Lipid metabolomic signature might predict subclinical atherosclerosis in patients with active rheumatoid arthritis

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Abstract

Objective

Although ^{1}H-nuclear magnetic resonance (NMR)-based lipid/metabolomics has been used to detect atherosclerosis, data regarding lipid/metabolomic signature in rheumatoid arthritis (RA)-related atherosclerosis are scarce. We aimed to identify the distinct lipid/metabolomic profiling and develop a prediction score model for RA patients with subclinical atherosclerosis (SA).

Methods

Serum levels of lipid metabolites were determined using ^{1}H-NMR-based lipid/metabolomics in 65 RA patients and 12 healthy controls (HCs). The occurrence of SA was defined as the presence of carotid plaques revealed in ultrasound images.

Results

*Compared with HC, RA patients had significantly higher levels of phenylalanine and glycoprotein acetyls (GlycA) and lower levels of leucine and isoleucine. RA patients with SA had significantly higher levels of phenylalanine, creatinine, and glycolysis_total and lower levels of total lipid in HDL(HDL_L) than RA patients without SA. The Lasso logistic regression analysis revealed that age, creatinine, HDL_L, and glycolysis_total were significant predictors for the presence of SA. The prediction scoring algorithm was built as (-0.657 + 0.011*Age + 0.004*Creatinine -0.120*HDL_L + 0.056*glycolysis-related measures), with AUC 0.90, sensitivity 83.3%, and specificity 87.2%. Serum phenylalanine levels were significantly decreased, and the levels of HDL_L and HDL_Particle were significantly increased in 20 RA patients, paralleling the decrease in disease activity score for 28-joints.*

Conclusion

With ^{1}H-NMR-based lipid/metabolomics, distinct profiling of lipid metabolites was identified between RA patients and HC or between RA patients with and without SA. We further developed a scoring model based on lipid/metabolomics profiling for predicting RA-associated SA.

Key words

lipoproteins, metabolomics, inflammation, biomarkers, rheumatoid arthritis, subclinical atherosclerosis

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Competing interests: none declared.

Introduction

Atherosclerosis, a chronic inflammatory process, is characterised by vascular plaque build-up and associated with an elevated risk of atherosclerotic cardiovascular disease (ASCVD) (1). Rheumatoid arthritis (RA) is a chronic inflammatory articular disease complicated by a high ASCVD burden (2), which would be explained by traditional risk factors and systemic inflammation in this disease (3, 4). Besides, the common use of disease-modifying anti-rheumatic drugs (DMARDs) may lead to a major remodelling in lipid metabolism that can be detected by lipid/metabolomics (5).

Recent advances in lipid/metabolomics could characterise lipoprotein subfractions and quantify the lipid species and metabolites from blood samples or vascular plaques (6, 7). ^{1}H-nuclear magnetic resonance (NMR)-based lipid/metabolomics has been used for diagnosis of subclinical atherosclerosis in young adults, prediction of 5-year mortality among a general population, or follow-up of the response to treatment, such as metabolic diseases (8). Therefore, the lipid/metabolomic signature would be a promising biomarker for ASCVD and may provide new therapeutic targets (8). Coelewij *et al*. recently demonstrates that serum metabolites are promising biomarkers to predict subclinical atherosclerosis (SA) in patients with systemic lupus erythematosus (SLE) (9). Wang *et al*. also revealed that serum lipid metabolites could be biomarker to discriminate SLE from HC and as SLE activity indicators (10). However, the association between serum lipid/metabolomic signature and RA-related subclinical atherosclerosis has yet to be explored.

Ultrasonography (US) of the carotid artery is a non-invasive method for identifying atherosclerotic plaques, which reflect severe SA and may predict the emergence of ASCVD (11-13). A recent study also revealed that the evidence of carotid plaque could predict the development of ASCVD in RA patients (13). Therefore, the presence of carotid plaques could be a gold standard for identifying the emergence of SA in patients at high risk of ASCVD (11-13). The European League Against Rheumatism (EULAR) guidelines recommend using carotid US to screen for asymptomatic atherosclerotic plaque as part of the ASCVD risk assessment in RA patients (14).

In this pilot study, we used ^{1}H-NMR-based lipid/metabolomics to identify the significantly differential lipid metabolites between RA patients and healthy control (HC) subjects, or between RA patients with and without SA, defined by the presence of carotid plaques. Our major aim was to develop a scoring model for predicting RA-associated SA, and we finally established one based on a combination of demographic data with lipid/metabolomics profiling.

Material and methods

Study population

In this prospective and single-centre study, 65 patients who met the 2010 revised criteria of the American College of Rheumatology for RA and were in active disease status were consecutively enrolled (15). Disease activity was assessed by the 28-joint disease activity score (DAS28)-erythrocyte sedimentation rate (ESR) (DAS28-ESR) (16), and active status was defined as DAS28 X3.2 (17). After baseline investigation for lipid/metabolomics, 59 biologic-naïve, conventional synthetic DMARDs (csDMARDs) treated, and active RA patients started biologic DMARDs (bDMARDs) or Janus kinase inhibitors (JAKi) therapy according to the guidelines (18). The other six patients continued with csDMARDs therapy alone. Twenty patients were available for evaluation of circulating lipid metabolites after 6–12 months of treatment with tofacitinib (one of JAKi, n=11) or tocilizumab (interleukin-6 receptor inhibitor, n=9). Patients with a recent history (*i.e*. within one year before enrollment) of coronary heart disease or ischaemic stroke were excluded. Twelve sex-matched healthy volunteers who had no rheumatic disease were enrolled as healthy controls (HC). The China Medical University & Hospital Research Ethics Center approved this study (CMUH109-REC3-161, approval date 13 December 2020), and

each participant's written consent was obtained according to the Declaration of Helsinki.

Determination of lipid profiles and atherogenic index (AI)

All the blood samples were collected from the participants in the early morning after an overnight fast for 12 hours. Plasma levels of total cholesterol (TC), triglyceride, high-density lipoprotein (HDL) cholesterol (HDL-c), and low-density lipoprotein (LDL) cholesterol (LDL-c) were measured using enzymatic methods with a chemistry analyzer AU5800 (Beckman Coulter, California, USA) according to the manufacturer's instructions. The AI was calculated as the ratio of TC/HDL-c.

Determination of serum lipid metabolites by ^{1}H-NMR lipid/metabolomics

All serum samples were analysed using the ^{1}H-NMR lipid/metabolomics (Nightingale Health, Helsinki, Finland) as in previous studies (7, 8). In detail, 100 µl serum and phosphate buffer (prepared with 5.5 mM sodium 3-trimethylsilyl [2,2,3,3-d4] propionate, 0.075 M Na_2HPO_4*$7H_2O$, 5 mL NaN_3 (4%) adjusted to pH 7.4 with 1 M HCl) mixed in an Eppendorf tube. The samples were transferred to a 3 mm NMR tube (Bruker Match system) and measured at 310 K in Bruker Avance III NMR spectrometers operating at 600.13 MHz equipped with a maximum gradient strength of 53 G/cm. Each sample tube was equilibrated at 310 K for 5 mins before acquisition. Each data set was automatically processed using a line broadening of 1Hz, with the Nuclear Overhauser effect spectroscopy (NOESY) data aligned to the alanine signal at 1.49 ppm. Thought Bayesian modelling to convert spectrometers signal to absolute concentrations of the metabolic measures, as described previously (18). The metabolomics platform provides 250 metabolites (Supplementary Table S1) from the following substance classes: lipoprotein subclasses, lipoprotein particle sizes, apolipoproteins, fatty acids and saturation, cholesterol, glycerides and phospholipids, amino acids, ketone bodies, inflammation, glycolysis-related metabolites, and fluid balance. Because some single metabolites were too fragmented in analysis and lacked the basis for comparison with other literature, we integrated the same type of metabolites into metabolites classes according to the category (Suppl. Table S2) as reported in a previous study (8). All metabolites have been turned into a total of 90 metabolite classes. (The list of metabolite class names and their descriptions was illustrated in Supplementary Table S2)

Construction of prediction model

All samples were analysed for lipid/metabolomic signature using R software as described previously (9). We performed the sparse partial least squares discriminant analysis (sPLS-DA) of the R package mixOmics on NMR metabolomic data to compare RA with SA and RA without SA. The chosen number of the components of the sPLS-DA result was determined by the lowest overall estimation error rate of 21.1%. Those components were further used in the Lasso logistic model. We then used non-normalised metabolomics data to construct a predictive model and performed a Lasso logistic model with the training set and test set at an 8:2 ratio. The performance of the Lasso logistic model was assessed by the receiver operating curve (ROC), and the area under the curve (AUC) was calculated. A two-sided p-value <0.05 was considered significant.

Ultrasound vascular imaging of carotid arteries

Ultrasound vascular imaging of the carotid arteries was used to detect the presence of focal plaque in the longitudinal view of the extracranial carotid tree, including the far wall of the common carotid arteries along a 10 mm section of the artery proximal to the carotid bifurcation, and the proximal 15 mm-long segment of the internal and external carotid arteries. Carotid plaque was defined as a localised thickening >1.2 mm that protrudes into the arterial lumen when both the far and near walls had sharp edges, or when the protrusion was at least 2-fold of the nearby corresponding carotid-intima media thickness, according to recommendations (11-13). Herein, SA is defined by the presence of carotid plaque, similar to previous reports (12, 13) but with a minor modification (their criteria is the protrusions more than 1.5 mm into the lumen).

Statistical analysis

The total number of enrolled subjects sufficient for the statistic power of 80% and alpha error of 0.05 for lipid/metabolomics investigation was calculated to be 58–75 (47 in the RA without SA group, 18 in the RA with SA group, and 12 healthy control participants). Therefore, we enrolled a total of 77 participants. The data were presented as the mean ± standard deviation (SD) or the median (interquartile range, IQR). We used the independent student's t test for a two-group comparison, and one-way ANOVA test with Bonferroni as post-test for comparing three groups. The correlation coefficient was obtained through the non-parametric Spearman's rank correlation test. The Wilcoxon matched-pairs signed-rank test was used in RA patients before and after treatment. A two-sided p-value <0.05 was considered statistically significant. The plots and statistical analysis were performed using IBM SPSS Statistics v. 25 (IBM, New York, USA) and GraphPad Prism v. 9.3 (GraphPad Software, San Diego, USA).

Results

Patient characteristics

Based on the findings of carotid ultrasonography, 18 (27.7%) of RA patients had SA defined by the presence of carotid plaque (Table I). RA patients with SA were older than patients without SA or HC participants (each p<0.05). Significantly higher ESR values and proportion of statins use were observed in RA patients with SA compared with those without SA. There were no significant differences in the proportion of females, disease duration, DAS28 scores, positivity for rheumatoid factor (RF) or anti-citrullinated peptide antibodies (ACPA), plasma lipid profile, the prescribed other medications, or comorbidities between RA patients with and without SA. Besides, significantly

Table I. Demographic data and laboratory findings in RA patients with or without SA and healthy controls[a].

	RA with SA (n=18)	RA without SA (n=47)	Healthy controls (n=12)
Age at study entry, years	69.6 (6.7) [b,c]	56.5 (13.1) [c]	50.8 (13.8)
Gender (female), n (%)	11 (61.1%)	39 (83.0%)	8 (66.7%)
RA duration, months	90.0 (27.6)	81.2 (26.6)	NA
BMI, kg/m^2	24.6 (3.6)	23.7 (4.3)	23.8 (1.9)
RF positivity, n (%)	14 (77.8%)	31 (66.0%)	NA
ACPA positivity, n (%)	14 (77.8%)	32 (68.1%)	NA
ESR, mm/1st hr,	35.9 (25.7) [b]	23.2 (16.0)	NA
CRP, mg/dL	3.41 (5.69)	1.65 (2.82)	NA
Tender joint count	10.3 (6.4)	13.0 (8.4)	NA
Swollen joint count	8.8 (4.6)	9.6 (5.1)	NA
DAS28 at study entry	6.52 (1.20)	6.25 (1.05)	NA
Lipid profile at study entry			
TC, mg/dl	185.2 (34.5)	203.6 (46.6)	179.8 (45.6)
HDL-c, mg/dl	53.9 (12.3) [b]	63.4 (16.0)	57.5 (15.5)
Triglyceride, mg/dl	120.4 (58.7)	96.0 (68.0)	86.0 (55.6)
LDL-c, mg/dl	113.1 (33.2)	117.2 (38.9)	115.0 (39.1)
Atherogenic index	3.6 (1.1)	3.4 (1.1)	3.4 (0.7)
Corticosteroid dose, mg/day	4.3 (1.9)	4.5 (1.6)	NA
Statins	8 (44.4%) [b]	8 (17.0)	NA
csDMARDs at study entry, n (%)			
Methotrexate	12 (66.7%)	28 (59.6%)	NA
Hydroxychloroquine	8 (44.4%)	19 (40.4%)	NA
Sulfasalazine	5 (27.8%)	11 (23.4%)	NA
Biologics used after baseline investigation, n (%)			
TNF-α inhibitors	4 (22.2%)	8 (17.0%)	NA
Abatacept	1 (5.6%)	5 (10.6%)	NA
Rituximab	0 (0.0%)	1 (2.1%)	NA
Tocilizumab	5 (27.8%)	11 (23.4%)	NA
JAKi (Tofacitinib)	6 (33.3%)	18 (38.3%)	NA
Comorbidities, n (%)			
Hypertension	8 (44.4%) [c]	18 (38.3%) [c]	0 (0.0%)
Diabetes mellitus	2 (11.1%)	3 (6.4%)	0 (0.0%)
Current smoker, n (%)	3 (16.7%)	4 (8.5%)	1 (8.3%)

[a] Continuous values are presented as the mean (S.D.), and binomial values presented as the number (%).

[b] $p<0.05$, *vs*. RA without SA, [c] $p<0.05$, *vs*. HC, as determined by one way ANOVA test and Bonferroni as post-test (continuous value) or Fisher's test (binomial value).

ACPA: anti-citrullinated peptide antibodies; Atherogenic index calculated by TC /HDL-c; BMI: Body Mass Index; CRP: C-reactive protein; csDMARDs: conventional synthetic disease-modifying anti-rheumatic drugs; DAS28: Disease Activity Score for 28-joints; ESR: erythrocyte sedimentation rate; HDL-c: High-density lipoprotein cholesterol; JAKi: Janus kinase inhibitor; LDL-c: low-density lipoprotein cholesterol; NA: not applicable; RA: rheumatoid arthritis; RF: rheumatoid factor; SA: subclinical atherosclerosis; TC: total cholesterol; TNF: tumour necrosis factor.

higher levels of triglyceride and atherogenic index at baseline were found in 6 patients receiving csDMARDs alone compared to those receiving bDMARDs or JAKi (Suppl. Table S3). There was no significant difference in the proportion of females, body mass index, or plasma lipid profile between RA patients and HC participants.

Metabolomic signature between RA and HC

We classified the individuals as RA or HC based on molecular profiling using the sPLS-DA method. It demonstrated that the mapping of PC-2 and PC-1 could significantly discriminate the RA group from the HC group (Fig. 1A). With the discriminating capability, the metabolomic classes were formed and illustrated in Fig. 1B; the top five discriminating metabolites were higher levels of glycoprotein acetyls (GlycA) and phenylalanine and lower levels of leucine, isoleucine, and HDL_S_particle_prop (small HDL particle proportion of all HDL) in RA patients compared to HC (Fig. 1C-G).

Metabolomic signature between RA patients with and without SA

As shown in Fig. 2A, the sPLS-DA analysis revealed that the mapping of PC-2 and PC-1 could significantly discriminate between RA patients with SA [RA-SA(+)] and without SA [RA-SA(-)]. As illustrated in Figure 2B, the top five significantly differential metabolites are higher levels of phenylalanine, creatinine, and glycolysis_total (glycolysis-related measures, including glucose, lactate, pyruvate, citrate, and glycerol), and lower HDL_P (HDL_particle) and HDL_L (total lipids in HDL) in RA-SA (+) patients compared to RA-SA (-) patients (Fig. 2C-G).

Establishment of prediction score model for the presence of SA in RA patients

To reach the best accuracy, we build up the lipid/metabolomic signature-based prediction model using Lasso logistic regression for the presence of SA, with the regression coefficient used as the weight for age and each significantly differential metabolite in the scoring system: the SA prediction score = (-0.657 + 0.011*Age + 0.004*Creatinine - 0.120*HDL_L + 0.056*Glycolysis_total) (Fig. 3A). The ROC analysis of this score for predicting SA showed a high AUC of 0.90 ($p<0.001$) at the cutoff value of 0.3469, with the sensitivity of 83.3% and specificity of 87.2% (Fig. 3B).

Change of serum levels of the significantly differential metabolites between RA patients with and without SA after treatment with tofacitinib or tocilizumab

Twenty RA patients were available for blood investigation for lipid metabolites at both the beginning and the end of the 6-12 months of treatment with tofacitinib (n=11) or tocilizumab (n=9). The DAS28 scores were significantly decreased after effective treatment (median 6.78, IQR 6.19-7.19 *vs*. 3.77, IQR 3.57–4.38, $p<0.001$, Fig. 4A). Besides, serum levels of phenylalanine were significantly decreased (0.088 mmol/L, IQR 0.073–0.099 mmol/L *vs*. 0.076 mmol/L, IQR 0.068–0.089 mmol/L, $p<0.05$, Fig. 4B), while the levels of

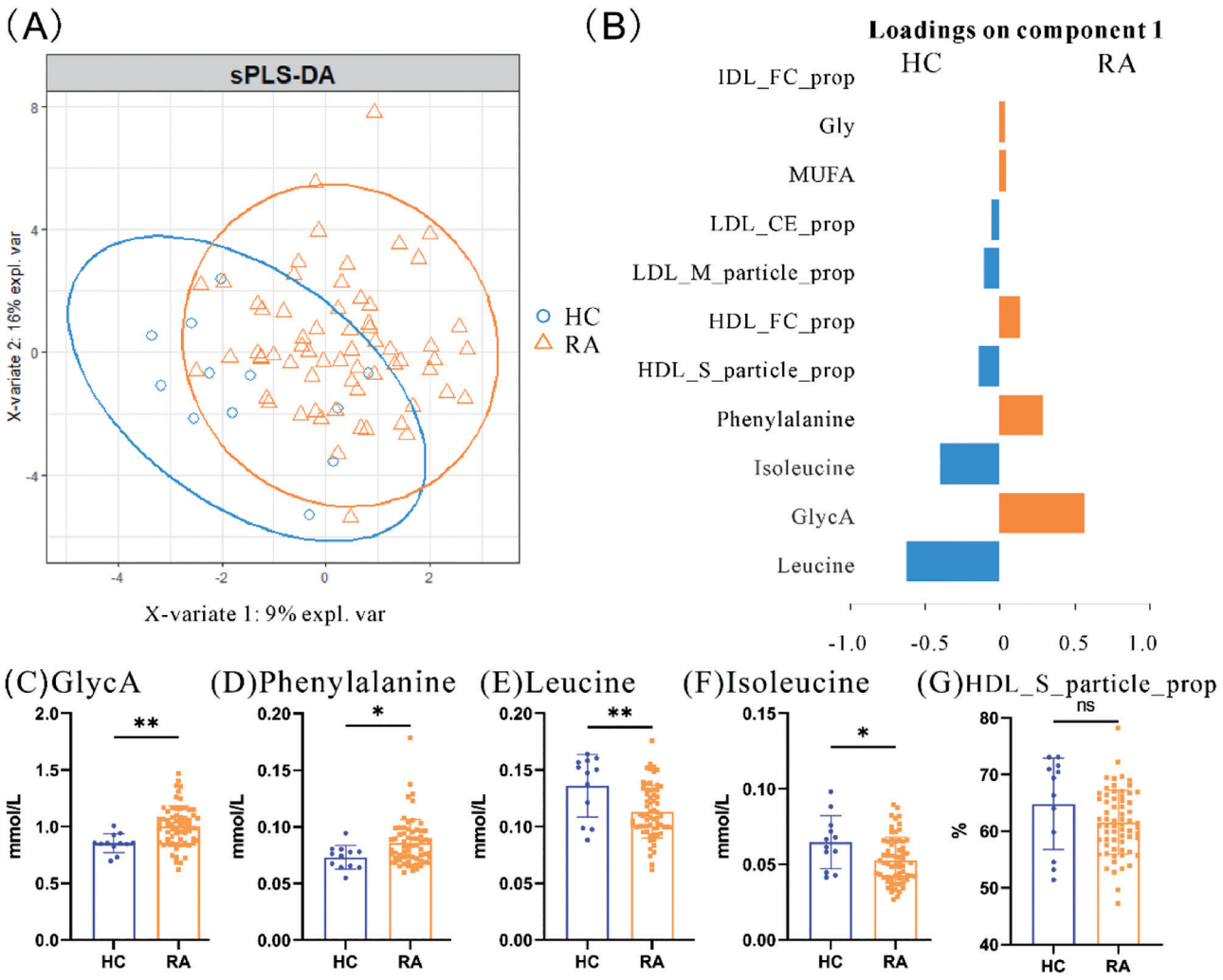

Fig. 1. The sPLS-DA could differentiate rheumatoid arthritis (RA) patients from healthy control (HC).
(**A**) The sPLS-DA plot of lipidomic profile between RA patients and HC. (**B**) Top eleven metabolites included in the sPLS-DA were plotted based on their loading value. Comparison of (**C**) GlycA, (D) phenylalanine, (**E**) leucine, (**F**) isoleucine, (**G**) HDL_S_particle_prop (small HDL particle proportion) in the top five loading value of sPLS-DA between RA and HC. Bars and error bars indicate mean and standard deviation, respectively.
* $p<0.05$, ** $p<0.005$, was determined by the student' s t-test.

HDL_L and HDL_P were significantly increased (3.204 mmol/L, IQR 2.957–3.676 mmol/L v *vs*. 3.678 mmol/L, IQR 3.094–3.850 mmol/L, $p<0.005$; 0.016mmol/L, IQR 0.015-0.019 mmol/L *vs*. 0.019 mmol/L, IQR 0.016–0.020 mmol/L, $p<0.001$; respectively, Fig. 4C-D). However, there was no significant change of glycolysis_total levels (7.528 mmol/L, IQR 7.123–8.056 mmol/L *vs*. 7.644 mmol/L, IQR 7.236–7.890 mmol/L, Fig. 4E) or creatinine levels (58.04mmol/L, IQR 49.87–69.95 mmol/L *vs*. 58.30 mmol/L, IQR 52.27–81.39 mmol/L, Fig. 4F).

Discussion

The lipid metabolomic signature has recently been reported to be a promising biomarker for ASCVD (8, 9). We would like to apply it to the investigation of SA in RA patients. First, we revealed different lipid/metabolomics signatures between RA patients and HC subjects. Furthermore, RA patients with SA, defined by the existence of carotid plaque, had a discriminative metabolomics signature from those without SA. The Lasso logistic regression analysis revealed that age, creatinine, HDL_L, and glycolysis_total were the significant predictors for SA in RA patients. We then developed a prediction scoring formula based on these significantly differential variables. At the cutoff value of >0.3469, this prediction score model showed a high AUC of 0.90, with high sensitivity of 83.3% and specificity of 87.2%, for predicting RA-associated SA. Besides, the serum levels of phenylalanine, HDL_L, and HDL_P changed significantly after effective therapy, paralleling the decrease in DAS28 scores. Our findings suggest that these lipid metabolites might be involved in the pathogenesis of RA-related SA and potentially serve as predictive markers.

RA patients appear at a significantly higher risk of ASCVD or SA than HC subjects (2, 19). Similar to previous studies (20), we found that the lipid/metabolomics signature differed between our RA patients and HC. Since RA-related inflammation may cause a change in lipid profiles (3, 4, 21), our RA patients had significantly higher

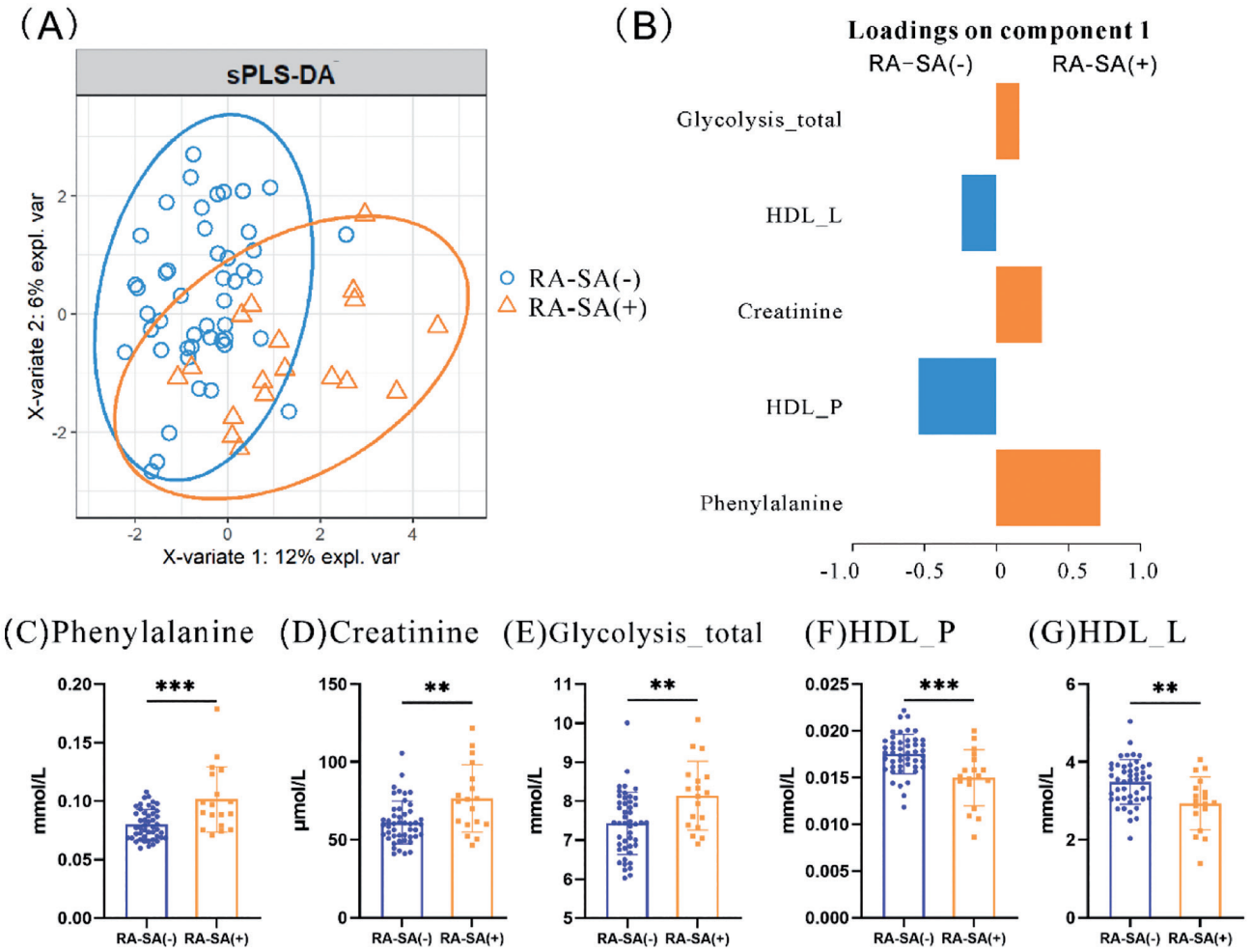

Fig. 2. The sPLS-DA could differentiate RA patients with subclinical atherosclerosis (SA) from those without SA.
(**A**) The sPLS-DA plot of lipid/metabolomics profile between RA patients with and without SA. (**B**) Top five metabolites included in the sPLS-DA were plotted based on their loading value. Comparison of serum levels of (**C**) phenylalanine, (**D**) creatinine, (**E**) glycolysis_toal (glycolysis-related measures), (**F**) HDL_P (HDL particle), and (**G**) HDL_L (total lipids in HDL) between RA with SA [RA-SA (+)] and without SA [RA-SA(-)]. Bars and error bars indicate mean and standard deviation, respectively.
* $p<0.05$, ** $p<0.005$, *** $p<0.001$, was determined by the student' s t-test.

(A)

Lasso Logsitic Regression	
Features Included	Beta Coefficient
(Intercept)	-0.657
*Age	0.011
Gender	0.000
*Creatinine	0.004
*HDL_L	-0.120
*Glycolysis_total	0.056
Phe	0.000
HDL_P	0.000

(B)

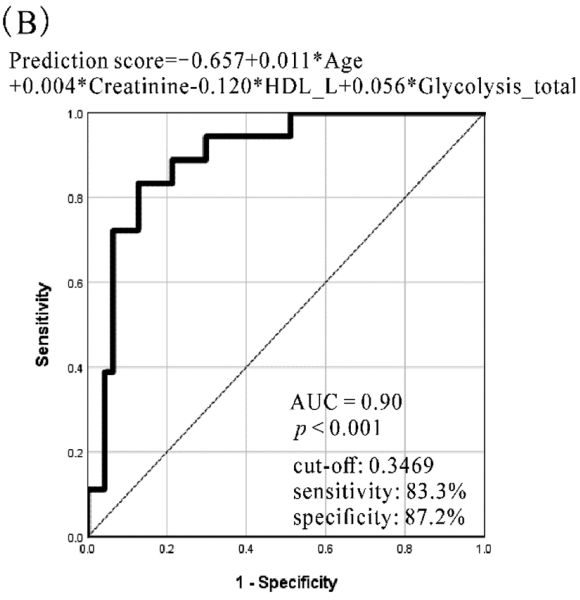

Fig. 3. The Lasso logistic regression analysis and ROC analysis of predictive formula.
(**A**) Lasso logistic regression analysis results with beta coefficient. (**B**) Establishment of the prediction score for the presence of SA. Receiver operating characteristic (ROC) curve analysis of the SA prediction score derived from age and three differential metabolites, creatinine, HDL_L,and glycolysis_total. Area under the curve (AUC)= 0.90 at the cut-off score 0.3469, with sensitivity of 83.3% and specificity of 87.2%.

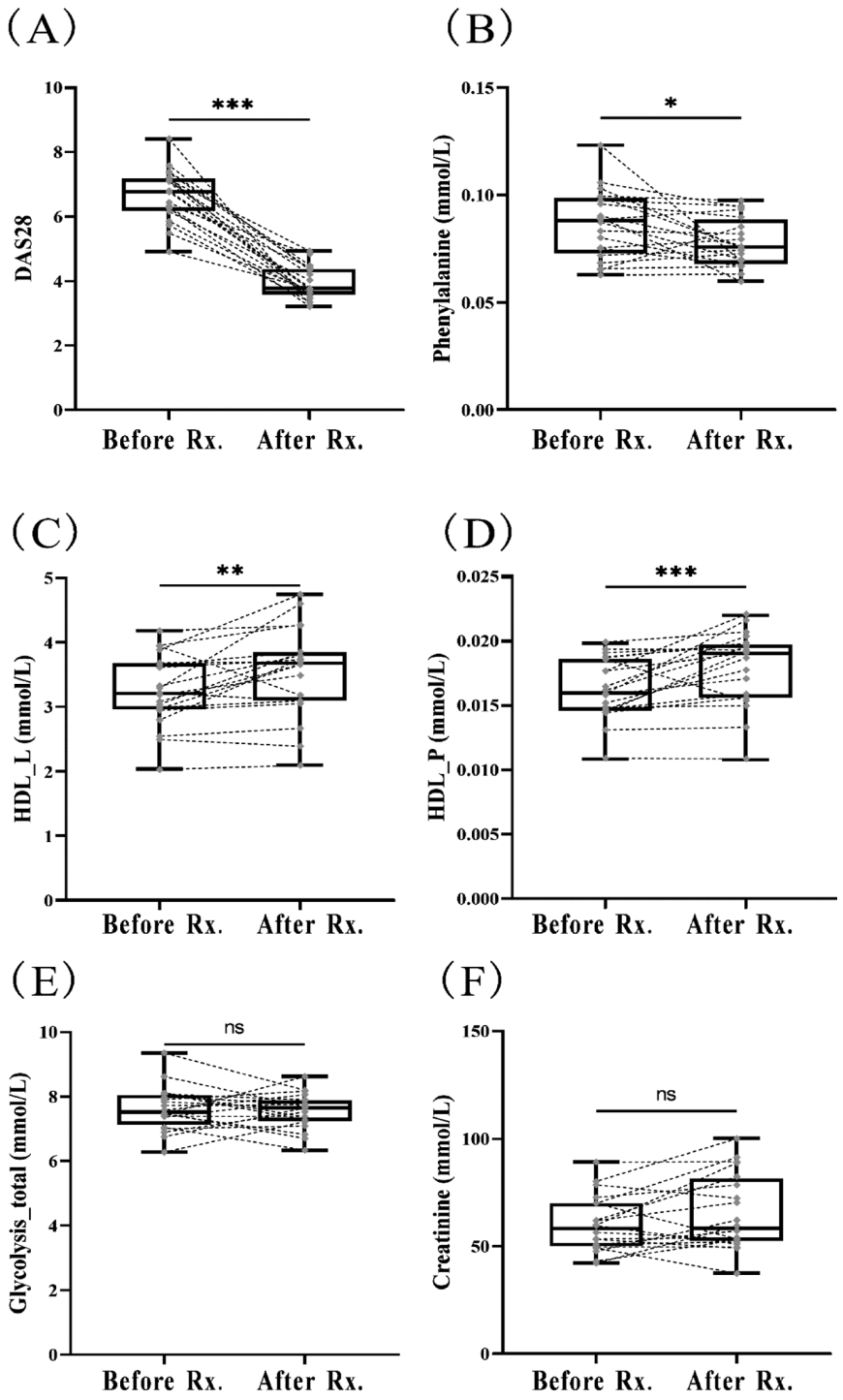

Fig. 4. The change of disease activity score and lipid metabolites after treatment in 20 RA patients. The change of (**A**) DAS28, (**B**) phenylalanine, (**C**) HDL_L, (**D**) HDL_P, (**E**) glycolysis_total, and (**F**) creatinine in patients after treatment.
* $p<0.05$, ** $p<0.005$, *** $p<0.001$, was determined by the Wilcoxon signed rank test.

levels of inflammation-related metabolites such as phenylalanine and GlycA (20, 22, 23). High phenylalanine levels were robustly associated with a high risk of coronary artery disease (24). The significantly lower levels of leucine and isoleucine in our RA patients compared with HC subjects support the findings that inflamed tissues would consume leucine and isoleucine in RA (25-27). Because HDL-c possesses anti-inflammatory and anti-thrombotic effects (28), it is a well-known protective factor for ASCVD. Accordingly, our RA patients at an elevated ASCVD risk had significantly lower levels of HDL_S_particle_prop compared to HC.

Since carotid plaques are significantly associated with future ASCVD risk and poor outcomes in RA patients (29, 30), the detectable carotid plaques would be a good indicator of SA (14, 31, 32). By this definition, we revealed a distinct lipid/metabolomics signature, in terms of phenylalanine, creatinine, glycolysis_total, HDL_P, and HDL_L, in RA patients with SA. Previous studies have used NMR-based metabolomics to reveal that high phenylalanine levels were a significant biomarker for future ASCVD events, with a hazard ratio of 1.18 (34). Resonated with these findings (24, 33), our RA patients with SA had significantly higher levels of phenylalanine than those without SA. Creatinine is a traditional ASCVD risk factor (34), and serum creatinine levels significant positively correlated with carotid intima-media thickness in RA (35). In our study, serum levels of glycolysis_total (glycolysis-related measures), including glucose, lactate, pyruvate, citrate, and glycerol, were significantly higher in RA with SA compared to those without SA. It supports previous findings that elevated serum glucose, lactate, and citrate levels were associated with greater ASCVD risk or poor prognosis (36-38). Given the link between a lower HDL-c level and higher ASCVD risk shown in several large clinical studies (39-41), the levels of HDL_P and HDL_L would negatively predict RA-related SA.

To achieve high accuracy, we used the Lasso logistic regression analysis to build a model for predicting SA in RA. The result showed that age, creatinine, HDL_L, and glycolysis_total were significant predictors for SA in RA. Age is a well-known predictor of ASCVD risk in inflammatory arthritis (12, 43). Gathering the observations from previous studies and ours, we combine age with three significant metabolites into a scoring model for predicting RA-associated SA, with a high AUC, sensitivity, and specificity. However, the external validity of our preliminary results needs further confirmation.

Interestingly, serum phenylalanine (an atherogenic metabolite) levels were

significantly decreased in twenty RA patients receiving IL-6R inhibitor or JAKi therapies. In contrast, serum levels of HDL_L and HDL_P, both atherogenesis-protective metabolites, were significantly increased after effective therapy in RA patients. Our findings suggest that these circulating lipid metabolites are related to SA and may be potential RA activity indicators. Given the small sample size and short follow-up period in our study, there is a need for future studies with a larger cohort recruiting more RA patients treated with bDMARDs or JAKi.

Despite the novel findings in this pilot study, there were still some limitations. First, we did not limit patients' diet or dietary patterns that may change metabolite levels (44). Second, the baseline or concomitant corticosteroids or csDMARD treatment may affect lipid metabolites (45-47). Although statins have been shown to affect lipid metabolites (48), we did not evaluate the relation between lipid metabolite levels and statin therapy. The RA cohort size (n=65) and the number of RA patients with SA may be too small to draw a definitive conclusion regarding the optimal cut-off level of the scores for predicting the occurrence of SA. Given no available blood samples for investigating the change of lipid metabolites at both before and after treatment with other biologics such as tumour necrosis factor -α inhibitors, we do not evaluate the effects of the different therapeutics on lipid metabolites. A long-term study of a larger group of RA patients is needed to confirm our findings.

In conclusion, we demonstrated different lipid/metabolomics signatures between RA and HC or between RA patients with and without SA. We also established a SA prediction scoring algorithm based on age and significantly differential metabolites, including creatinine, HDL_L, and glycolysis_total. Hopefully, these findings might aid in the early detection of RA-associated SA and the stratification of ASCVD risk. Further investigations are needed to explore the mechanism behind the link between differential lipid/metabolomics profiling and RA-associated atherogenesis.

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