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Differences in the serum metabolome and lipidome identify potential biomarkers for seronegative rheumatoid arthritis versus psoriatic arthritis

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ABSTRACT

Objectives The differential diagnosis of seronegative rheumatoid arthritis (negRA) and psoriasis arthritis (PsA) is often difficult due to the similarity of symptoms and the unavailability of reliable clinical markers. Since chronic inflammation induces major changes in the serum metabolome and lipidome, we tested whether differences in serum metabolites and lipids could aid in improving the differential diagnosis of these diseases.

Methods Sera from negRA and PsA patients with established diagnosis were collected to build a biomarker-discovery cohort and a blinded validation cohort. Samples were analysed by proton nuclear magnetic resonance. Metabolite concentrations were calculated from the spectra and used to select the variables to build a multivariate diagnostic model.

Results Univariate analysis demonstrated differences in serological concentrations of amino acids: alanine, threonine, leucine, phenylalanine and valine; organic compounds: acetate, creatine, lactate and choline; and lipid ratios L3/L1, L5/L1 and L6/L1, but yielded area under the curve (AUC) values lower than 70%, indicating poor specificity and sensitivity. A multivariate diagnostic model that included age, gender, the concentrations of alanine, succinate and creatine phosphate and the lipid ratios L2/L1, L5/L1 and L6/L1 improved the sensitivity and specificity of the diagnosis with an AUC of 84.5%. Using this biomarker model, 71% of patients from a blinded validation cohort were correctly classified.

Conclusions PsA and negRA have distinct serum metabolomic and lipidomic signatures that can be used as biomarkers to discriminate between them. After validation in larger multiethnic cohorts this diagnostic model may become a valuable tool for a definite diagnosis of negRA or PsA patients.

Key messages

What is already known about this subject?

- Clinical symptoms of (seronegative) rheumatoid arthritis (RA) and psoriatic arthritis (PsA) can be similar. Biomarkers for a correct diagnosis do not exist. Since immunosuppressive drugs have different therapeutic effects on both diseases, a correct diagnosis is important for the success of treatment.

What does this study add?

- The study presents evidence that chronic inflammatory diseases with similar clinical symptoms have significant differences in their metabolomes and lipidomes at systemic level.
- The study identifies novel biomarkers for the differential diagnosis of seronegative RA and PsA.

How might this impact on clinical practice or future developments?

- Better and earlier attribution of patients with arthritis to the correct diagnosis, which will help a faster correct choice of drugs.
- The expansion of nuclear magnetic resonance-based metabolomic and lipidomic analyses to other cohorts of clinically and demographically well-characterised patients with chronic autoimmune diseases may unveil new biomarkers to improve differential diagnosis, therapy response or disease relapses.

INTRODUCTION

The diagnosis of rheumatoid arthritis (RA) is mostly based on clinical symptoms and the serological positivity of rheumatoid factor (RF) and/or anticitrullinated peptide antibodies (anti-CCPs), whereas for psoriasis arthritis (PsA), only clinical and imaging features help in diagnosing the disease. Although most patients with RA are seropositive for RF and/or anti-CCP, in about 15%–20% of cases, the levels of RF and anti-CCP are not elevated, and since the symptoms between RA and PsA can be very similar, making a differential diagnosis between seronegative RA (negRA) and PsA is often difficult. Since the therapeutic strategies for the two diseases are different, early recognition and correct choice of treatment are essential to attain remission or low disease activity and to prevent, or at least to limit, joint damage as well as systemic manifestations.[1–7] Therefore, innovative tools for a reliable diagnosis of negRA versus PsA are needed.

In patients with chronic inflammatory diseases, an altered action of cytokines and other proinflammatory effector molecules added to a prolonged intake of immunomodulatory drugs leads to major remodelling in cellular and tissue metabolism. Such metabolic modifications also have a systemic impact that can be monitored by analysing the changes in the metabolome of biofluids. Assessing several metabolites simultaneously can potentially locate differences between disease profiles, thereby allowing the identification of potential

biomarkers and the discovery of altered metabolic pathways. ^{1}H nuclear magnetic resonance (NMR)-based metabolomic studies of serum, urine and synovial fluid obtained from patients with chronic arthritis have been used for diagnostic, prognostic and following the response to treatment. The development of RA in patients with early arthritis has been associated with increased serum levels of certain metabolites that correlated with the C reactive protein (CRP) titre.[8] High serum levels of lactate, acetylated glycoprotein and cholesterol differentiated healthy individuals from patients with RA regardless of anti-tumor necrosis factor α (TNF) therapy.[9] The urine metabolome from anti-TNF-treated patients with RA identified high levels of histamine, glutamine, thymine, creatinine and xanthine as predictors of a good response to TNF-α blockade.[10] An effective response to methotrexate (MTX) in patients with RA appears to be linked to elevated serum levels of uric acid, taurine, histidine, hypoxanthine and methionine.[11] However, a comparison of the aromatic, sugar and aliphatic regions in the ^{1}H NMR spectra of synovial fluid samples could not distinguish groups of patients with different types of arthritis.[12]

In all the studies comparing the metabolome of patients with RA to the metabolome of other patients with chronic inflammatory arthritis, there is no separate analysis of the negRA group, eventually due to the small size of the studied RA cohorts. Therefore, the potential of using metabolomic and lipidomic profiling to improve the differential diagnosis of PsA and negRA remains largely unexplored. Hence, we carried out ^{1}H NMR-based metabolomic and lipidomic analysis of serum samples from a large cohort of PsA and negRA patients, followed by a validation cohort analysis in order to identify and confirm serum metabolome-based biomarkers as a diagnostic multivariate model for these two pathologies.

PATIENTS AND METHODS

A detailed description of the patient selection, the experimental and statistical methods can be found in the online supplementary materials file 1.

Serum samples were collected from 49 patients with negRA and 73 with PsA at the Division of Rheumatology outpatient clinic of the University Hospital Heidelberg. Clinical and demographic characteristics of the cohort are summarised in table 1.

Study approval and patient and public involvement

Besides their voluntary participation in donating samples patients had no further involvement in the planning or execution of this study.

NMR spectroscopic analysis and metabolite identification

Metabolic analysis of the serum samples was carried out on a Bruker 600MHz NMR spectrometer following previously described procedures.[13] Metabolite identification was performed using the resonance assignments, chemical shifts and coupling patterns published for human serum samples.[14 15] Further details on the NMR analysis can be found in the online supplementary materials file 1.

RESULTS

Metabolomic and lipidomic profile of blood samples from negRA and PsA patients

In the ^{1}H single-pulse NMR spectrum, peaks from both small molecules and macromolecules are observed, resulting in an uneven baseline and the overlap of the signals originating from different compounds. Nonetheless, due to their characteristic spectral profiles, it is possible to use ^{1}H NMR to identify and quantify lipids in the serum (figure 1A). By suppressing the broad signals from lipids and proteins, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence allows the peaks from low-molecular weight compounds to not be overshadowed by the signals arising from macromolecules. Since they are characterised by sharp signals and a well-defined baseline, CPMG NMR spectra allow better identification and analysis of signals arising from small molecules (figure 1B).

Table 1 Clinical and demographic data of the study participants

	negRA (n=49)	PsA (n=73)	P value
Female:Male	39:10	29:44	<0.0001 (χ^2)
Age (minimum–maximum in years)	64.2 (32–83)	56.2 (30–78)	0.003
Disease duration (minimum–maximum in years)	11.6 (1–41)	9.0 (0–24)	0.042
DAS28-CRP (minimum-maximum)	2.6 (0–6.2)	2.3 (0.96–4.09)	*0.093*
% active (DAS28 >3.2)	30.6%	28.8%	
% remission (DAS28 <2.6)	55.1%	57.5%	
CRP (±SD) (mg/L)	5.7±7.4	6.7±13.8	*0.642*
Rheumatoid factor positive	None	None	
Anti-CCP positive	None	None	
MTX (±SD) (mg)*	13.2±4.8	14.1±3.2	*0.368*
% from total patients	44.9%	50.7%	*0.531* (χ^2)
Glucocorticoid (±SD) (mg)	6.2±5.0	5.6±3.5	*0.651*
% from total patients	38.8%	34.2%	*0.610* (χ^2)
Leflunomide (±SD) (mg)	15.8±4.7	13.6±4.5	*0.266*
% from total	24.5%	15.1%	*0.192* (χ^2)
On immunotherapy	13	35	0.018 (χ^2)
Anti-TNF	15.1%	27.8%	
Anti-IL-6R	3.8%	0%	
Anti-CTLA-4	1.9%	0%	
Anti-IL-12/IL-23	0%	6.3%	
JAK-blockade	5.7%	0%	

p-values above 0.05 are indicated in italic.
*The average dosage of each medication (MTX, glucocorticoid and leflunomide) was calculated only for the patients taking that medication. The p-values bove 0.05 are indicated in italic.
anti-CCP, anticyclic citrullinated peptide antibodies; CRP, C reactive protein; CTLA, cytotoxic T-lymphocyte-associated protein 4 inhibitor; DAS28, disease activity score; IL, interleukin; JAK, janus kinase inhibitor
; MTX, methotrexate; nd, not determined; TNF, anti-tumor necrosis alpha.

Untargeted analysis

Multivariate statistical analysis was performed on the bucketed ^{1}H single-pulse and CPMG NMR spectra in order to determine whether there were characteristic spectral patterns or peaks that distinguished between the two diseases.

Based on the results partial least squares discriminant analysis (PLS-DA) and random forest models, there was no evidence that any of the clinical and demographic covariates could influence the metabolomic patterns of the patients' sera (online supplementary figure SF1). Additionally, there were no significant correlations between the clinical or demographic covariates and the regions of the ^{1}H (figure 1C) and CPMG (data not shown) spectra.

Since principal components analysis-based clustering of metabolomics data is often difficult,[16–18] the ^{1}H and CPMG spectral data-sets were evaluated by PLS-DA. Even though the principal components 1–5 explained 96.9% of the variance of the ^{1}H data or 84.6% of the variance in the CPMG data, a clear clustering that could distinguish between the negRA and

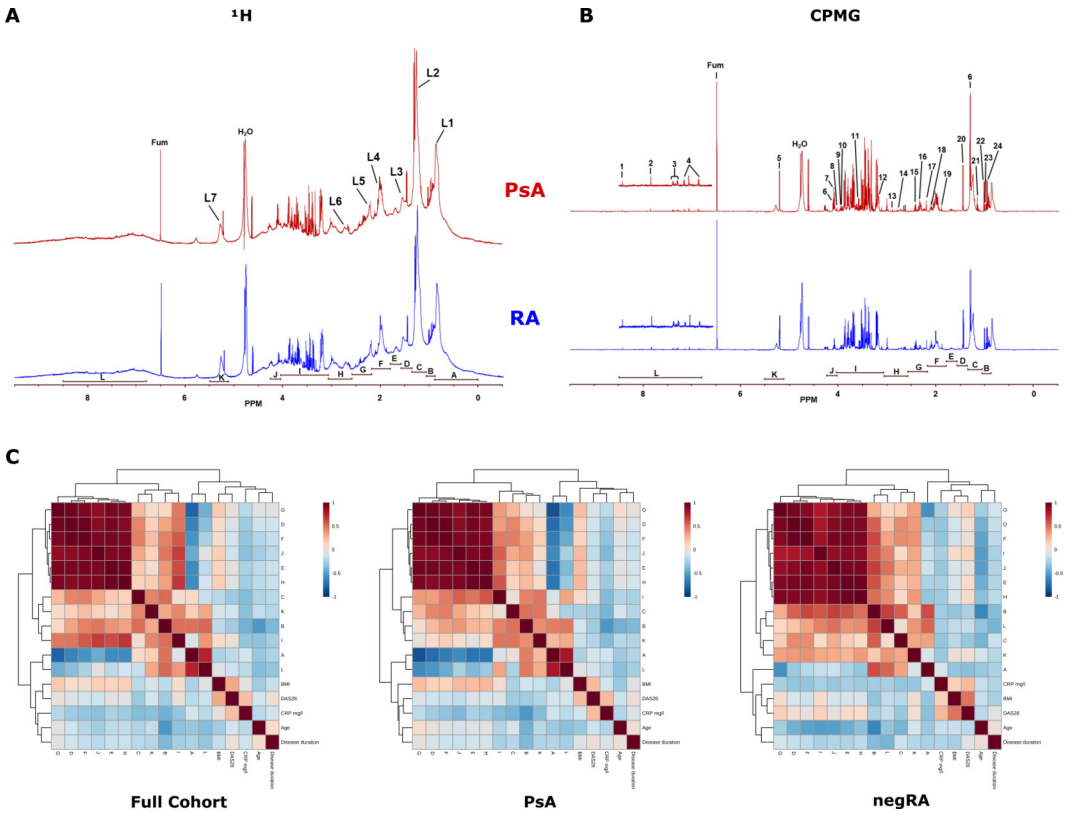

Figure 1 PsA and negRA patients have distinct spectral profiles that do not correlate with clinical and demographic covariates. Representative water-suppressed and baseline-corrected (A) ^{1}H single-pulse and (B) CPMG NMR spectra of blood serum from patients with PsA and negRA assigned with the regions and metabolites and lipid groups included in the untargeted and targeted analysis: (1) formate, (2) histidine, (3) phenylalanine, (4) tyrosine, (5) α-glucose, (6) proline, (7) lactate, (8) creatinine, (9) creatine, (10) creatine phosphate, (11) threonine, (12) choline, (13) sarcosine; (14) citrate, (15) glutamine, (16) succinate, (17) acetoacetate, (18) glutamate, (19) acetate, (20) alanine, (21) β-hydroxybutyrate, (22) valine, (23) isoleucine and (24) leucine. (L1) Lipid methyls, (L2) lipid aliphatic chain, (L3) lipid β-methylenes, (L4) lipid allylic methylenes, (L5) lipid α-methylenes, (L6) lipid polyunsaturated allylic methylenes and (L7) lipid alkenes. Fumarate (10 mM in 99.9% D_2O) was used as an internal standard. (C) Correlograms showing the Pearson correlation coefficients between the clinical or demographic variables and the ^{1}H spectral regions, and hierarchical clustering with Euclidean distance metric for the full discovery cohort, and the split PsA and negRA groups. negRA, seronegative rheumatoid arthritis; NMR, nuclear magnetic resonance; PsA, psoriasis arthritis.

PsA patients was not evident (figure 2A). When assessing the quality of the PLS-DA models, their accuracy was ≤65%, and both the R^2 and Q^2 values were very low (^{1}H-spectra: R^2=0.13, Q^2=0.06; CPMG spectra: R^2=0.16, Q^2=0.08). To improve the diagnostic accuracy based on spectral patterns, we used a random forest classification algorithm, due to its robustness for high dimensional data analysis. In both ^{1}H and CPMG spectra, the algorithm identified similar regions that classified negRA and PsA patients with an out-of-bag error of 0.361 for the classification based on the ^{1}H, and of 0.336 based on the CPMG (figure 2B,C). Nonetheless, the significant spectral regions identified by the random forest algorithm were used to focus the targeted analysis.

Targeted analysis

For the lipidomic analysis, the ^{1}H single-pulse NMR-spectra were used. Due to the broad character of the lipid signals, seven groups of lipid signals (L1–L7) were assessed and designated by the moieties present within[19]: L1: lipid methyls; L2: lipid aliphatic chain; L3: lipid β-methylenes; L4: lipid allylic methylenes; L5: lipid α-methylenes; L6: lipid polyunsaturated allylic methylenes and L7: lipid alkenes (figure 1A). Due to the broadness of the lipid signals, lipid groups were compared in patients as ratios relative to the lipid methyl group L1. In the metabolomic analysis using the CPMG spectra, we chose 24 metabolites that could be clearly identified and quantified and are present in the healthy human sera and have been reported to be altered in chronic arthritis[8 9 11 14 20–24] (figure 1B).

After quantifying the concentration of metabolites and lipid groups, the differences between patient groups were determined by univariate analysis. Among the 24 metabolites, nine had significantly different concentrations between both patient groups, namely the amino acids (AA) alanine, leucine, phenylalanine, threonine and valine and the organic compounds acetate, choline, creatine and lactate. In our analysis, lipid ratios L3/L1, L5/L1 and L6/L1 were found to be statistically different between negRA and PsA patients (figure 3A). There was a clear enrichment of certain metabolic pathways when comparing both groups (figure 3B).

Correlation between serum metabolites and lipids and clinical data of the patients

Age, gender and therapeutic regimen can influence the concentration of metabolites in biological fluids in different diseases,[25 26] thus impacting the definition of the biomarkers to be used in therapy-naïve patients or in patients of different ages. To analyse whether any of the clinical or demographic parameters could

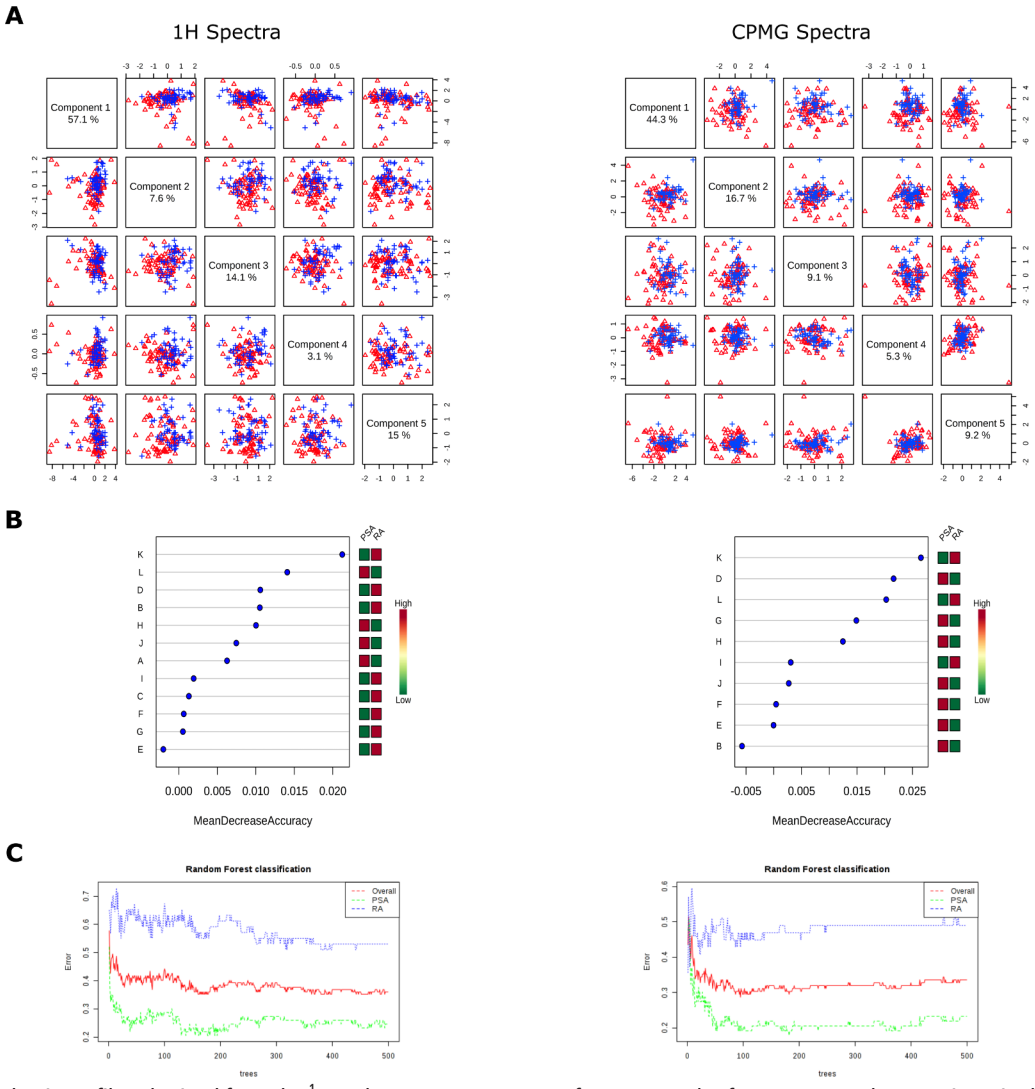

Figure 2 Metabolomic profiles obtained from the ^{1}H and CPMG NMR spectra of serum samples from negRA and PsA patients in the disovery cohort after supervised PLS-DA analysis and random forest analysis. (A) Pairwise scores plots between the five principal components with the corresponding variances shown in the diagonal. (B) Significant features identified by random forest. The features are ranked by the mean decrease in classification accuracy when they are permuted. (C) Cumulative error rates by random forest classification. The overall error rate is shown as the red line; the blue and green lines represent the error rates for each disease. negRA, seronegative rheumatoid arthritis; NMR, nuclear magnetic resonance; PLS-DA, partial least squares discriminant analysis; PsA, psoriasis arthritis.

have influence on the serum concentration of the 24 metabolites or the lipid groups, we carried out a one-way and multiway multivariate analysis of variance (MANOVA) of the associated metabolites and the potential clinical and demographic confounders (see online supplementary tables ST1–ST4). Disease activity was associated with changes in choline concentration and L2/L1 and L7/L1, while disease duration was associated with changes in the concentration of citrate, phosphocreatine, glucose, histidine, tyrosine and valine. Changes in metabolite concentrations and lipid ratios were equally seen when combining age and body mass index classes with the disease groups. Even though RA is a disease mainly affecting women, which contrasts with PsA, the MANOVA analyses combining disease groups and gender did not present any significant differences in the associated metabolites. The same was true when disease and therapy were combined. Univariate analyses did not present any significant correlations between metabolites' concentration or lipid ratios and clinical and demographic variables (figure 3C).

Multivariate diagnostic model for patient classification

Receiver operating characteristic (ROC) analyses of the single metabolites or lipid ratios yielded area under the curve values (AUC) lower than 70% (online supplementary table ST6). Thus, univariate models did not present enough sensitivity and specificity to classify PsA and negRA patients. In order to reach the highest diagnostic accuracy, we built three different machine learning algorithms: random forest, naive Bayes and multivariate logistic regression on the metabolomic and lipidomic profile of 73 PsA and 49 negRA patients. The random forest had an accuracy of 73.3% (Cohen's kappa 40.1%) and the naïve Bayes accuracy was 63.7% (Cohen's kappa 26.5%) to predict the probability of a patient having PsA (ROC curves not shown).

By the stepwise forward–backward selection algorithm, the following diagnostic predictors were included into the diagnostic model: age, gender, L6/L1, L5/L1, L2/L1, alanine, succinate and creatine phosphate.

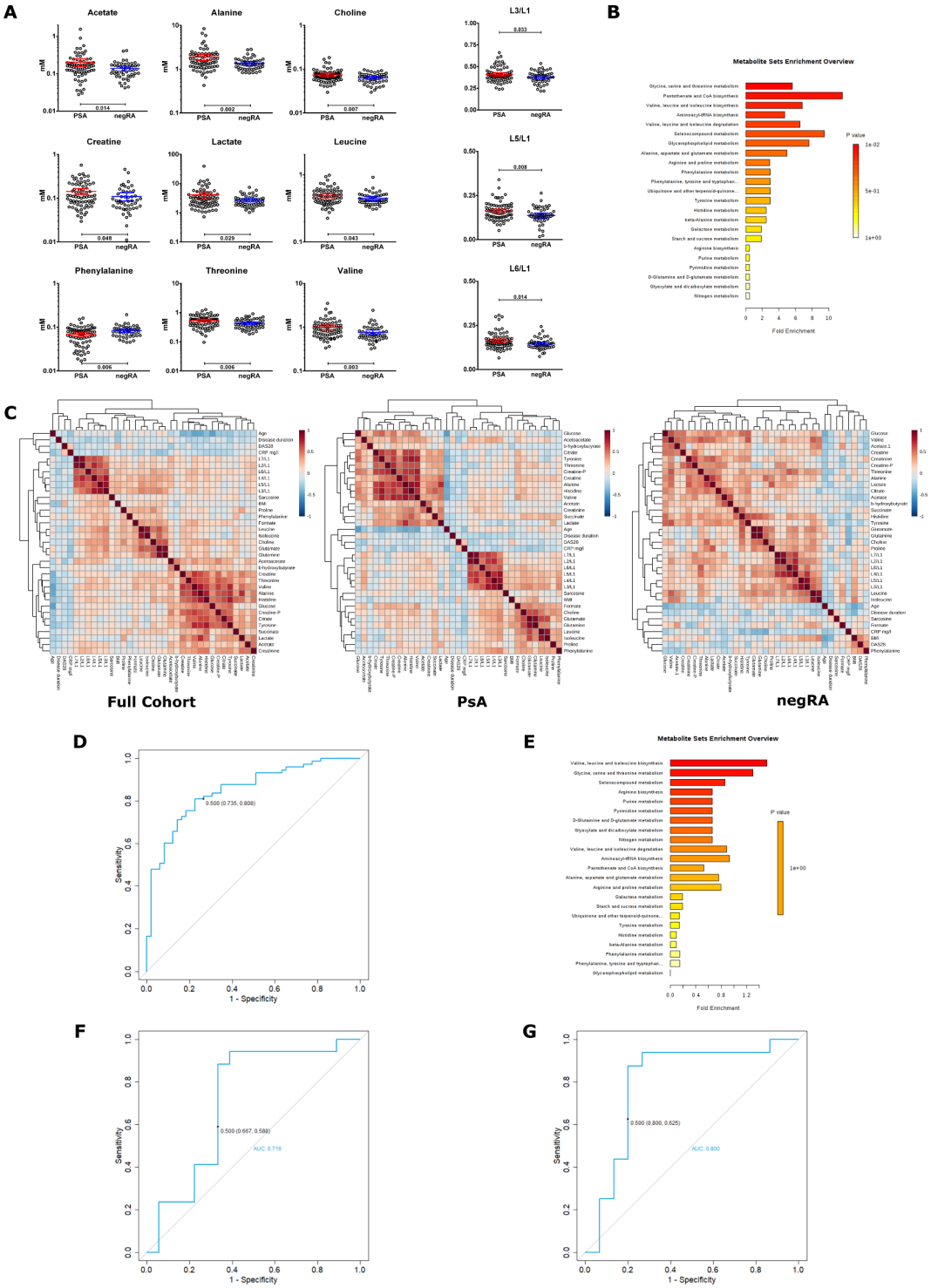

Figure 3 The concentrations of several metabolites and lipid groups allow the distinction between negRA and PsA patients. (A) Dot plots of the metabolites and lipid ratios included in the targeted analysis and that present significant differences between the two patient groups in the discovery cohort. Lines indicate the mean and 95% CI. (B) Summary bar graph for quantitative enrichment analysis showing the changes between negRA and PsA metabolomes in the discovery cohort. (C) Correlograms showing the Pearson correlation coefficients between the clinical or demographic variables and the metabolites, and hierarchical clustering with Euclidean distance metric for the full discovery cohort, and the split PsA and negRA groups. (D) ROC curve for the modelled probability p_{PsA} based on the cross-validation in the discovery cohort. (E) Summary bar graph for quantitative enrichment analysis showing the changes between negRA and PsA metabolomes in the blinded validation cohort. (F) ROC curve for the modelled probability p_{PsA} based on the blinded validation cohort. (G) ROC curve for the modelled probability p_{PsA} based on the reassessed validation cohort. negRA, seronegative rheumatoid arthritis; PsA, psoriasis arthritis; ROC, receiver operating characteristic.

In a first validation procedure, the resulting model was evaluated using a 10-fold cross-validation (CV), which yielded the coefficient estimates in table 2.

Employing these estimates into the regression model yields the following formula:

Table 2 Estimates of the model coefficients

	Estimate	SE	Test statistics*	P value	ORs
(Intercept)	1.046	2.018	0.518	0.604	.
Age	−0.055	0.025	−2.177	0.029	0.947
Gender male	2.412	0.640	3.767	<0.0001	11.155
L6/L1	16.653	8.676	1.919	0.055	17074068.923
L5/L1	16.639	6.820	2.440	0.015	16829326.675
Alanine	2.475	0.756	3.630	<0.0001	15.572
Succinate	−48.819	17.246	−2.831	0.005	0.000
Creatine phosphate	−11.231	4.818	−2.331	0.020	0.000
L2/L1	−1.619	0.681	−2.378	0.017	0.198

*The test statistic and the p value correspond to the Wald test, that is, test if the coefficient is equal to zero.

$$(F1)\log\left(\frac{p_{PsA}}{(1-p_{PsA})}\right) = x = 1.046 - 0.055 \times Age + 2.412 \times Male + 16.653 \times \left[\frac{L6}{L1}\right] + 16.639 \times \left[\frac{L5}{L1}\right] + 2.475 \times [Alanine] - 48.819 \times [Succinate] - 11.231 \times [Creatine\ Phosphate] - 1.619 \times \left[\frac{L2}{L1}\right]$$

The concentrations of each metabolite, age and gender (male=1, female=0) are substituted into the formula. The probability of belonging to the PsA group is then calculated by substituting the result × obtained in F1:

$$(F2)p_{PsA} = \frac{e^x}{(1+e^x)}$$

The probability of a patient belonging to the negRA group is given by:

$$(F3)p_{negRA} = 1 - p_{PsA}$$

To classify patients into the two groups, a cut-off value for the calculated probability (F2) has to be chosen. Usually, the cut-off value 0.5 is applied and a subject is classified to PsA if its estimated probability of having PsA is larger than 0.5, which reflects the idea to classify a subject to the diagnosis that is more likely. The following ROC plot shows the sensitivity and specificity of the model for this cut-off value and an overall area under the ROC curve (AUC) of 84.5%(figure 3D).

To further validate the model retrieved from the cross-validation procedure in a prospective way, a separate blinded sample of 35 new arthritis patients was collected (online supplementary table ST7). This cohort had a similar pathway distribution as the cohort used to build the diagnostic model (figure 3E). Evaluation of the cohort using the cut-off 0.5 yielded a correct prediction of 62.9% of the patients (table 3), and an ROC analysis was performed in which the AUC dropped to 71.6% (figure 3F).

After diagnosis prediction, there was a clinical re-evaluation of the 13 patients for which the predicted diagnosis was different from the one initially done by the rheumatologist. For four of those patients, a definite diagnosis could still not be assigned, as they kept lacking distinctive clinical parameters. Taking this into consideration, we removed those four individuals from the validation cohort and recalculated the prediction match, which increased to 71.0%, and performed a new ROC analysis that resulted in increased sensitivity (62.5%) and specificity (80.0%) (figure 3G).

Table 3 Classification table of the blinded validation cohort (numbers in parenthesis indicate how many individuals lacked distinctive clinical parameters after reassessment)

	Prediction	
Diagnosis	PsA	RA
PsA	10	7 (1)
RA	6 (3)	12

PsA, psoriasis arthritis; RA, rheumatoid arthritis.

DISCUSSION

A definite differential diagnosis between negRA and PsA is often impossible due to lack of clear clinical, serological or radiological parameters. As therapy differs, a reliable diagnosis is important to prescribe the correct treatment. Additionally, the chronic inflammatory processes leading to the characteristic joint destruction in RA and PsA patients may cause major and variant alterations in the metabolism of cells, tissues and organs.[27–29] Such metabolic alterations result in changes in the serum metabolome and lipidome that we were able to quantify with the aim of discovering biomarkers to improve the clinical differential diagnosis between PsA and negRA patients and learn more about the specific metabolomics processes in these chronic arthritides. By means of ^{1}H NMR-based metabolomics and lipidomic analyses, we were able to identify metabolites and lipid groups that differed in concentrations in the sera of negRA and PsA patients. A model was derived from these data to classify the patients into one of the two disease categories and was subsequently validated on separate blinded cohort of patients.

Even though we used different technical and metabolite identification approaches, we reached similar conclusions pertaining to the differences in the levels of the AAs alanine, leucine, threonine and valine between negRA and PsA patients as those reported in a metabolomic analysis of serum samples of healthy individuals, and PsA and total RA patients by mass spectrometry.[22] Serum alanine and valine levels in RA have been associated with synovial B-lymphocyte stimulator expression, and the serum levels of threonine, phenylalanine and leucine associated with synovial expression of IL-1β and IL-8.[24] Free serum AA can be the result of disease-related protein catabolism, but they can also regulate cell functions by controlling intracellular signalling cascades and gene expression.[30] In HeLa cells, alanine, valine and threonine act on the mammalian target of rapamycin complex 1 (mTORC1) in a two-step process in which they prime, and then activate, mTORC1 leading to the phosphorylation of its downstream targets.[31] On activation, mTORC1 is a major inducer of aerobic glycolysis in several cell types.[32 33] It is therefore not surprising that we found strong correlations between lactate levels (the product of aerobic glycolysis) and certain AA, particularly valine and alanine in PsA sera and to a lesser extent

in negRA sera. Furthermore, lactate has been shown to reshape $CD4^+$ T cell phenotype in arthritis towards a proinflammatory profile.[34] The high-energetic demand caused by chronic inflammation in the joint and skin could be related to the higher levels of serum creatine found in PsA patients. Creatine plays a major role in T cell proliferation and cytokine secretion by securing a continuous replenishment of the adenosine triphosphate (ATP) pool.[35]

Short-chain fatty acids (SCFAs), such as acetate, originate from microbiota in the gastrointestinal tract and are involved in a plethora of essential cellular, tissue and organ functions. However, disease-induced dysbiosis leads to altered local and systemic concentrations of SCFA resulting in functional modifications that contribute to disease exacerbation and development of comorbidities. Dysbiosis of the gut microflora has been reported for RA and PsA patients affecting bacteria families that are major SCFA producers.[36–38] However, the role of acetate and other SCFA in inflammatory diseases is still not fully understood as different animal models yield contradictory results. In experimental autoimmune encephalopathy, a model for multiple sclerosis, and in collagen-induced arthritis, a model for autoimmune polyarthritis, dietary supplementation with acetate leads to amelioration of disease scores. However, in another model of polyarthritis, acetate supplementation resulted in increased inflammation and joint destruction[39] and dietary supplementation of healthy mice with acetate resulted in kidney disease with increased serum levels of creatinine and urea, elevated systolic pressure and higher IL-17A and IFN-γ secretion by T-lymphocytes.[40] Nonetheless, SCFAs have a positive effect on increasing bone mass by suppressing osteoclastogenesis.[41]

Choline and acetyl-coenzyme A (CoA) build the neurotransmitter acetylcholine, which is found in the RA synovium.[42] Moreover, on action of choline kinase, choline is used to synthesise the cell membrane phospholipid phosphatidylcholine, which is present in synovial fibroblasts and associates with TNF-α production and migration.[43] Thus, it was not surprising that we detected changes in the serum concentrations of choline, which have been associated to the expression of synovial markers.[24]

The lipid groups L3, L5 and L6 show significant differences between PsA and negRA patients, being all higher in PsA. While L3 and L5 can mostly associate with changes in levels of lipids in the sera, since they reflect the lipid β-methylenes and α-methylenes, common to most medium and long-chain fatty acids, the L6 group reflects polyunsaturated allylic methylenes due to the presence of polyunsaturated fatty acids (PUFAs), which are known to play a central role in the homeostasis of the immune system. PUFAs have been associated with both proinflammatory (ω6-PUFAs) and anti-inflammatory (ω3-PUFAs) features.[44–46]

Even though the univariate analysis pointed to differences in the serum metabolome and lipidome between PsA and negRA patients, none of the identified compounds for itself could clearly and accurately distinguish between the two groups. Therefore, a multivariate approach was pursued that also accounts for possible interactions between the covariates, and a variable selection was performed for noise reduction. Even though the model was able to reach more than 70% prediction match in the blinded validation cohort, there were still four patients with a mixed diagnosis that could not be assigned to any of the groups. Consequently, we must accept that this proposed model will still fail to identify patients presenting clinical features of both diseases. Moreover, for a translation into clinical practice, it still needs to be tested in a larger multinational/multiethnic cohort for its validation in genetically heterologous populations. Nonetheless, our data propose expanding ^{1}H NMR-based metabolomic and lipidomic analyses as a biomarker discovery tool to other autoimmune diseases, for which differential diagnosis, response to therapy or disease prognosis are still hard to determine or predict.[47] Supported by several reports on successful implementation of ^{1}H NMR-based metabolomics as a routine diagnostic tool in clinical settings for non-autoimmune diseases,[23 48 49] our study helps paving the way to extend this technique to the routine diagnostic techniques for autoimmune pathologies. Moreover, our data once again highlight that the metabolomic processes associated with inflammatory rheumatic diseases are different between diseases even when clinical feature are similar. Consequently, metabolomics and lipidomics are starting to feed a completely new field of research in autoimmunity.

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Contributors Conceptualisation: MS-C, RAC and H-ML; methodology: MS-C, RAC, KDK and RB; formal analysis: LT, RB, KU, MS-C and RAC; investigation: LT, KU and KDK; resources: H-ML, MS-C, RAC and KDK; writing – original/ draft: LT, RB, KU, H-ML, MS-C, KDK and RAC; writing – review and editing: RB, MS-C, RAC, H-ML and KDK; supervision: MS-C, RAC and H-ML; funding acquisition: MS-C, RAC and H-ML.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. Original NMR spectra files are available on reasonable and justified request. Please contact the corresponding author.

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