 0.074	0.038	646.875	96.90	37.50	67.20
Asn	0.984 ± 0.011	0.000	2.640	90.60	96.90	93.75
His	0.871 ± 0.047	0.000	10.900	90.60	75.00	82.80
Lys	0.989 ± 0.008	0.000	2255.000	87.50	100.00	93.75
Trp	0.857 ± 0.050	0.000	89.550	81.30	75.00	78.15
Val	0.664 ± 0.070	0.028	254.000	93.80	34.40	64.10
LN + SLEvs.**NC**						
Arg	0.986 ± 0.009	0.000	455.938	90.60	100.00	93.60
Asn	0.601 ± 0.058	0.124	2.450	51.60	83.30	61.72
His	0.733 ± 0.051	0.000	13.150	48.40	100.00	64.87
Lys	0.676 ± 0.055	0.007	3305.000	57.80	80.00	64.89
Trp	0.818 ± 0.045	0.296	93.800	57.80	93.30	69.13
Val	0.838 ± 0.041	0.000	230.500	65.60	93.30	74.44
SLE + NCvs.**LN**						
Arg	0.827 ± 0.043	0.000	646.875	96.90	67.70	77.64
Asn	0.848 ± 0.040	0.000	3.665	84.40	72.60	76.62
His	0.904 ± 0.035	0.000	12.350	84.40	88.70	87.24
Lys	0.978 ± 0.012	0.000	2150.000	87.50	96.80	93.63
Trp	0.905 ± 0.031	0.000	94.900	87.50	79.00	81.89
Val	0.796 ± 0.046	0.000	230.500	78.10	69.40	72.36

Legend: *P** values were determined by analyses of ROC curves under a nonparametric assumption. $P < 0.05$ (two-tailed) was regarded statistically significant. Accuracy=(A×sensitivity + B×specificity)/(A + B); where A is the participant number of the corresponding disease group; and B is the participant number of the corresponding control group. ROC, receiver operating characteristic.

downregulation of glucogenic and ketogenic AAs in their peripheral blood (Yan et al. 2016; Bengtsson et al. 2016). During the active period of metabolic activation, lysosome catabolism is increased to secure AAs from starvation through autophagy of proteins and organelles, resulting in the accumulation of branched chain AAs (Val, Leu, and Iso) (Proud 2002), glutamine (Choi et al. 2018), and His. The depletion of glutathione and Cys stimulates the mTOR complex 1 (MTORC1) (Gergely et al. 2002), which promotes the proliferation and differentiation of T lymphocyte subsets as well as reactive activation of inflammatory T lymphocytes. AAs play an important role in the occurrence and development of systemic autoimmune and organ inflammation in SLE patients.

SLE is a heterogeneous disease that shares features with various rheumatic disorders, including acute gout (AG), rheumatoid arthritis (RA), spondyloarthritis (SpA), primary Sjögren's syndrome (pSS), and systemic sclerosis (SSc). Altered AA metabolism plays a significant role in these conditions. AAs such as Iso, Lys, and Ala have been identified as key differentiators between AG patients and those with hyperuricemia (AHU) and healthy controls (Luo et al. 2018). Distinct serum profiles of Ala, Leu, Thr, and Val have also been observed in seronegative RA and psoriatic

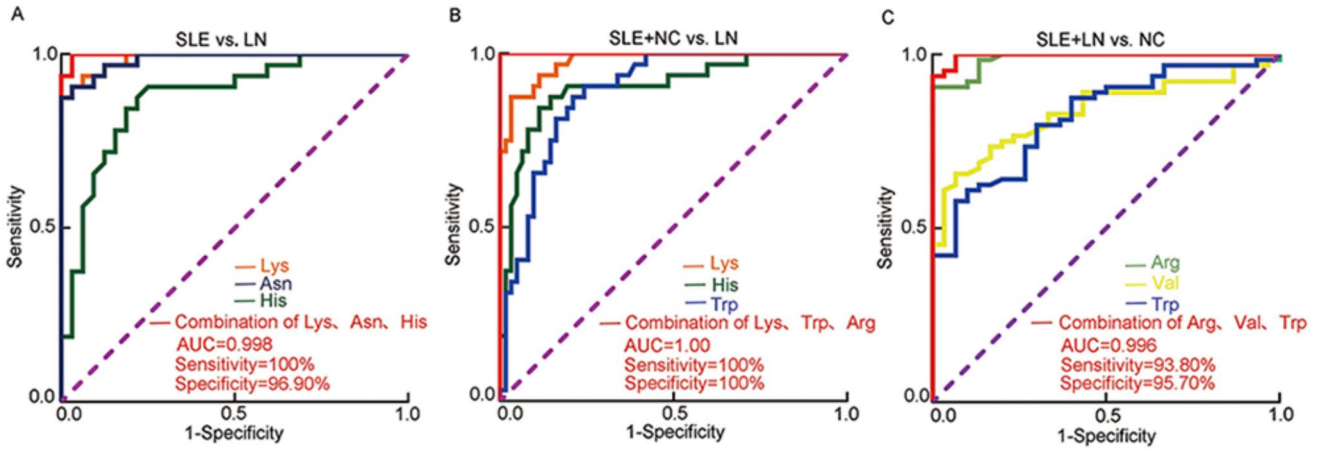

Fig. 6 ROC curves of individual AAs and joint forecast model. (**A**) ROC curves of Asn, His, and Lys as well as the joint forecast model for the LN vs. SLE comparison. (**B**) ROC curves of His, Lys, and Trp as well as the joint forecast model for the SLE + NC vs. LN comparison. (**C**) ROC curves of Arg, Val, and Trp as well as the joint forecast model for the SLE + LN vs. NC comparison

arthritis (PsA) (Souto-Carneiro et al. 2020). In SpA patients, decreased levels of Trp and Glu have been consistently reported (Huang et al. 2022). Comparative metabolic profiling indicates that SLE shows over 67% specificity when distinguished from pSS, SSc, and matched healthy controls, with Trp identified as a particularly discriminative metabolite. Alterations in Trp levels correlate with changes in aromatic amino acid decarboxylase (AADC) activity and kynurenine pathway activation (Bengtsson et al. 2016). Systemic autoimmune diseases (SADs), characterized by immune dysfunction, include SLE, SSc, pSS, RA, primary antiphospholipid syndrome, and mixed connective tissue disease. Multivariate metabolomics models effectively differentiate these SADs from healthy controls (AUC > 0.7). Differential metabolites related to AA metabolism, including Val, Met, and Leu, highlight the dysregulation of Trp metabolism and its association with immune activity and inflammatory responses (Fernández-Ochoa et al. 2020).

At present, accumulating evidence shows that AA metabolisms are also closely related to the pathogenesis of LN. The development of LN is a multistage development process. Stimulated by various internal and external factors, the clearance mechanism of apoptotic cells is inhibited resulting in the continuous exposure of autoantigens to the immune system, thus activates the local immune response and inflammatory response and aggravate the injury of renal tubulointerstitium and blood vessels, resulting in impaired renal function and chronic disease progression in LN patients (Frangou et al. 2020). In addition, there is evidence that pathogenic anti-double-stranded DNA antibodies bind to renal intrinsic cells and induce apoptosis, autophagy, inflammation, and oxidative stress, thereby aggravating renal fibrosis damage in LN (Yung et al. 2020). The antibody-mediated inflammatory renal disease may be inhibited by AA metabolism and a protective autophagic response (Chaudhary et al. 2015). Increasing kidney Indoleamine-2,3-dioxygenase (IDO1) activity or treating with a general control nonderepressible 2 (GCN2) agonist protects antibody-mediated inflammatory renal disease mice from nephritic kidney damage (Chaudhary et al. 2015). As recently reported, GCN2 drives AA starvation-induced autophagy in vitro by inducing autophagy-related genes (B'chir et al. 2013). Therefore, IDO-GCN2 pathway may limit renal pathological changes caused by inflammatory and immune responses through driving AA starvation-induced autophagy in LN patients.

The present metabonomic analysis showed that the plasma His level was increased in patients with LN. His have anti-inflammatory and antioxidant effects based on their ability to scavenge free radicals and chelate bivalent metal ions (Babizhayev et al. 1994; Lee et al. 2005). The present study identified the expression of His in LN patients was increased, which is consistent with a previous study (Li et al. 2017). Related comprehensive metabolome studies have suggested that the plasma His level may reflect organic injury accumulation in LN patients and participate in the pathogenesis of disease (Iwasaki et al. 2023). In the literature, the reports on LN metabonomics are often inconsistent, which may be due to the heterogeneity of disease manifestations, differences in the characteristics of subjects, the use of different drugs, and other unexcluded confounding factors. There may be an unclear benign compensatory protection mechanism in the pathological state of LN that produces protective His from inhibiting the hyperactive oxidative stress response.

The plasma Lys level in the LN group was significantly lower than that in the NC and SLE groups. Some foreign studies have reported that the plasma Lys level is decreased

in SLE patients (Yan et al. 2016; Bengtsson et al. 2016). Lys is an essential AA that is involved in many important physiological processes as promoting human growth and metabolism; promoting protein absorption and fat oxidation; improving immunity and preventing osteoporosis (Flodin 1997). Reduced Lys in LN patients is due to various catabolic pathways, such as participating in the biosynthesis and metabolism of carnitine, which helps to reduce the level of oxidative stress (Pekala et al. 2011). Lys is relatively abundant in some animal protein-rich foods, and the changes of plasma AAs may be related to diet, lifestyle, environment, and genetic variation. Since this was not a strictly diet-controlled study, it was difficult to rule out the possibility that dietary differences may lead to changes in plasma AAs. However, all participants were non-vegetarians, which avoided the differences between vegetarians and non-vegetarians.

In the present study, we observed that the expression of plasma Trp in LN patients was significantly lower than that in normal and SLE subjects. Trp is an essential AA for the biosynthesis of key compounds, and its main metabolites include serotonin and kynurenine. The degradation of Trp is increased in SLE patients, which is significantly correlated with the high production of interferon-gamma and the disease activity of SLE (Muller et al. 2010; Pertovaara et al. 2007). High levels of dietary Trp exacerbates autoimmune phenotypes, whereas low levels of dietary Trp alleviates the disease in lupus prone mouse models (Choi et al. 2020). IDO is a rate-limiting enzyme responsible for Trp catabolism. IDO inhibitor treatment increases the autoantibody titer and accelerates the pathological damage of glomerulonephritis (Ravishankar et al. 2012). The Trp pathway may promote the pathogenesis of LN by activating the mTOR signaling pathway and affecting the metabolism of T cells, thus changing the immune state of the body or directly affecting the kidney cell function (Furie et al. 2020). Thus, plasma Trp and its metabolites may be used as potential predictors of renal injury in LN patients.

In the present study, the expression of Arg was increased in both LN and SLE patients compared to the NC group. Arg is a conditionally essential AA that is always nonessential in most mammalian healthy organisms, but Arg must be additionally supplemented after trauma or during illness (Barbul 1986; Heird 1998). Arg mainly comes from dietary supplements and protein turnover in human body, and degraded by nitric oxide synthase (NOS) and arginase (ARG). NOS catalyzes the conversion of Arg into nitric oxide (NO) and citrulline. ARG catalyzes the degradation of Arg into ornithine and urea (Morris 2007). At physiological concentrations, NO regulates renal hemodynamics as well as renin-angiotensin system balance and the tubuloglomerular feedback response (Baylis 2008). However, Arg may lead to the excessive production of NO, thus increasing the formation of peroxynitrite anions and hydroxyl radicals, which may promote the pathogenesis of LN (Peters et al. 2003, Baylis 2008 ; Popolo et al. 2014). Long-term dietary supplementation of Arg has been found to be associated with the functional decline of the kidneys and vasculature during aging (Huang et al. 2021). Therefore, increased plasma Arg may be detrimental in LN patients.

This study has several limitations. Firstly, patients with active SLE and LN often present with hematological manifestations (such as anemia or thrombocytopenia), hypoproteinemia, skin lesions, arthritis, and other complications. While we aimed to minimize confounding factors affecting metabolites, caution is needed when interpreting AA changes in SLE and LN patients, as factors like diet and inflammation may also influence metabolic pathways. Secondly, the sample size was relatively small, limiting the robustness and generalizability of the findings. Thirdly, as a single-center retrospective analysis, the results may not be broadly applicable. Additionally, we focused on targeted metabolomic profiling of only 20 AAs, incorporating both untargeted and targeted approaches could provide a clearer AA profile for LN patients. Our study aimed to explore the potential AA metabolic profile in LN patients and assess whether differential AAs could distinguish normal subjects, LN patients, and SLE patients without renal involvement. However, we did not investigate the specific mechanisms of abnormal AA metabolism in LN. To better understand LN progression related to His, Lys, and Trp levels, further prospective multi-center longitudinal studies and in vivo or in vitro interventions are needed.

Conclusion

In summary, this study provides valuable insights into the plasma AA metabolism profiles of LN patients, suggesting potential avenues for improved clinical practice. The proposed joint AA forecast models show encouraging promise in enhancing diagnostic accuracy for LN and lupus disease status, which may help inform patient selection and potentially reduce the financial burden of unnecessary renal biopsies. Looking ahead, exploring the combination of AA nutritional supplements with targeted biologics could represent a worthwhile direction for future research. This approach may hold the potential to optimize treatment outcomes while minimizing the side effects of conventional immunosuppressants for patients with SLE, LN, and other rheumatic diseases. Continued exploration in this field could ultimately contribute to better management strategies and improved patient experiences.

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Author contributions Z.G. and M.L. conceived and conducted the experiment, analyzed the experimental data and wrote manuscripts. D.L. participated in the execution of the experiment and review and revision the manuscript. C.Z., Q.Z. participated in the construction of the technical route of the experiment and was responsible for managing and coordinating the planning and execution of research activities. Q.Z. and Z.L. had the main supervision and leadership responsibility for the planning and implementation of research activities, and approval the final version. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Statement This study and the associated protocols for sample collection were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (the ethical approval number: NO. 2021-KY-0477-003). The research has been carried out in accordance with the World Medical Association Declaration of Helsinki, and informed consent was obtained from all individual participants included in this study.

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